

Performance of an antigen rapid test compared to RT-PCR for the detection of SARS-CoV-2

Abstract

Background

Rapid and accurate diagnosis of COVID-19 is critical for the management of patients and to limit the spread of the infection. real-time reverse transcription polymerase chain reaction (RT-PCR) is the gold standard for the diagnosis of SARS-CoV-2 infection, however, it is costly and require time to obtain a result. A number of alternative rapid test are available now to provide a faster and more convenient solution for the diagnosis of COVID-19. The aim of this work was to compare the performance of a SARS-Cov-2 Antigen Rapid Test (ART) Cassette to the RT-PCR conventional method.

Methods

Two nasopharyngeal swabs were taken from each of the 126 patients included in this study. Of those, 23 were healthy individuals, 9 were confirmed COVID-19 patients and 103 patients from COVID-19 isolation ward in hospital. For each patient, one sample were processed for RT-PCR and a second swab was used on the ART kit.

Results

Participants were 57.5 males and 42.5 females. The average age was 54.7 (± 14). Outpatient The QPCR swabs returned 67.9% positivity while the ART positivity was observed in 27.4% of COVID-19 patients. In 56.6% of patients the QPCR results concurred with the rapid test results. Using Fagan nomogram analysis, the 95% confidence interval was (2-20) with a negative likelihood of 0.18. Posterior probability was 0.1. Positive test (blue) prior probability was set at 26%. The 95% confidence interval was (31-41) with a positive likelihood of 1.56. Posterior probability was 0.6.

Conclusion

The ART is a useful and efficient test for diagnosing COVID-19, however, QPCR sensitivity is higher. It is recommended to use ART twice for confirming COVID-19 positivity, which will give a statistically more accurate finding.

Key words: SARS-CoV-2, diagnosis, rapid test, Antigen, RT-PCR

Introduction:

On March 2020, the World Health Organization declared that Coronavirus disease 19 (COVID-19) is a global pandemic (Lu, Stratton, & Tang, 2020). This pandemic outbreak was caused by the exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first appeared in December 2019 in Wuhan, Hubei Province, China then spread to the rest of the world (Hui et al., 2020). Infected patients were suffering primarily from acute atypical respiratory symptoms including fever, dry cough, dyspnea, and hypoxia. In addition, other organ systems were also involved (Wang et al., 2020).

Worldwide, approximately 241 million patients were infected with the virus with a total death of around 5 million (Worldometers.info). To control the spread of the infection and react quickly to new cases, faster and cheaper diagnostics are required. Currently, testing approaches fall into two main categories; either nucleic acid or serological (Esbin et al., 2020; Wolfel et al., 2020). Nucleic acid methods directly probe for the viral RNA of a swab taken from the patient throat or nasal cavity (Peeling et al., 2020). Real time polymerase chain reaction (RT-PCR) was retained as the gold-standard for clinical diagnosis by the Centers for Disease Control and Prevention (CDC). However, running this method requires the use of special equipment, reagents, and well-trained personnel (Fenollar et al., 2021).

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test (ART) Cassette (INV BIO) is an *in vitro* immunochromatographic membrane diagnostic test that detect coronavirus antigen using sensitive monoclonal antibodies (Harmon et al., 2021). Samples tested can be collected using throat swab, sputum sample, nasal swab, and nasal aspiration (Rubin, 2020). The ART can provide fast (takes less than 10 minutes to develop) and simple alternative to RT-PCR specially for routine screening. In this article, a side-by-side comparison was done to compare the sensitivity of ART to CDC-recommended RT-PCR protocol.

Materials and methods

The aim of the work is to evaluate the SARS-Cov-2 Antigen Rapid Test (ART) Cassette (INVBIO, Beijing, China) for the detection of SARS CoV-2 antigens in nasopharyngeal swabs, in comparison with the standard RT-PCR technique.

