Original Research Article

Correlation of COVID-19 antigen test and CBNAAT results at a single tertiary care hospital in North India: an experience

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ABSTRACT

Background: Nucleic acid amplification test (NAAT) is considered gold standard in the molecular diagnosis of CoV-2 infection but since it is costly, labor intensive and needs technical expertise, rapid chromatographic immunoassay for the qualitative detection of specific antigens to SARS CoV-2 have been devised. Objectives of this study was to compare the results of Antigen test and NAAT for CoV-2 infection carried out during the months of July and August 2020 by single tertiary care hospital in Lucknow, Uttar Pradesh and to determine the utility of rapid antigen test in the SARS CoV-2 diagnosis.

Methods: All the patients who came to our hospital seeking admission during July 2020 and August 2020 were included in the study. A total of 1000 patients were included in this study.

Results: Out of a total 1000 cases which were included in the study, 769 cases (76.9%) were found to be SARS CoV-2 negative by both antigen and CBNAAT, 100 cases (10.0%) were SARS CoV-2 positive by both antigen and CBNAAT tests. But in 131 cases (13.1%), antigen was not able to pick up the disease. It was also found that the Cycle Threshold (Ct) value for the discordant group was higher (Mean E= 28, Mean N2=33) when compared to the group where antigen was positive.

Conclusions: The present study establishes the role of rapid antigen tests in contributing to the quick, point of care diagnosis of SARS CoV-2. These assays are safe, simple, and fast and can be used in local clinics and hospitals. These tests are very important for real-time patient management and infection control decision.

Keywords: COVID-19, CBNAAT, Rapid Antigen Test, SARS-CoV-2

INTRODUCTION

The ongoing pandemic of the novel coronavirus (CoV-2) has posed a challenge for public health laboratories. The NAAT assays remain the test of choice for the etiologic diagnosis of SARS-CoV-2 infection. But NAAT is a costly, labor intensive and time taking procedure.[1,2] Hence, a rapid immunochromatographic immunoassay has been devised for the qualitative detection of specific antigens to SARS CoV-2 present in human nasopharynx.[3-5] This study was designed to compare the results of Antigen test and PCR for CoV-2 carried out by single tertiary care hospital in Lucknow, Uttar Pradesh and also to assess the utility of the Rapid Antigen test for SARS CoV-2.

METHODS

Patients and samples

The patient population comprise of all the cases who seeked admission to Sahara Hospital, a tertiary care hospital in Lucknow in July and August 2020. Study was performed at Department of Laboratory Medicine, Sahara Hospital, Lucknow. Since our hospital was not a COVID hospital then, therefore patients were not suffering from

symptoms suggestive of COVID (no febrile illness, shortness of breath, Influenza like illness). Data includes age and sex. All cases were subjected to both Antigen test and CBNAAT.

Collection of specimen

The person taking the specimen essentially wore personal protective equipment (PPE). To obtain Nasopharyngeal swab specimen, the swab must be inserted deeply into the nasal cavity. Patients will likely flinch, but that means the swab has hit the target. Swabs should be kept in place for 10 seconds while being twirled three to five times. Swabs should have flocked nontoxic synthetic fibers, such as polyester, as well as synthetic nylon handle.

Antigen test

The Antigen test was done using Standard Q COVID-19 Ag Test Kit manufactured by SD Biosensor, Korea and validated by ICMR. It is a rapid chromatographic immunoassay for the qualitative detection of specific antigens to SARS-CoV-2 present in human nasopharynx. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. During the test, SARS-CoV-2 antigen in the specimen interacts with monoclonal anti-SARS-CoV-2 antibody conjugated with color particles making antigen-antibody color particle complex.

This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen. The intensity of colored test line will vary depending upon the amount of SARS-CoV-2 antigen present in the specimen.

The kit comprises of test device, extraction buffer tube, nozzle cap, sterile swab. The specimen needed is nasopharyngeal swab. Immediately insert the swab into an extraction buffer tube. While squeezing the buffer tube, stir the swab more than 5 times. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab. Press the nozzle cap tightly onto the tube. Apply 3 drops of extracted specimen to the specimen well of the test device. Read the result in 15-30 minutes. Do not read the result after 30 minutes, it may give false results. A colored band will appear in the top section of the result window to show that the test is working properly. This band is control line (C). If no control line appears, the test is considered invalid. A colored band will appear in the lower section of the result window. This band is test line of SARS CoV-2 antigen (T). Even if the test line is faint, the test should be considered positive.

Nucleic acid amplification test (NAAT)

It was done by cartridge-based nucleic acid amplification test (CBNAAT) using Cepheid's Xpert Xpress SARS-CoV-2 test. The nasopharyngeal swab is immersed in Viral Transport Media (VTM) from HiMedia. The swab is broken at the breakpoint. The VTM is then transported to the lab and were stored at 40° C until processing. Further processing of the specimen is carried out in Biosafety cabinet class II.

Preparing the cartridge

Remove a cartridge from the package. The sample is mixed well with the media by rapidly inverting the VTM containing specimen 5 times. Remove the transfer pipette from the wrapper. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the VTM. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 μl) into the large opening (Sample Chamber) in the cartridge. Dispose of the used pipette. Close the cartridge lid. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Click Start Test (GeneXpert Dx). Open the instrument module door and load the cartridge. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge. The result is available in 1 hour 30 minutes.

