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Evaluation of Two Rapid Antigen Tests for Detection of SARS-CoV-2 Virus

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Abstract: Nucleic acid and antibody detection assays have been utilized in COVID-19 laboratory diagnosis. However, the use of viral antigenic proteins for diagnosis has not been successfully developed. Using viral antigen allows rapid direct viral detection earlier than production of antibodies. The present study was aimed at evaluating the performance of two COVID-19 rapid antigen detection tests, which are BIOCREDIT COVID-19 Ag (RapiGEN Inc., Korea) and Standard Q COVID-19 Ag (SD Biosensor, Korea), in comparison with RT-PCR. These tests were performed on 80 COVID-19 RT-PCR positive respiratory samples and 20 RT-PCR negative control samples. BIOCREDIT COVID-19 Ag and SD Biosensor RAD kits recorded total sensitivities of 52.5% and 68.7% and specificities of 46% and 96%, respectively. In high viral load samples, BIOCREDIT COVID-19 Ag and SD Biosensor RAD kits recorded higher sensitivities of 60% and 77%, compared to 45% and 60% in normal viral load samples, respectively. Sensitivity and specificity of the 2 antigen kits varied significantly with *P* values of <0.000001 and 0.0135, respectively. The evaluated RAD tests presented promising performance which was relatively better for SD-Biosensor than BIOCREDIT RAD tests, especially in high viral load samples. However, antigen tests are still considered substandard in comparison with RT-PCR in detecting SARS-CoV-2.

Keywords: COVID-19, SARS-CoV-2, Rapid Antigen Detection, RT-PCR

1. Introduction

COVID-19 disease is a viral respiratory infection that is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first described in China [1, 2]. It is proved that SARS-CoV-2 is a virus that spreads rapidly with strong human-to-human transmission and was declared by the World Health Organization (WHO) as an outbreak of public health emergency that would turn into a pandemic affecting 216 countries worldwide [3]. COVID-19 infection can result in adverse complications in immunocompromised patients or patients with chronic illnesses. The clinical symptoms that are overlapping with those of seasonal flu and other respiratory infections imply accurate and confident diagnosis [4, 5].

Development of well-performing laboratory tests, as well as implementing effective control measures, is obligatory for diagnosing and managing COVID-19-infected patients. Due to the lack of vaccine or antiviral treatment, speedy diagnostic tests with short-time assay, which can be employed as point-of-care tests, have become a matter of great concern [6]. Rapid assays confirm COVID-19 clinical suspicion promptly, which can aid in providing early isolation and appropriate care for infected patients. Although various serological antibody

tests have been developed, they do not fulfill the requirement of early detection of infection as the immune system takes on average 3 to 5 days to produce the antibodies [7].

Direct SARS-CoV-2 laboratory diagnosis can be achieved, through either viral nucleic acid or antigen detection in respiratory specimens. Currently, real-time reverse transcription polymerase chain reaction (RT-PCR) is the recommended molecular method for diagnosing COVID-19 acute infection. Although sensitive and specific, PCR assays are time-consuming and costly and require professional and skilled laboratory staff as well as specialized reagents and equipment. Thus, they are inconvenient when utilized on a large-scale population [8, 9].

Rapid antigen detection (RAD) tests rapidly detect certain COVID-19 viral proteins in respiratory samples and can yield results within minutes [10]. These tests are considered less expensive and faster than molecular tests. Moreover, they are more practical to be applied on a wide scale. However, antigen detection methods may suffer the same limitation that the PCR has an increased chance of false-negative results since various factors can affect the test sensitivity such as sample type, collection method, and transport conditions [11]. In order to meet the market needs, test developers were urged to rapidly introduce several laboratory COVID-19 diagnostic tests. However, this was challenged by limited chances for full performance review and process control [12]. Due to the current exceptional circumstances, an Emergency Use Authorization (EUA) policy has been issued by the Food and Drug Administration (FDA) on the 16th of March 2020 for SARS-CoV-2 laboratory tests to 100 µL be independently validated [13]. In this perspective, the current study was aimed at evaluating the performance of 2 commercially available COVID-19 RAD kits, BIOCREDIT COVID-19 Ag (RapiGEN Inc., Korea) and Standard Q COVID-19 Ag (SD Biosensor, Korea), compared to gold standard RT-PCR in terms of sensitivity, specificity, and accuracy.