The study was reviewed and approved by the general ethical committee for medical research in the ministry of health (MOH) in Madinah (approval number: IRB48-2021). A written consent was taken from all patients who agreed to participate in the study. All subjects were assigned a study identification number and stayed anonymous and information which identifies patients was not used in this work. The study was performed between May and July 2020.

To study the performance of the rapid antigen test compared to RT-PCR, two nasopharyngeal swabs were taken from 103 COVID-19 hospitalized patients, in addition to 9 positive controls (COVID-19 conformed cases) and 23 negative controls (healthy individuals). On the day when routine swabbing of patients is normally carried out for RT-PCR screening, an additional swab was taken from each patient to be used for the ART. Results for the first swab were obtained from the hospital record, while the second swab was used on the rapid test onsite.

The kit contains individually sealed strips with two lanes, a bottom small slot for applying the sample and a top lane where the appearance of two bands indicated a positive result, while one band indicated a negative result. To use the kit, the test device was removed from the sterile foil pouch and placed on a clean and level surface. The nasopharyngeal swabs taken from a patient were inserted in the supplied disposable dropper containing 10 drops of the provided extraction buffer, mixed by squeezing and shaking well, before applying three drops onto the strip. Results developed between 2 and 5 minutes. Strips were maintained for further 10 minutes before disposal, to make sure no further changes will occur.

Results

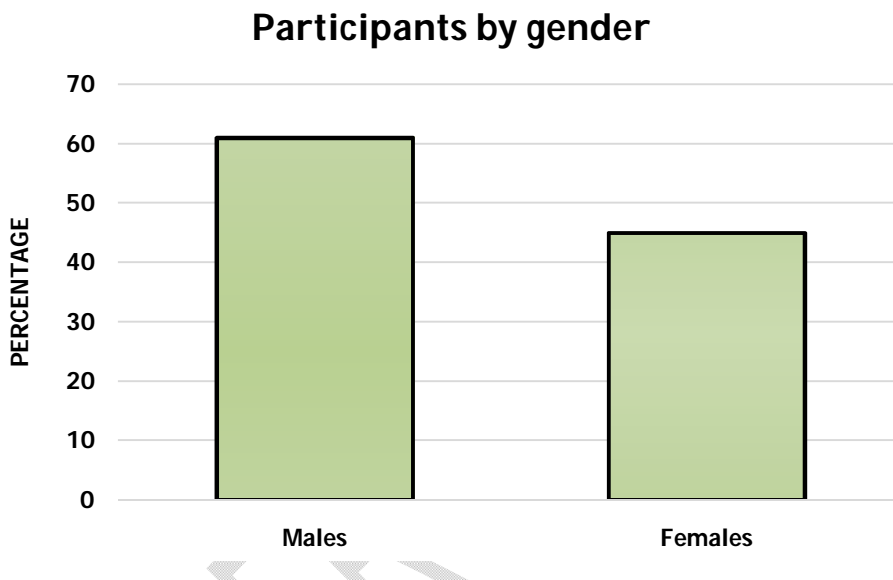
Patients QPCR and ART parallel tests

Participants in this study were 126 patients, including 23 negative healthy control individuals, 9 positive controls with confirmed COVID-19 positivity by QPCR and 103 patients all from the

COVID-19 isolation ward in the local COVID-19 reference hospital. Among those patients, 92.5% were in normal rooms, 4.7% in ICU and 2.8 on mechanical ventilators (MV) in ICU units. Two swabs were taken from each patient in hospital, out of which, one was sent for QPCR and the other for ART. 67.9% of QPCR results were positive while 27.4% of ART were positive (Figure 1). Comparing QPCR results to ART results, when both tests were negative or positive, this was recorded as 'agreement'. Agreement was found in 56% of cases showing a good correlation between the QPCR and ART results (Figure 1), however, ART was less sensitive.

