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Original Article

Comparison of COVID-19 Rapid Antigen Test Results with PCR Reactive/ Non-Reactive Cases

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ABSTRACT

Objective: To evaluate the analytical accuracy of the COVID-19 Ag Rapid Test Device by Abbott PanbioTM using RT-PCR as the reference assay and to detect any false-positive/negative reactions to assess the specificity of this analytical procedure.
Study Design: Cross-Sectional analytical study.
Place and Duration of Study: Department of Chemical Pathology, Chughtai Institute of Pathology, Lahore Pakistan, from Feb to May 2021.
Methodology: This study was comprised of a total of 105 samples. The result of these nasopharyngeal specimens was already established by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). Then the same (nasopharyngeal) specimens were analysed on Rapid Antigen Detection Test Device.
Results: Out of 105 specimens, we got 88(83%) PCR positive samples and 17(16%) PCR negative, which, when compared with the Abbott PanbioTM Rapid Antigen Test Device, showed 60(57%) positive and 45(42.8%) negative samples having a sensitivity of 75(86%) (CI 67.04%-83.32%) and specificity of 100%(80.49-100%). The accuracy of the test was found to be 78(95%). This assay was found convenient and more appropriate for outdoor settings.

Keywords: COVID-19, RT-PCR, Rapid antigen test, Specificity, Sensitivity.

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INTRODUCTION

The coronavirus shares similar features to seasonal influenza and viral pneumonia. However, its range of clinical symptoms and the spread characteristics are different.[1,2] Thus, effective control of the community spread of this infection is highly important. Presently, the standard method for diagnosing SARS-CoV-2 cases is considered to be RT-PCR.[3] However, in order to perform RT-PCR assays, specific equipment and services are required, and this test is costly with a high turnaround time.[4] Hence for better screening and isolation of such patients, a rapid antigen detection immunoassay (RAD) has been introduced and is particularly suitable for point-of-care testing (POCT).[5]

The sample requirement for rapid antigen tests is the same as for RT-PCR. However, the analytical technique used in this rapid antigen detection device is ''lateral flow immunoassay''. These devices are available in the form of disposable plastic kits.[6] These RDT cassettes already have viral antibodies on the test strips, bound to viral antigens in the patient sample.[7] These antibodies, already present on test strips, are either fluorescent-labelled or colloidal gold based. The antigen is detected as positive in the form of clear lines appearing on the test strip (Colloidal Gold-based Immuno-assays) or by fluorescence technique which can be analysed using" an immune-fluorescence analyser.[8]

However, before adopting this assay, we should validate this alternative method of rapid antigen detection test, which can detect the virus antigen in respiratory samples, despite the antibody tests which detect antibodies against the respective antigen.[9] The reported number of SARS-CoV-2 cases represents only a small population, including mostly symptomatic patients, but the real number involving such cases is much more.[10] For this purpose, we conducted a study comparing this newly introduced Rapid Antigen Detection test with RT-PCR, so this assay can be used to recognise infection with different presentations and easy approaches.

METHODOLOGY

The cross-sectional study was performed at Chughtai Institute of Pathology, Lahore from February to May 2021 after getting approval from Institutional Review Board (IRB certificate no. CIP/ IRB/ 1058).

Inclusion Criteria: Patients of COVID-19, PCR Reactive (Positive)/Non-reactive cases (Negative) irrespective of age and gender were included in the study.

Exclusion Criteria: Autoimmune Disorders, Other viral diseases like Hepatitis A, B, and C, Infectious

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diseases, PCR samples of all other viral diseases were excluded from the study.

