Original research article

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 requires time to obtain a result. A number of alternative rapid tests 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 the hospital. For each patient, one sample was 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). The QPCR swabs returned 67.9% positivity while the antigen rapid test returned 27.4% positivity. 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:

In the month of March 2020, the World Health Organization declared Coronavirus disease 19 (COVID-19) is a global pandemic (Lu, Stratton et al. 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, E et al. 2020). However, recently the WHO declared that COVID-19 is no longer a pandemic or an emergency. 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 (Alimohamadi, Sepandi et al. 2020, Wang, Hu et al. 2020).

Worldwide, approximately 241 million patients were infected with the virus with a total death of around 6.8 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, Whitney et al. 2020, Wolfel, Corman et al. 2020, Hussain, Adil et al. 2022). Nucleic acid methods directly probe for the viral RNA of a swab taken from the patient throat or nasal cavity (Peeling, Wedderburn et al. 2020). Real time polymerase chain reaction (RT-PCR) a was retained as the gold-standard for clinical diagnosis by the Centers for Disease Control and Prevention (CDC) (Tahamtan and Ardebili 2020, Figueroa, Freire-Paspuel et al. 2021). However, running this method requires the use of special equipment, reagents, and well-trained personnel (Fenollar, Bouam et al. 2021).

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test (ART) Cassette (INVBIO) is an *in vitro* immunochromatographic membrane diagnostic test that detect coronavirus antigen using sensitive monoclonal antibodies (Harmon, Chang et al. 2021). Samples 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 especially 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.

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

Materials and methods

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). 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 2021.

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 (INVBIO, Beijing, China). 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 trip. Results developed between 2 and 5 minutes. Strips were maintained for further 10 minutes before disposal, to make sure no further changes will occur. Statistical

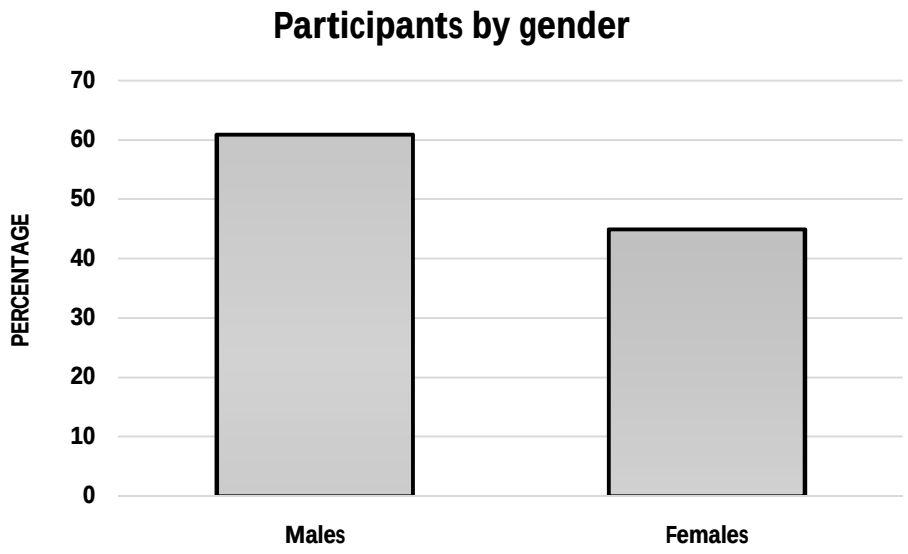
analysis was carried out with a confidence interval (CI) of 95%. The 95% confidence interval was used for the positive and negative likelihood ratios.

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 form 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.

A



B

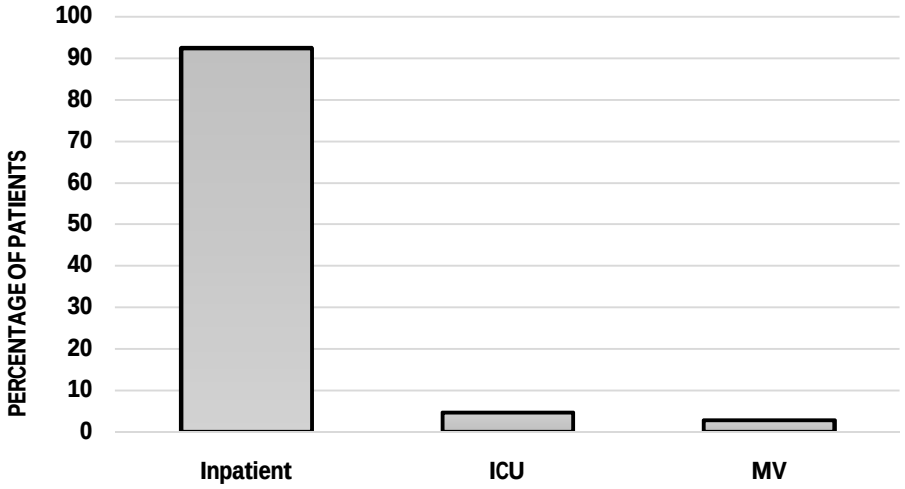


Figure 1: Participants by gender and percentage of patients

Figure 1/A represents the distribution of gender (males/females) participating in the study. Figure 1/B represents the distribution of patients according to ward and condition; inpatient are patients maintained at isolation rooms at normal room air, ICU represents patients with severe infections maintained in intensive care unit, while MV represents patients with critical conditions, maintained on mechanical ventilators.