Figure 1 / must written below the figures A and B with the title of figures

A



B

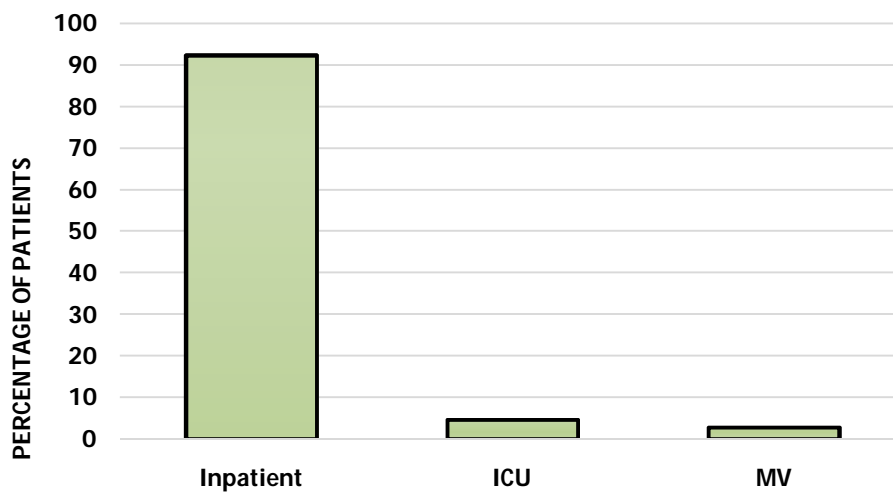


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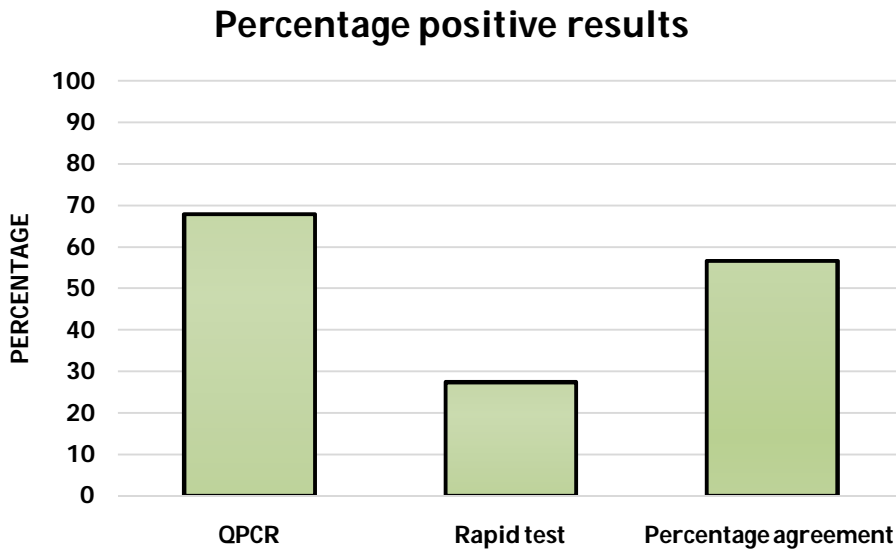


Figure 2: Positivity of QPCR and ART

Column representation of percentage positive results in all COVID-19 patients in QPCR and ART. The agreement percentage means to percentage of patients with QPCR and ART identical results, either both negative or both positive.

Analysis of agreement between QPCR and ART

The first hypothesis to be tested was to measure whether QPCR test results are confirmatory of COVID-19 not clinical features. Clinical features included any signs or symptoms of COVID-19, which were seen in all hospitalized patients in this study. The likelihood of agreement is shown in figure 3 using Fagan nomogram. The figures show the change in posterior probability after the NIRS VOT. The test was considered positive if the delta tissue oxygen index was < 15.2 . The positive and negative log-likelihood ratios were 3.67 and 0.51. Panel A. The 'prior' was set at 0.8. The 95% confidence interval for the positive and negative log-likelihood ratios were (1.1-12) and (0.35-0.73). Panel B. The 'prior' was set at 0.001. The 95% confidence interval for the positive and negative log-likelihood ratios were (0.01-1901) and (0.00-1904). Results are shown in table 1.