The study comprised of 105 nasopharyngeal swabs of COVID-19 patients, including positive and negative samples. The All these samples were first analysed on RT-PCR. Then these nasopharyngeal swab samples were stored at adequate temperature for further analysis by COVID-19 Rapid Antigen Test Device (Abbott Diagnostics, Germany), an immunochromatographic assay using lateral flow technique. This assay can detect SARS-CoV-2 antigens and strengthen the PCR reactive and non-reactive results in nasopharyngeal specimens of COVID-19 patients. This RDT kit has two lines on the strips: control (C) and test (T), which are pre-coated with antibodies. The control (C) region is covered with mouse monoclonal antibodies, and the test (T) region is covered with mouse monoclonal antibodies against the SARS-CoV-2 antigen. A visible line in the result window shows a positive result for human IgG antibodies against SARS-CoV-2 Ag gold conjugate. Hence, this visible control line is mandatory for the test to be authentic. The software used was EP Evaluator to compare RT-PCR and Rapid Antigen Detection tests. Study variables were the presence or absence of disease, gender and stability of the sample.

Statistical Package for Social Sciences (SPSS) version 23:00 was used for statistical analysis. Percentages and frequencies along with negative predictive value and specificity were calculated, an EP evaluator was used to study the agreement between the two tests and 95% CI was calculated by the scoring method. The test agreement >75% showed a high agreement. McNemar Test has been used for symmetry. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

In this study, 105 cases were analysed on RT-PCR and lateral flow rapid antigen tests. In our study, the mean age of the participants was 42.8±15.6 years, and most cases were male (53%). In addition, already established PCR reactive and non-reactive samples were evaluated using rapid antigen tests. A total of 60(57.1%) were positive, and 17(16.1%) came out negative on both PCR and COVID-19 Ag Rapid Test, while 28(26.6%) were positive on PCR and negative on COVID-19 Ag Rapid Test Device (Table-I).

EP Evaluator evaluated the results for Method Comparison between RT-PCR and COVID-19 Ag Rapid Test Device by Abbott PanbioTM. There is an agreement of 73.3% between the two methods. The result shows a sensitivity of 68% with a Confidence Interval of 67.04%–83.32% and a specificity of 100% with a Confidence interval of 80.49%-100%. The positive predictive value of the disease is 100%, and the negative predictive value is 37.78% (Table-II). The accuracy of the results was about 78.95%. Simple linear regression was used to test if rapid antigen tests significantly predicted PCR results. The fitted regression model was Y=mX+b, where Y was the response (dependent) variable, X WASs the predictor (independent) variable, m WAS the estimated slope, and b is the estimated intercept. The overall regression was statistically significant (R=0.508, F (df 1, df 104)=35.73, *p*= <0.001). The symmetry between COVID-19 Ag Rapid Test Device and RT-PCR was shown in the Table-III.

Table-I: Results of Nasopharyngeal Swabs analyzed on RT-PCR and Rapid antigen Device (n=105)

Parameters	Reactive	Non-Reactive
RT-PCR	88(83.8%)	17(16.1%)
COVID-19 Ag Rapid Test Device	60(57.1%)	45(42.8%)

Table-II: Diagnostic Parameters Showing Outcomes of Study

	RT-PCR Positive	RT-PCR Negative
COVID-19 Ag Rapid Test Device Positive	60(57.1%)	0(0%)
COVID-19 Ag Rapid Test Device Negative	28(26.6%)	17(16.1%)

*Sensitivity= TP/(TP+FN)=60/(60+28)*100=68%, Specificity=TN/ (TN+FP)= 17/(17+0)*100=100%, Positive Predictive Value=TP/(TP+FP)* 100=60/ (60+0)= 100%, Negative Predictive Value=TN/(TN+FN)*100=17/(17+28)= 37.7%, Diagnostic Accuracy=(TP+TN)/All patients*100= (50+6)/58=96%*

Table-III: Symmetry between COVID-19 Ag Rapid Test Device and RT-PCR.