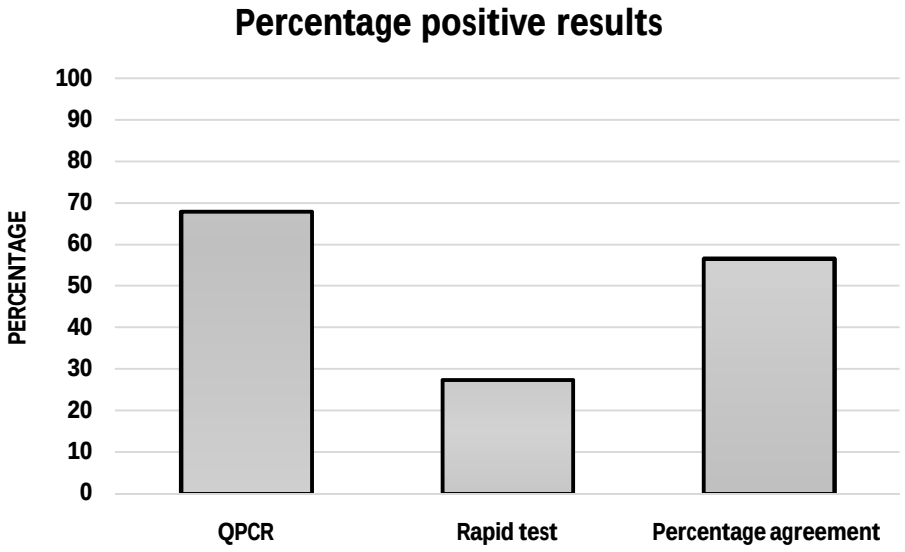


Figure 2: Positivity of QPCR and ART

Column representation of percentage positive results in all COVID-19 patients in QPCR and ART. The agreement percentage of patients with QPCR and ART identical results is shown in the third column. The percentage included two negative or two positive results for the same patient using QPCR and ART.

Analysis of agreement between QPCR and ART

The first hypothesis to be tested was to measure weather QPCR test results is confirmatory of COVID-19 not clinical features. Clinical features included any signs or symptoms of COVID19, 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 loglikelihood ratios were (0.01-1901) and (0.00-1904). Results are shown in table 1.

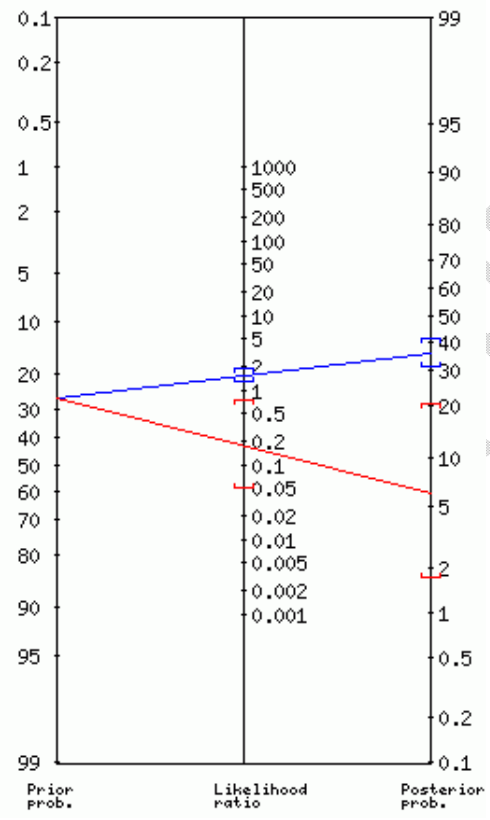


Figure 3. Fagan nomogram.

