IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
, Journal Volume 11, Iss 11, 2022

Assessing the Efficacy and Responsiveness of the Laboratory-Based Rapid Antigen Diagnostic Test for Detecting SARS - CoV-2 Antigens

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Abstract
Objectives: To detect severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and evaluate the diagnostic precision of the GenBody COVID-19 Antigen kit.
Methodology: Swabs were taken from individuals suspected of having COVID-19, specifically from the nose and throat. These swabs were then evaluated using the RT- the GenBody Rapid antigen kit. The performance characteristics of the antigen kit were computed.
Results: We performed analyses on samples collected from both the nasopharynx and oropharynx, with a total sample size of 50000. The rapid antigen test yielded a positive result for 21108 cases, showing an overall sensitivity rate of 42.21%. The mean cycle threshold (Ct) values in individuals who tested positive for COVID-19 were 27.3. In addition, among the overall number of infected cases, 27,550 were men, accounting for 55.1% of the total, while 22,450 were females, making up 44.9%. Furthermore, 50.15% of all the individuals who were affected had symptoms. Subsequent examination revealed that 36% of patients who received a positive result on the Rapid Diagnostic Test (RDT) were within the age range of 15-47, whereas 50% of RDT-positive patients were in the age bracket of 48-63.
Conclusion: The fast antigen kit performed well in detecting high viral load samples, however, it missed detecting samples with lower levels of the virus. Regrettably, the antigen kit's insufficient sensitivity precludes its use as the sole primary diagnostic kit for diagnosing COVID-19.
Keywords: *COVID-19, Cycle threshold, Rapid Diagnostic Test, SARS-CoV-2, Viral load*

1. Introduction

Since it was first documented in December 2019 in Wuhan, the capital city of Hubei province in China, the pandemic caused by the coronavirus disease 2019 (COVID-19) has caused widespread destruction all over the world (Mohan and Nambiar, 2020; Yang et al 2020). SARS-CoV-2, also known as the severe acute respiratory syndrome coronavirus, is a virus that is composed of a single strand of RNA and is classified under the genus Beta of the family Coronaevirideae (Chauhan. 2020). This newly discovered coronavirus disease, known as COVID-19, has rapidly spread across the entire world due to its high rate of transmission (Zhao

et al., 2020). It was on March 11, 2020 that the World Health Organisation (WHO) announced that COVID-19 had been classified as a pandemic (Zheng et al., 2020).

Viral culture and reverse transcription-polymerase chain reaction (RT-PCR) assays are considered to be the gold standard methods for diagnosing SARS-CoV-2 infections. Even though real-time polymerase chain reaction (RT-PCR) takes a few hours to identify the nucleic acid and to isolate the virus from materials takes a few days, these tests require a specialized instrument and individuals who have been trained in that particular sector (Zhang et al., 2020, Sethi and Chakraborty, 2021). It has been observed that the number of instances has been increasing, particularly in nations such as the United States of America, Brazil, and India (Wu et al., 2020). Therefore, quick testing for SARS-CoV2 could potentially play a significant role in mass screening (Younes et al., 2020), which would help to facilitate the prevention and control of the disease as well as screening before any surgical treatment (Yan et al., 2020).

Rapid antigen detection (RAD) tests are immunochromatographic tests that are commercially available as lateral flow (Kim et al., 2021; Daoud et al., 2020). These tests detect viral antigens by using an immobilized SARS-CoV-2 antibody that is coated on the device (Kim et al., 2021). These lateral flow assays, which make use of monoclonal antibodies that are specific to SARS-CoV-2 antigens and are retrieved from nasopharyngeal and oropharyngeal swabs, have the potential to be utilized as screening tests if their diagnostic accuracy is comparable to that of real-time RT-PCR assays (Guan et al., 2020). In addition to requiring no specialized equipment, the RAD test can be completed in a shorter amount of time and can provide results in as little as thirty minutes (Cao et al., 2019). As a result, RAD tests have the potential to significantly increase turnaround time and reduce the amount of work that is required in diagnostic hospitals and laboratories that are already under burden (Ai et al., 2020). According to the World Health Organisation (WHO), the usage of RAD tests to detect antigens for SARS-CoV-2 is not indicated for clinical diagnosis. Further evaluation of this role is required.