Methods	Results (Different on both methods)	Results (Same on both methods)
Test<reference	28(26.7%)	17(16.1%)
Test>Reference	0(0%)	60(57.1%)

(Test method=Rapid Device) (Reference method=PCR)

DISCUSSION

Rapid Antigen Detection tests aim to aid in confirming or screening COVID-19 patients with or without symptoms, but their analytical sensitivity and specificity may vary from PCR. This correlates with a study published in 2020 which comprised 138 nasopharyngeal samples, out of which 94(68.8%) were PCR reactive for SARS-CoV-2. Out of these 94 PCR-reactive samples, with rapid antigen detection test, only 47 specimens came positive, thus, resulting in a sensitivity of 50.0%. Thus, the sensitivity and specificity of the rapid antigen detection were found to be less than PCR assay.[11]

However, the rapid antigen detection test could be a rapid and easier method to perform for separating symptomatic SARS-CoV-2 individuals from asymptomatic individuals.[12] These Rapid Antigen detection devices have many advantages, as they are user-friendly and transportable and can be used for patient ease at the bedside and even in outdoor settings.[13] Also, these devices do not have complicated steps, are cost-effective and provide results in a short period.[14]

In another study conducted in the year 2020, a total of 368 COVID-19 PCR reactive samples were received in adequate quantity, and then the same samples were analysed by BIOCREDIT COVID-19 Ag test. During testing, it was observed that the detection ability of the RAD test was 1000-fold less compared with RT-PCR. Although the manufacturer of this rapid antigen test kit mentioned in the data that this test can identify the SARS-CoV-2 virus in nasopharyngeal specimens but with diverse sensitivity.[15] Rapid antigen detection tests might be associated with the stability of the nasopharyngeal specimen as it was found to be more sensitive, and the results were more accurate in the initial phase of symptomatic infection when antigen tests were performed on these specimens.[16]

Another study was performed in Korea in April 2021, where 38 symptomatic COVID-19 patients were engaged, and a total of 200 nasopharyngeal specimens were collected serially. The result showed that RATs (Rapid Antigen Tests) showed a sensitivity of 91.3-100% and specificity was 98.7-98.9% depending upon the time and period of sampling to the patient's symptoms.[17]

In a survey conducted in France, a total of 204 PCR-reactive samples was included, and a raid antigen test was performed on these samples; among these samples, 154 were found to be positive on the RAD kit with a sensitivity of 75.5%. On the other hand, seven samples of asymptomatic patients showed positive results on the RAD kit, which were negative on PCR (specificity, 94.9%).[18]

Similarly, in one of the research projects conducted in South Africa, the Rapid Test device by Abbott-PanbioTM was validated in 535 participants. The test yielded a sensitivity of 85.5% (95% CI: 78.0–91.2), with 106 positive cases on the Rapid antigen test device and 124 positives for RT-PCR. While out of 411 RT-PCR negative individuals, specificity was 100.0% (95% CI: 99.1–100).[19] Showing a strong co-relation with our study.

However, using an adapted sample proved to be an advantage of this test that helped compare RT-PCR & RDT from the same material without possible errors induced through separate swabs. On the other hand, sample stability is of prime importance, which should be considered for conducting a Rapid antigen test.

LIMITATION OF STUDY

This test has a few limitations; first, the specimen should be collected and transported properly; otherwise, a false negative test result may arise. False results may occur if the test reading is taken at most 15 minutes or after 20 minutes. A negative test result does not exclude the possibility of SARS-CoV-2 infection, and the result must be established after viral culture or other molecular assays.

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CONCLUSION

We concluded in this study that the samples of the symptomatic patients of COVID-19 presented in the first week of clinical symptoms, when analysed on antigen-coated immunofluorescence rapid antigen detection test kit, manifested a high degree of sensitivity and specificity. Thus, a relation of sample stability was also established with the RDT kit for reliable results for diagnosing COVID-19. Therefore, this assay is of dynamic importance for the initial diagnosis/screening of COVID-19. Moreover, for people who are highly clinical suspects of COVID-19 and have negative rapid antigen detection tests, RT-PCR tests would still be considered a preferred option.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NZ & MDK: Data acquisition, conception, study design, approval of the final version to be published.

HB & ORC: Data analysis, data interpretation, approval of the final version to be published.

ASC & MS: Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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