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:

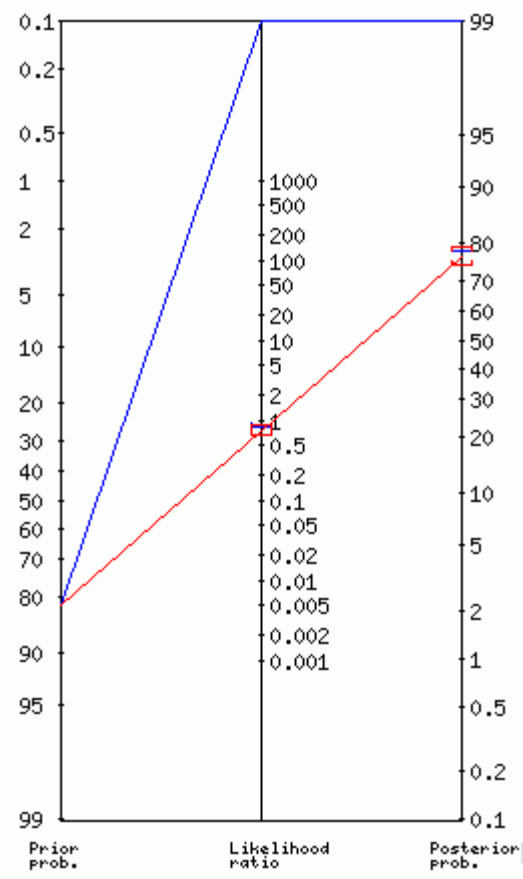
Positive Likelihood ratio:	1.56
95% confidence interval:	[1.26,1.94]
Posterior probability (odds):	36% (0.6)
95% confidence interval:	[31%,41%]

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 determine 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.



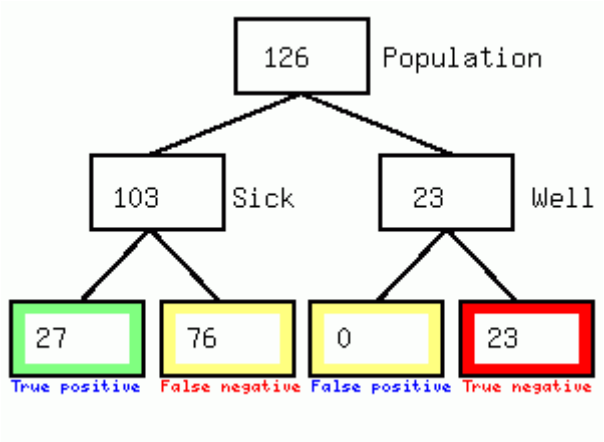


Figure 4: Results of the second hypothesis to investigate weather clinical features

Discussion

SARS-CoV-2 is a member of a large family known as coronavirus causing the COVID19 pandemic. The pandemic is now over as the virus has infected around 841 thousand in Saudi Arabia according to the ministry of health statistics. 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 et al. 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, Torres 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 (Bustin and Mueller 2005, VirgilioParadiso, De Summa et al. 2020). These limitations make RT-PCR inappropriate for use when rapid and simple diagnosis is necessary especially in the case of screening healthcare workers, travelers, and patients. Rapid and onsite detection methods can significantly improve the outbreak containment effort (Cassaniti, Novazzi et al. 2020, Park, Won 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, Winkel et al. 2021). The test is simple and takes 10 minutes to get results. 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 in the emergency room (Ghaffari, Meurant et al. 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, Whitney et al. 2020). Samples taken from a swab of the nasopharyngeal cavity can harbor approximately 1 million viral particles (Gambarini, Galli et al. 2020). In addition, serological tests quantify antibodies in the patient's serum, which tend to be high during the first few days after infection (Sidiq, Hanif et al. 2020).

Conclusion

The rapid test used in this research has many advantages over PCR due to its has high sensitivity and accuracy. Besides, the cost of the test is much cheaper than PCR and it does not need training to collect or run the sample.

References

Albert, E., I. Torres, F. Bueno, D. Huntley, E. Molla, M. Á. Fernández-Fuentes, M. Martínez, S. Poujois, L. Forqué and A. Valdivia (2021). "Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres." Clinical Microbiology and Infection **27**(3): 472. e477-472. e410.

Alimohamadi, Y., M. Sepandi, M. Taghdir and H. Hosamirudsari (2020). "Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-analysis." Journal of preventive medicine and hygiene **61**(3): E304.

Bustin, S. A. and R. Mueller (2005). "Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis." Clinical Science **109**(4): 365-379.

Cassaniti, I., F. Novazzi, F. Giardina, F. Salinaro, M. Sachs, S. Perlini, R. Bruno, F. Mojoli and F. Baldanti (2020). "Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department." Journal of medical virology.

Crozier, A., S. Rajan, I. Buchan and M. McKee (2021). "Put to the test: use of rapid testing technologies for covid-19." bmj **372**.

Esbin, M. N., O. N. Whitney, S. Chong, A. Maurer, X. Darzacq and R. Tjian (2020). "Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection." RNA **26**(7): 771-783.