Interpretation of results

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC). The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets; E gene and N2 gene according to the algorithm shown in Table 1.

Table 1: Xpert xpress SARS-COV-2.

E gene	N2 gene	SPC	Result	Further Action
+/-	+	+/-	Positive	-
+	-	+/-	Presumptive Positive	Retesting Required
-	-	+	Negative	-
-	-	-	No Result	Retesting Required

RESULTS

Thus, out of a total 1000 cases which were included in the study, 769 cases (76.9%) were found to be SARS CoV-2 negative by both antigen and CBNAAT, 100 cases (10.0%) were SARS CoV-2 positive by both antigen and CBNAAT tests.

But in 131 cases (13.1%), antigen was negative but CBNAAT was positive. The false negative rate by antigen is 13.1%. Thus overall senstivity of the antigen test is 43.3%, specificity is 100%, positive predictive value is 100% and negative predictive value is 14.5%. The spectrum of cases is summarised in Table 2.

Table 2: Spectrum of cases.

100 (True positive)	0 (False positive)
131 (False negative)	769 (True negative)

We further studied this group where antigen and CBNAAT results were discordant. The mean age of patients was 49.7 years with range from 05 months to 84 years. Male:female ratio was 1:2.5. We also studied the Cycle Threshold (Ct) value for these positive cases as shown in Table 3.

Table3: Cycle threshold (CT) value for positive cases by CBNAAT.

Antigen negative and CBNAAT positive			Antigen positive and CBNAAT positive		
	E gene	N2 gene		E gene	N2 gene
Minimum value	0	0	Minimum value	16	16
Maximum value	46	44	Maximum value	28	28
Mean	28	33	Mean	20	22

DISCUSSION

Coronaviruses have single-stranded RNA genome that can be detected using nucleic acid amplification tests.[6] The tests detects various structural proteins, including envelope glycoproteins spike (S), envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N).[7] In addition, there are species-specific accessory genes that are required for viral replication; RNA-dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE), and open reading frames ORF1a and ORF1b.[6-8]

Rapid antigen tests detects the envelope glycoprotein spike (S) whereas CBNAAT detects E and N2 gene. Studies prove that Rapid antigen tests provide the advantage of quick results and low-cost but have poor sensitivity.

Two clinical studies conducted at ICMR and the All India Institute of Medical Devices, Delhi evaluated the antigen test and reported a 50.6%-84% sensitivity and 99.3%-100% specificity. Thus there is a concern that antigen detection may miss cases due to low infectious burden or sampling variability.

We observed that Rapid antigen tests (Standard Q COVID-19 Ag Test Kit manufactured by SD Biosensor, Korea) detected a substantial number of cases. Our senstivity is 43.3% which is relatively lower in comparison to other researchers, this can be attributed to the asymptomatic population on which we have based our study. We also observed rapid antigen tests gives positive result even in asymptomatic or non-specific symptoms cases who do not have a positive contact history. This is the first study so far in asymptomatic or non-specific symptoms cases.

We also concluded that antigen test failed to detect the cases only if Ct values were high, mean Ct values being 28 for E gene and 33 for N2 gene. These findings are similar to the findings of Scohy et al who have observed that the rapid antigen detection test is able to detect SARS-CoV-2 with high sensitivity in nasopharyngeal samples with high viral load equivalent at least to 1.7×105 copies/mL (Ct < 25), but the sensitivity declines substantially when the viral load decreases with Ct values over 30, equivalent to 9.4×103 copies/mL.[11]

Khairat et al have evaluated two rapid antigen tests; BIOCREDIT COVID-19 Ag (RapiGEN Inc., Korea) and Standard Q COVID-19 Ag (SD Biosensor, Korea), their study showed sensitivities of 52.5% and 68.7% and specificities of 46% and 96% respectively.[9] Hirotsu et al claimed the antigen test (LUMIPULSE based on CLIA) sensitivity as 55.2% and 99.6% specificity.[10]

Scohy et al tested COVID-19 Ag Respi-Strip (Coris Bioconcept, Gembloux, Belgium) on 148 nasopharyngeal swabs.[11] Amongst the 106 positive RT-qPCR samples, 32 were detected by the rapid antigen test, given an overall sensitivity of 30.2%. All the samples detected positive with the antigen rapid test were also positive with RT-qPCR. They concluded that overall poor sensitivity of the COVID-19 Ag Respi-Strip does not allow using it alone as the frontline testing for COVID-19 diagnosis.

To the best of our knowledge, this is the first study from India evaluating the role of rapid antigen test available for COVID 19. Also this study is one of its kind as we have evaluated patients who were not suffering from symptoms suggestive of COVID (no febrile illness, shortness of breath, Influenza like illness).

CONCLUSION

The ongoing outbreak of SARS-CoV-2 infections has emphasized the importance of the quick and accurate laboratory diagnosis in order to limit the spread as well as appropriately treat those patients who have a serious infection.

The present study establishes the role of rapid antigen tests in contributing to the quick, point of care and accurate diagnosis of SARS CoV-2. These tests are very important for real-time patient management and infection

control decisions. These assays are safe, simple, and fast and can be used in local clinics and hospitals.

Our studies also establishes the fact that rapid antigen test fails to pick up the cases with high Ct values and thus low viral loads and hence low infectivity or less serious infection. But has a good detection rate in high viral load samples. Thus in present times when there is high infectivity among community, Rapid Antigen Test proves to be very promising and can be utilized on a large population.

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