2. Methods

2.1. Sample Collection and Study Population

A total number of hundred nasopharyngeal (NP) specimens were utilized in the present study. The hundred specimens were in the form of 80 samples collected from real-time polymerase chain reaction (RT-PCR) COVID-19 positive patients, which were received at the Virology Department of Central Public Health Laboratories (CPHL), Ministry of Health in Egypt, and twenty samples obtained from normal healthy individuals tested negative for RT-PCR-COVID-19 as control group for test specificity. The eighty RT-PCR COVID-19 positive samples were further categorized into 40 high viral load and 40 normal viral load samples.

2.2. COVID-19 Rapid Antigenic Detection (RAD) Tests

All samples were tested for SARS-CoV-2 antigens using two commercially available rapid antigen detection (RAD) kits in Egypt: BIOCREDIT COVID-19 Ag (RapiGEN Inc., Korea) and Standard Q COVID-19 Ag (SD Biosensor, Korea) that were provided to the CPHL in order to assess the performance of these tests before introduction to routine.

COVID-19 RAD kits are rapid and qualitative tests that allow detecting SARS-CoV-2 antigen in nasopharyngeal specimens. These tests utilize monoclonal antibodies conjugated to colloidal gold nanoparticles in order to target SARS-CoV-2 antigen. These antibodies are fixed onto nitrocellulose membrane. Antigen detection was conducted as per the manufacturers' instructions through mixing 100 µL of nasopharyngeal secretions with 4 drops of dilution buffer in a tube, which is then added to the strip, where solubilized conjugate together with the sample flows with passive diffusion and reacts with SARS-CoV-2 antibodies fixed onto the nitrocellulose membrane. The strip includes a control line in order to ensure correct flow of the sample. The results were interpreted visually after 15 minutes [5]. Sensitivity, specificity, and accuracy were considered when evaluating the performance of both kits, in comparison with the gold standard viral nucleic acid detection using RT-PCR.

2.3. Performance Evaluation and Statistical Analysis

We evaluated the performance of these tests as per the guidelines of the Clinical and Laboratory Standards Institute [14]. Sensitivity, specificity, and accuracy were considered when evaluating each of the tests. Sensitivity represents how frequently the antigen test yields true positive results for truly affected patients. Specificity measures the proportion of how frequently the antigen test is negative when the COVID-19-free volunteer is accurately identified as PCR negative for COVID-19. Binomial 95% confidence intervals (CI) were calculated for proportions. Cohen Kappa index was calculated between both test kits. We compared the differences in performance of both kits using Chi-square (X^2) test. *P* value less than 0.05 was considered statistically significant. Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows (Microsoft Corp., Redmond, WA) were utilized for conducting all statistical analyses.

2.4. Ethical Statement

This research received an institutional approval and was conducted upon a request from the Central Public Health Laboratories (CPHL) of the Ministry of Health, Egypt, in order to assess the performance of 2 commercial COVID-19 rapid antigen detection kits. No clinical trials or invasive procedures or laboratory animals were involved in this study.

3. Results

The present study enrolled 100 respiratory samples which were classified into 80 RT-PCR COVID-19 positive samples and 20 RT-PCR-COVID-19 negative NP specimens as control group. The eighty RT-PCR-COVID-19 positive respiratory samples were classified into 40 high viral load samples (Ct

values <18.57) and 40 normal viral load samples (Ct values >18.57).

As presented in Table 1, the total sensitivity for BIOCREDIT COVID-19 Ag and SD Biosensor RAD kits was found to be 52.5% (95% CI: 0.68-0.91) and 68.7% (95% CI: 0.62-0.83), respectively. In high viral load samples, the sensitivity for BIOCREDIT COVID-19 Ag and SD Biosensor RAD kits was 60% (95% CI: 0.54-0.86) and 77% (95% CI: 0.47-0.75), while in normal viral load samples, it was 45% (95% CI: 0.46-0.82) and 60% (95% CI: 0.40-0.70), respectively. The recorded specificity was 46% (95% CI: 0.14-0.41) and 96% (95% CI: 0.002-0.28) for BIOCREDIT COVID-19 Ag and SD Biosensor, respectively. The 2 antigen kits varied significantly in sensitivity and specificity with P values of <0.000001 and 0.0135, respectively. The 2 antigen kits recorded fair agreement of 77% (kappa: 0.22).