In the current research work the GenBody COVID-19 Antigen test, was utilized to evaluate its effectiveness in laboratory confirmation of cases. The chief purpose of the research was to determine the performance parameters of the RAD test to identify SARS-CoV-2.

2. Methodology

2.1 Materials

2.1.1. Data sources and mode of ascertainment

Data of all SARS-CoV-2 (COVID-19) registered under the OPD and IPD in MMG district hospital area was collected. Data was collected for a two-year period between September 2020 to September 2022. Data was collected from SARS-CoV-2 (COVID-19) registers and lab reports from a major SARS-CoV-2 (COVID-19) Unit in the MMG district hospital, Ghaziabad. Data obtained from the lab reports was cross checked by verification of cases from SARS-CoV-2 (COVID-19) registers for the respective years.

2.1.2. Data entry and variables

Data was entered into Microsoft Excel worksheet (version 2019). Data of a two period September 2020 to September 2022 was collected for the following:

a. Total number of chests symptomatics registered in the MMG district hospital, Ghaziabad between September 2020 to September 2022.

b. Total number of SARS-CoV-2 (COVID-19) cases diagnosed in the MMG district hospital, Ghaziabad based on Nasooropharyngeal swab Rapid Antigen Detection (RAD) test September 2020 to September 2022.

2.1.3 Analysis of data

The diagnostic and screening efficiency of current routinely used diagnostics was determined through this analysis. Screening efficiency was defined as the number of SARS-CoV-2 (COVID-19) cases diagnosed using a given diagnostic from among all symptomatic. Diagnostic efficiency was defined as the number of SARS-CoV-2 (COVID-19) cases diagnosed based on positive smear results or other diagnostics in the diagnostic paradigm amongst all SARS-CoV-2 (COVID-19) cases (Zhao et al., 2020).

2.2. Methods

2.2.1. Ethical considerations

Before testing of clinical samples brainstorming sessions with the Chief Medical Officer (SARS-CoV-2 (COVID-19)), Chief Pathologist, and Radiologists of the SARS-CoV-2 (COVID-19) Unit were organized. Necessary permission from the MMG district hospital, Ghaziabad authorities was taken to test the sample after it had been processed for all investigations at the SARS-CoV-2 (COVID-19) Unit. Diagnostic decisions were made by site physicians according to the COVID-19 guidelines (Zou et al., 2020).

2.2.2 Inclusion Criteria

15-47years (Youth group) and 48-63years (Middle age group) were included in the present study.

2.2.3 Exclusion Criteria

The pediatric group (0-14 years), old age group (>64 years), pregnant women, and patients suffering from other life-threatening diseases were not included in the present study.

2.2.2 Laboratory Analysis

2.2.2.1 Specimen collection

The Nasooropharyngeal swab was placed in the sterile viral transport media (total volume 3 mL) and sealed securely. Sample (n=50,000) was stored at -4-to -20°C for 24 hours and -80°C for long time. Virus concentrations in samples were estimated from cycle threshold (Ct) value. All the specimens were processed in biosafety cabinet level 2 (BSL 2 Advanced) following all infection control practices.

2.2.2.2 Rapid Antigen Detection Test

The antigen test is a quick and reliable way to detect the presence of SARS-CoV-2 nucleocapsid (NP) antigen in human respiratory samples. To conduct the test, 400 µL of the sample is added to the extraction buffer provided in the GenBody COVID-19 Antigen kit. The nasopharyngeal (and oropharyngeal) swab sample(s) is then inserted into the extraction solution and mixed 8-10 times. After that, the swab is removed and pressed against the solution tube to extract most of the specimen (Yang et al., 2020). Four drops (~100 µL) of the extracted solution are then dropped into the sample well (S). The test results can be interpreted after 15-20 minutes as per the instructions provided with the kit.

2.2.2.3 Statistical analysis

For statistical analysis of the patient demographics, descriptive statistics were used. The continuous data were presented in mean, standard deviation (SD), median, and range (Xiong et al., 2020). To calculate sensitivity, specificity, positive predictive value (PPV), and negative

predictive value (NPV), IBM SPSS Statistics for Windows software v22 (IBM Corp., Armonk, NY) was used.