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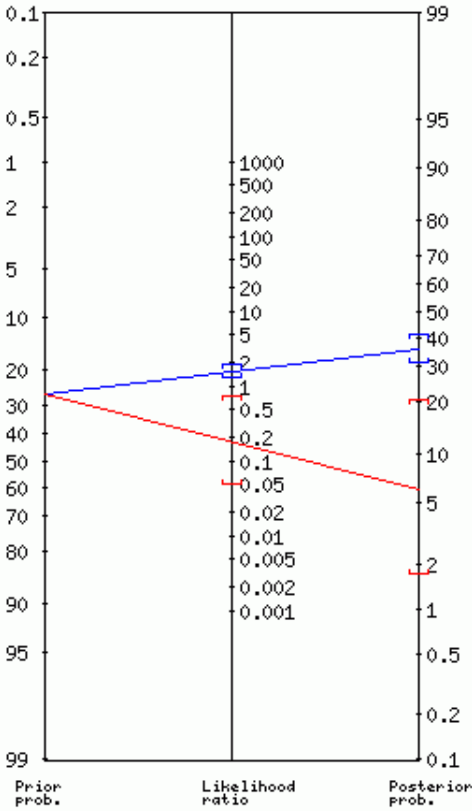


Figure 3. Fagan nomogram.

Graphical representation showing prior and posterior probability of COVID-19 test results and the likelihood ration. Fagan nomogram negative test (red) prior probability was set at 26%. The 95% confidence interval was (2-20) with a negative likelihood of 0.18. Posterior probability was 0.1. Positive test (blue) prior probability was set at 26%. The 95% confidence interval was (31-41) with a positive likelihood of 1.56. Posterior probability was 0.6

Table 1:

Prior probability (odds): 26% (0.4)

POSITIVE TEST:

Positive Likelihood ratio: 1.56
 95% confidence interval: [1.26,1.94]
 Posterior probability (odds): 36% (0.6)
 95% confidence interval: [31%,41%]

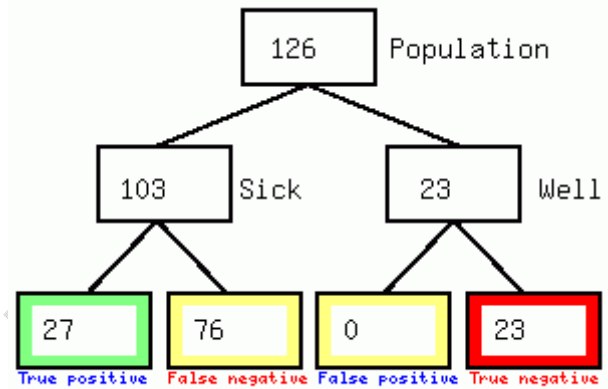
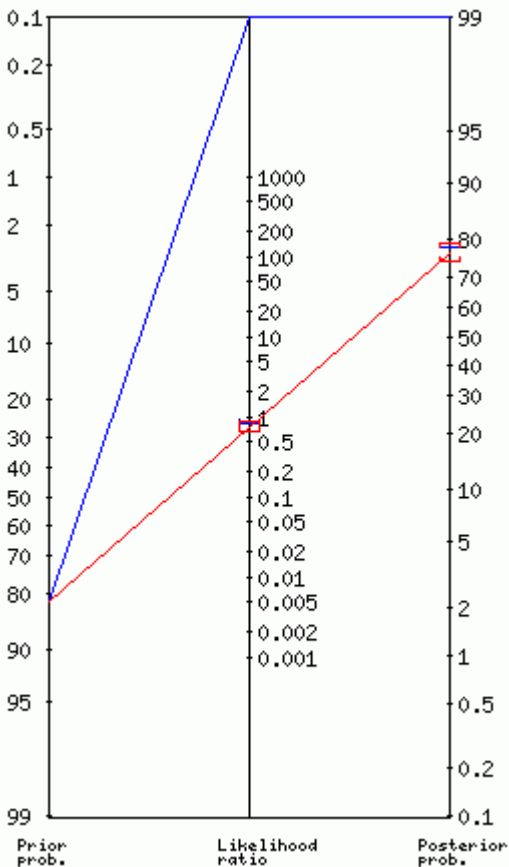
NEGATIVE TEST:

