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REVIEW

Performance and application evaluation of SARS-CoV-2 antigen assay

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid detection is the gold standard for the laboratory diagnosis of coronavirus disease 2019 (COVID-19). However, this method has high requirements for practitioners' skills and testing sites, so it is not easy to popularize and promote the application in places other than large hospitals. In addition, the detection flux of SARS-CoV-2 nucleic acid is small, and the whole detection process takes much time, which cannot meet the actual needs of rapid screening in large quantities. The WHO conditionally approved a batch of SARS-CoV-2 antigen reagents for clinical application to alleviate this contradiction. SARS-CoV-2 antigen detection offers a trade-off among clinical performance, speed and accessibility. With the gradual increase in clinical application, the accumulated clinical data show that the sensitivity and specificity of the SARS-CoV-2 antigen assay are over 80% and 97%, respectively, which can basically meet the requirements of the WHO. However, the sensitivity of the SARS-CoV-2 Antigen Assay among asymptomatic people in low prevalence areas of COVID-19 cannot meet the standard, leading to a large number of missed diagnoses. In addition, the detection ability of SARS-CoV-2 antigen reagent for different SARS-CoV-2 mutant strains differs greatly, especially for those escaping the COVID-19 vaccines. In terms of results interpretation, it is highly reliable to exclude SARS-CoV-2 infection based on the high negative predictive value of the SARS-CoV-2 antigen assay. However, in the low prevalence environment, the probability of false positives of the SARS-CoV-2 antigen assay is high, so the positive results need to be confirmed by the SARS-CoV-2 nucleic acid reagent. The SARS-CoV-2 antigen assay is only a supplement to SARS-CoV-2 nucleic acid detection and can never completely replace it. To date, SARS-CoV-2 nucleic acid detection continues to be the standard laboratory method for COVID-19 diagnosis.

KEYWORDS
COVID-19, mutant strain, SARS-CoV-2 antigen, sensitivity, specificity

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1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a severe infectious respiratory virus that can cause damage to almost all human organs, including the lungs, heart, kidneys, liver, and brain, and can even cause death in severe cases.[1–9] It is well known that the basic principles of controlling infectious diseases are controlling the infection source, cutting off the transmission route, and protecting the susceptible population. However, the existing vaccines and monoclonal antibodies are less effective because the virus mutates so quickly that immune escape is severe.[10–15] Therefore, it is difficult to contain the coronavirus disease 2019 (COVID-19) epidemic simply from the perspective of patient prevention and treatment. There is also a great necessity to control the source of infection further and cut off the transmission route of SARS-CoV-2 to completely control the epidemic situation. For the above reason, timely and accurate diagnoses and identification of COVID-19 patients are pivotal. At present, the gold standard of COVID-19 diagnosis is nucleic acid detection of SARS-CoV-2, which needs to be performed by specialized personnel in a polymerase chain reaction (PCR) laboratory with a second-class biosafety level.[16–19] Therefore, it cannot be widely promoted due to the high requirements of staff skills and site requirements, especially in some grassroots medical institutions. In addition, it takes 4–6 h to complete the whole detection process of SARS-CoV-2 nucleic acid detection, and it takes a longer time when the number of specimens is large, leading to delays in the identification of COVID-19 patients in some cases. The simultaneous gathering of COVID-19 patients and non-COVID-19 patients in medical institutions not only increases the risk of cross-infection but also occupies a large number of medical resources, which places tremendous pressure on preventing and controlling COVID-19 epidemics in medical institutions. Fortunately, many in vitro diagnostic companies have developed point-of-care SARS-CoV-2 antigen assays to accelerate the detection process. This paper provides a comprehensive and detailed analysis of the product performance, specimen types, applicable population, and clinical application of point-of-care SARS-CoV-2 antigen assays to guide their correct use.

1.1 | The applicable population of SARS-CoV-2 antigen assay

The WHO recommends a minimum of 80% sensitivity and 97% specificity for rapid antigen diagnostic tests, which can be used for patients with symptoms consistent with COVID-19. However, the sensitivity and specificity recommended by the WHO are the performance data of the detection kit obtained in a specific prevalence population. The actual application results show that sensitivity from individual studies ranged from 37.7% to 99.2% and specificity from 92.4% to 100.0%.[20] For the first time, the SARS-CoV-2 antigen test, which was first authorized for emergency use, reported 96.6% for positive percent-agreement (PPA) and 99.3% for negative percent-agreement (NPA). Pray et al. studied the performance of this platform against nucleic acid detection of SARS-CoV-2 and reported that the PPA and NPA of asymptomatic people were 41.2% and 98.4%, respectively.[21] The detection performance improved in symptomatic people, with 80.0% PPA and 98.9% NPA. The results of many studies have shown that the actual use effect of the kit cannot achieve what it claims.[22] In addition to possible technical problems such as reagent quality, the most important thing is that the actual effectiveness of these kits will vary depending on the prevalence of COVID-19 in the population being used in the real world. The prevalence rate of COVID-19 determines the applicability of the SARS-CoV-2 antigen assay in this population. Which groups of people are suitable for using these SARS-CoV-2 antigen assays? To date, researchers have performed many explorations in this field.