3. Results and Discussion

According to a current study that tested suspected COVID-19 cases using a rapid antigen kit, 42.21 % were positive for SARS-CoV-2 nucleocapsid antigen. The median age of COVID-19 cases was 35 years, ranging from 15 to 63 years. Out of the total infected cases, 27550 were males (55.1%) and 22,450 were females (44.9%). Moreover, 50.15% of the total infected cases showed symptoms.

Table 1: Detection of SARS-CoV-2 by COVID-19 rapid Antigen Detection Kit

Sample Type	**N=50000**				
	Ct value				
Nasooropharyngeal	**Mean**	**Range**	**Tested**	**Positive**	**Sensitivity**
	27.3	11.76-34.65	50000	21108	42.21%
Viral Load					
Ct<17.48		12.74-17.48	11102	9804	88.3%
Ct>17.48		17.48-30.01	38898	11304	29%

The average cycle threshold (Ct) values in COVID-19 positive cases were 27.3 (minimum 11.76, maximum 34.65). The sensitivity of RAD was found to be 42.21%. Based on the Ct cut-off value, samples with Ct< 17.48 were considered as high viral load and Ct > 17.48 were considered as normal viral load. The corresponding Ct values, mean Ct value, Ct value range, and sensitivity were calculated as depicted in table 1. A significant difference in the performance of the rapid detection test (RDT) was found in patients with 'high viral load' and 'normal viral load' (p-value = .000022).

Table 2: Performance of RDT in different age groups

Age group (years)	**Sample tested**	**RDT Positive**	**Percentage Positive**
15-47	27503	9900	36%
48-63	22497	11208	50%

Upon further analysis, it was found that 36% of patients who tested positive for RDT were between the ages of 15-47, while 50% of RDT-positive patients were aged between 48-63.

In this study, we evaluated the performance of the RAD test, a rapid antigen kit, in detecting the SARS-CoV-2 virus in respiratory samples. Prompt diagnostic testing for SARS-CoV-2 is crucial for managing infected patients and limiting the spread of the virus. With the ongoing pandemic, rapid antigen immunoassays can speed up the screening process. Several rapid tests have been developed by companies based on SARS-CoV-2 proteins in respiratory samples, but their performance is affected by various factors such as viral load, specimen quality, the method of processing the sample, stage of infection, and the setting of the patient.

The swift dissemination of SARS-CoV-2 necessitates the presence of a prompt, precise, and cost-effective diagnostic examination. Swift diagnosis can effectively curb the transmission of the infection during its early phases and prove highly advantageous for individuals who have come into touch with symptomatic patients and those who are highly contagious. Conducting RT-PCR testing for SARS-CoV-2 in a densely populated country such as India poses significant challenges. Therefore, the RAT can be employed as a quick point-of-care test for the detection of SARS-CoV-2. The virus's nucleocapsid protein is highly abundant in the nasopharyngeal samples, making it the chosen target for antigen detection.

A recently licensed fast antigen test was tested for sensitivity and specificity using 137 clinical samples from two universities. Test sensitivity was 88.2–89.6% for infectious patient samples with viral loads. Of 32 rRT-PCR-positive samples, 19 showed cell culture infectivity and 84% were antigen reactive. This antigen test found seven full-genome sequenced SARS-CoV-2 isolates and SARS-CoV-1 without cross-reactivity to other respiratory viruses (Toptan et al., 2021).

The current Indian recommendations and advisory bodies suggest that asymptomatic patients should undergo initial testing using only the fast antigen detection test (Kanaujia et al., 2021). The test findings can be classified as either true positive or true negative. However, the RAD test is less sensitive than RT-PCR as in RT-PCR a small amount of viral genetic material can be amplified. In the current study, RAD recognized SARS-CoV-2 in all samples with low Ct, but its sensitivity decreased with higher Ct values. Thus, RAD may misdiagnose SARS-CoV-2. Very viscous materials might induce false-positive results even when viscosity is unknown. Despite precautions, handling many patient samples could cross-contaminate.

Conclusion

The rapid antigen test (RAT) demonstrated a high level of sensitivity in individuals who presented with symptoms at the early stages of the disease they were experiencing. Considering the vast population and the fact that the majority of SARS-CoV-2 patients do not exhibit any symptoms, the testing method that was developed by the Indian Council of Medical Research (ICMR) at the national level was efficient in terms of cost. Therefore, RDTs have the potential to play a crucial part in the early diagnosis, policy formation, and surveillance of the SARS-CoV-2 virus.

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