Table 1. *Results of COVID-19 RAD tests' analytical sensitivity, specificity, and accuracy.*

COVID-19 RAD tests	Sensitivity% (95% CI)	NPV	Specificity% (95% CI)	PPV%	Accuracy%
BIOCREDIT COVID-19 Ag test (RapiGEN)	52.5 (0.68-0.91)	21.9	45 (0.14-0.41)	81.3	51
Standard Q COVID-19 Ag (SD Biosensor)	68.7	43.1	95 (0.002-0.28)	98.4	74

PPV: Positive Predictive Value, NPV: Negative Predictive Value, 95% CI: 95% Confidence Interval, and RAD: Rapid Antigen Detection.

4. Discussion

COVID-19 diagnostic testing plays a vital role in managing SARS-CoV-2 infected patients appropriately and controlling the spread of the virus. Currently, molecular and immunoassays are the most commonly known diagnostic categories related to COVID-19 that overlap in the COVID-19 pandemic state [8]. Several diagnostic tests have been developed in order to detect SARS-CoV-2 viral antigens in respiratory samples. These tests have been independently validated by test developers and rapidly introduced to the market. The current study evaluated the performance of 2 commercially available COVID-19 RAD tests. SD Biosensor had better performance than BIOCREDIT antigen tests showing significantly higher sensitivity (68.7% vs. 52.5%, respectively) with a *P* value <0.0000001 and higher specificity (95% vs. 45%, respectively) with a *P* value <0.0135. To date, published data regarding the performance of COVID-19 antigen tests are still limited. However, performance data concerning various SARS-CoV-2 diagnostic methods from different laboratories worldwide are shared on an electronic platform by the Foundation of Innovative New Diagnostics (FIND), a nonprofit global organization [15]. According to FIND, the evaluation of BIOCREDIT antigen test showed higher sensitivity (62%) and specificity (100%) in comparison with our study. Few studies evaluated the performance of various COVID-19 RAD tests but for manufacturers other than those presented in our study. Among these studies, Mak *et al*. [16] conducted a study in which they recorded a total sensitivity of 30.2% for Resp strip COVID-19 antigen test. Mertens *et al*. [5] conducted another study in which they evaluated the performance of Resp strip COVID-19 antigen test and reported sensitivity and specificity of 57.6% and 99.5%, respectively. In the current study, the sensitivity of BIOCREDIT and SD Biosensor was observed to be higher in high viral load RT-PCR positive samples (Ct values <18.57) (60%, 77.5%) compared to low viral load samples (Ct values >18.57) (45%, 60%). This observation agreed with that of Scohy *et al*. [10] who reported that COVID-19 antigen tests show high sensitivity in high viral load samples (Ct values <25) which declines when viral load decreases (Ct values >30). Likewise, Mertens *et al*. [5] demonstrated that higher sensitivity of 74.2% was observed in the subpopulation with high viral load (Ct values <25) of SARS-CoV-2. Being rapid, easy to use, and of low cost with no demand for special equipment of skilled personnel are of the main advantages of COVID-19 RAD tests; thus, these tests are more preferable than PCR, especially when applied on a wide scale [10]. However, the analytical performance of COVID-19 RAD tests depends on several factors related to specimen quality, processing, and inhered viral load. Similar to many of the developed commercially available lateral flow immunoassays, COVID-19 rapid antigen detection tests have challenging sensitivity in comparison with molecular assays [5]. Furthermore, although COVID-19 RAD tests can detect actively infected patients, they cannot identify people who had been previously infected or developed immunity [9]. This is also one limitation of molecular assays as they cannot identify patients with past infection.

To the best of our knowledge, this is the first study from Egypt evaluating the 2 currently available COVID-19 rapid antigen detection tests in the market in order to provide potential guidance about their analytical performance. Further researches are needed to enrich the field with evidence-based data and to offer guidance for well-performing commercially available COVID-19 antigen tests in the market.

5. Conclusion

Either COVID-19 nucleic acid detection by PCR or antigen detection tests can be utilized in respiratory samples for direct detection of SARS-CoV-2. The overall performance of the 2 evaluated RAD tests was promising. SD Biosensor showed relatively better performance than BIOCREDIT. Higher sensitivities were recorded in high viral load samples compared to normal ones. However, so far antigen tests do not yet live up to RT-PCR in SARS-CoV-2 detection.

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