Negative Likelihood ratio: 0.18
 95% confidence interval: [0.05,0.71]
 Posterior probability (odds): 6% (0.1)
 95% confidence interval: [2%,20%]

Investigating clinical features versus QPCR

To investigate whether clinical features will necessarily result in positive QPCR result, the second hypothesis was to study if clinical features are confirmatory to COVID-19 rather than QPCR tests. Figure 4 shows the results of the second hypothesis to investigate whether clinical features are confirmatory of COVID-19 infection and not QPCR. It shows a higher percentage within the 95% confidence level.

Figure 4: Results of the second hypothesis to investigate whether clinical features / must moved to below the figure



Discussion

SARS-CoV-2 is a member of a large family known as coronavirus causing the current COVID19 pandemic. The pandemic is ongoing as the virus has infected around 357 thousand in Saudi Arabia with 600 reported deaths. The gold standard for diagnosis of infected patients with the virus is PCR test, however, faster, and cheaper methods are urgently required to help control the spread of the infection. The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (INVBIO) is an *in-vitro* diagnostic test for qualitative detection of coronavirus antigens in nasal Swab and nasal aspirate samples, using the rapid immunochromatographic method (Crozier,

Rajan, Buchan, & McKee, 2021). The identification is based on coronavirus antigen specific monoclonal antibody. The assay will provide an easy and fast option especially for healthcare workers routine screening and is a promising tool for combatting the infection (Albert et al., 2021). While PCR is currently the gold standard for the detection of the infection, new testing platforms were introduced based on the detection of antigens in nasopharyngeal swabs. Those tests are cheaper and can provide results within minutes. The PCR tests require certified laboratories, expensive equipment, and well-trained technicians to operate the instrument. In addition, false negative results have been reported when RT-PCR was used in detecting SARS-CoV-2. These limitations make RT-PCR inappropriate for use when rapid and simple diagnosis is necessary specially in the case of screening healthcare workers, travelers, and patients. Rapid and onsite detection methods can significantly improve the outbreak containment effort (Cassaniti et al., 2020). Therefore, there is an urgent need for a rapid, simple, sensitive, and accurate test to identify infected patients of SARS-CoV-2 to prevent virus transmission and to assure timely treatment of patients. The novel coronavirus (SARS-Cov-2) antigen rapid test cassette concept is to employ monoclonal antibodies with specificity for the novel coronavirus antigen (Gremmels et al., 2021). The test is simple and takes 10 minutes to see the result. Point-of-care diagnostic tests (POCTs) for detecting viral antigens in clinical samples would be very helpful for the diagnosis of COVID-19 either as mass-screening or first aid tests at the emergency room (Ghaffari, Meurant, & Ardakani, 2020).

The SARS-Cov-2 ART kit used to detect SARS-CoV-2 antigens in nasopharyngeal swabs employs a rapid immunochromatographic method to identify the viral antigens using specific monoclonal antibodies. The relative sensitivity of the test was around 96.17% and the accuracy 98.79% as reported by the manufacturer. To get precise results using the CDC protocol, the test takes about three hours to complete and costs about \$10 (Esbin et al., 2020). Samples taken from a swab of the nasopharyngeal cavity can harbor approximately 1 million viral particles (Gambarini et al., 2020). However, it takes the body about ten days to produce IGM and IGG antibodies after initial symptoms emergence (Wolfel et al., 2020). On the other hand, serological tests quantify antibodies in the patient's serum, which tend to be high during the first few days after infection.

As a conclusion, the rapid test used in research has many advantages over PCR since it has high sensitivity and accuracy. Besides that, the cost of the test is much cheaper than PCR and it does not need training to collect or run the sample.

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