Fenollar, F., A. Bouam, M. Ballouche, L. Fuster, E. Prudent, P. Colson, H. Tissot-Dupont, M. Million, M. Drancourt and D. Raoult (2021). "Evaluation of the Panbio Covid-19 rapid antigen detection test device for the screening of patients with Covid-19." Journal of clinical microbiology **59**(2): e02589-02520.

Figueroa, S., B. Freire-Paspuel, P. Vega-Mariño, A. Velez, M. Cruz, W. B. Cardenas and M. A. Garcia-Bereguiain (2021). "High sensitivity-low cost detection of SARS-CoV-2 by two steps end point RT-PCR with agarose gel electrophoresis visualization." Scientific Reports **11**(1): 21658.

Gambarini, E., M. Galli, D. Di Nardo, G. Miccoli, S. Patil, S. Bhandi, M. Giovarruscio, L. Testarelli and G. Gambarini (2020). "A survey on perceived COVID-19 risk in dentistry and the possible use of rapid tests." J Contemp Dent Pract **21**(7): 718-722.

Ghaffari, A., R. Meurant and A. Ardakani (2020). "COVID-19 serological tests: how well do they actually perform?" Diagnostics **10**(7): 453.

Gremmels, H., B. M. Winkel, R. Schuurman, A. Rosingh, N. A. Rigter, O. Rodriguez, J. Ubijaan, A. M. Wensing, M. J. Bonten and L. M. Hofstra (2021). "Real-life validation of the Panbio™ COVID-19 antigen rapid test (Abbott) in community-dwelling subjects with symptoms of potential SARS-CoV-2 infection." EClinicalMedicine **31**: 100677.

Harmon, A., C. Chang, N. Salcedo, B. Sena, B. B. Herrera, I. Bosch and L. E. Holberger (2021). "Validation of an at-home direct antigen rapid test for COVID-19." JAMA network open **4**(8): e2126931-e2126931.

Hui, D. S., I. A. E, T. A. Madani, F. Ntoumi, R. Kock, O. Dar, G. Ippolito, T. D. McHugh, Z. A. Memish, C. Drosten, A. Zumla and E. Petersen (2020). "The continuing 2019-nCoV epidemic

threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China." Int J Infect Dis **91**: 264-266.
Hussain, N., M. Adil, M. Mumtaz and M. Waseem (2022). "The Biological Causes and Consequences of COVID-19: ACE I/D Polymorphism and In-Silico Screening of Potential Bioactive Phytochemicals Against COVID-19." Bioinformatics and Biology Insights **16**: 11779322221139061.
Lu, H., C. W. Stratton and Y. W. Tang (2020). "Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle." J Med Virol **92**(4): 401-402.
Park, M., J. Won, B. Y. Choi and C. J. Lee (2020). "Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID-19) using PCR and real-time PCR." Experimental & molecular medicine **52**(6): 963-977.
Peeling, R. W., C. J. Wedderburn, P. J. Garcia, D. Boeras, N. Fongwen, J. Nkengasong, A. Sall, A. Tanuri and D. L. Heymann (2020). "Serology testing in the COVID-19 pandemic response." The Lancet Infectious Diseases **20**(9): e245-e249.
Rubin, R. (2020). "The challenges of expanding rapid tests to curb COVID-19." Jama **324**(18): 1813-1815.
Sidiq, Z., M. Hanif, K. K. Dwivedi and K. Chopra (2020). "Benefits and limitations of serological assays in COVID-19 infection." indian journal of tuberculosis **67**(4): S163-S166.
Tahamtan, A. and A. Ardebili (2020). "Real-time RT-PCR in COVID-19 detection: issues affecting the results." Expert review of molecular diagnostics **20**(5): 453-454.
VirgilioParadiso, A., S. De Summa, D. Loconsole, V. Procacci, A. Sallustio, F. Centrone, N. Silvestris, V. Cafagna, G. De Palma and A. Tufaro (2020). "Clinical meanings of rapid serological assay in patients tested for SARS-Co2 RT-PCR." MedRxiv: 2020.2004. 2003.20052183.
Wang, D., B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, Y. Zhao, Y. Li, X. Wang and Z. Peng (2020). "Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China." JAMA **323**(11): 1061-1069.
Wolfel, R., V. M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M. A. Muller, D. Niemeyer, T. C. Jones, P. Vollmar, C. Rothe, M. Hoelscher, T. Bleicker, S. Brunink, J. Schneider, R. Ehmann, K. Zwirglmaier, C. Drosten and C. Wendtner (2020). "Virological assessment of hospitalized patients with COVID-2019." Nature **581**(7809): 465-469.
Worldometers.info. "Worldometer." Retrieved 20 October, 2021, from https://www.worldometers.info/coronavirus/.