Some researchers have reported SARS-CoV-2 antigen screening in asymptomatic populations without epidemiological history. Turcato et al.[23] detected 2419 subjects attending the emergency department for a non-COVID-19 condition by a standard Q COVID-19 AG SD biosensor, and 50.0% sensitivity and 99.6% specificity were obtained. Another study published by Okoye et al.[24] used Abbott BinaxNOW COVID-19 antigen detection reagent to screen 2645 asymptomatic college students, and a similar low detection rate was obtained. From October 28 to November 20, 2020, a SARS-CoV-2 test in the Bordeaux University Health Campus showed that the overall sensitivity and specificity of the Abbott Pan Bio SARS-CoV-2 antigenic rapid test were 63.5% and 100%, respectively, while in the asymptomatic subgroup, the above date changed to 35.0% and 100%, respectively.[25] In a community screening in Taiwan Province, 70 out of 2096 antigen rapid test specimens were confirmed to be positive for nucleic acids, with a positive rate of 3.33%.[26] The sensitivity and specificity of rapid antigen detection were 76.39% and 99.26%, respectively. These studies have shown that the sensitivity of reagents is generally low in low prevalence areas.

In addition, there are significant differences in the sensitivity of the SARS-CoV-2 antigen assay between symptomatic and asymptomatic patients. A systematic evaluation of the accuracy and effectiveness of 64 different SARS-CoV-2 antigen assay studies showed a significant difference in sensitivity (72.0% vs. 58.1%) but equal specificity (99.5% vs. 98.9%) between symptomatic and asymptomatic patients.[22] There are also considerable differences in antigen detection between clinically suspected COVID-19 patients and close asymptomatic contacts.[27] All tests showed invariably 100% specificity. Sensitivity in clinically suspected patients was 68.9% (SGTI-Flex), 71.1% (Panbio™), and 84.6% (NovaGen) and 84.6%, 33.3%, and 55.6% in close asymptomatic contacts, respectively.[27] A group of data from hospitals showed a sensitivity of 60.5% and specificity of 99.5% for antigen detection among 1732 paired samples from asymptomatic patients. Among them, the sensitivity and specificity of 307 symptomatic subjects were 72.1% and 98.7%, respectively.[28] Other studies found that the overall sensitivity in symptomatic and asymptomatic children was 73% and 43%, respectively, lower than the 80% critical value recommended by the World Health Organization.[29] In conclusion, the SARS-CoV-2

antigen assay has low sensitivity in asymptomatic populations and easily causes missed diagnoses.

In addition, the detection rate is related to the viral load and the time of onset. In a prospective observational study conducted in Spain, it was found that the sensitivity of the SARS-CoV-2 antigen assay increased with increasing SARS-CoV-2 RNA load, reaching 95.6% in samples with a virus load ≥ 7.5 log10 copies/ml ($C_t \le 20$).[30] Further stratified analysis showed that when the critical value of the real-time (RT)-PCR cycle threshold (C_t) was reduced to 24, the sensitivity of the SARS-CoV-2 antigen assay was significantly improved.[31] Additionally, the detection rate is related to the time of onset. The sensitivity of the samples collected 0–1 days after symptom onset increased from 79.6% to 86.4% when the samples collected 4–5 days after symptom onset were grouped.[30] In another study, symptom onset within 3 days and between 4 and 7 days showed a sensitivity above 80%, while the onset of symptoms between 8 and 14 days was associated with a far lower sensitivity.[32] From the above results, it appears to be acceptable to screen patients with SARS-CoV-2 at the early stage of symptoms and high viral load.

Is the SARS-CoV-2 antigen assay suitable for use in medical institutions? A study of SARS-CoV-2 rapid antigen detection (Roche diagnosis) in an emergency department and primary health care center showed that the overall sensitivity of the reagent was 80.3%, and the overall specificity was 99.1%.[32] Hospital outbreaks were defined as the existence of three or more epidemiological-related cases. A total of 541 samples of antigens and nucleic acids were detected in parallel in 17 hospital epidemics in 11 hospitals. The results of six cases of SARS-CoV-2 antigen assay were inconsistent with those of RT–PCR, among which five cases of SARS-CoV-2 antigen assay were negative, one case of SARS-CoV-2 antigen assay was positive, and RT–PCR was negative. The sensitivity of the SARS-CoV-2 antigen assay was 83.3%, and the specificity was 99.8%. The positive predictive value (PPV) was 96.2%, and the negative predictive value (NPV) was 99%.[33] Thus, healthcare providers must always be aware of the low sensitivity of antigen detection and consider conducting confirmatory SARS-CoV-2 nucleic acid detection, as false-negative results may lead to the failure of infection control and preventive measures and delays in diagnosis, isolation, and treatment.[28]

In summary, screening of SARS-CoV-2 antigen reagent in a high-risk population is ideal, but it is still unavoidable to miss diagnosis when screening patients with SARS-CoV-2 antigen reagent in asymptomatic populations in low prevalence areas of COVID-19 due to the limitation of the methodology itself. In addition, although the specificity of antigen reagents is generally good, the problem of false positives caused by cross-reactions or interfering substances cannot be ignored.

1.2 | Detection ability of SARS-CoV-2 antigen reagent for the SARS-CoV-2 mutant

The variant carries one or more NP gene mutations, which may affect the sensitivity of the SARS-CoV-2 antigen assay.[34] Some clinical studies think that antigen reagents are less sensitive to detect Omicron.[30,34,35] None of the kits consistently detected either Delta or Omicron at the lowest dilutions (5.23 log10 copies/ml, with a C_t of 28.8 [Delta]; 5.33 log10 copies/ml, with a C_t of 28.8 [Omicron]).[36,37] It compromises the diagnosis of SARS-CoV-2 (K417N/T, E484K, and N501Y) associated with the beta or gamma SARS-CoV-2 variants.[38] The Rapid Antigen Detection Test for SARS-CoV-2 underestimates the identification of COVID-19 positive cases.

The performance of the SARS-CoV-2 antigen assay in vaccine-escaped COVID-19 patients is also a concern. During the delta wave, a total of 692 samples from vaccinated individuals were tested for COVID-19 nucleic acid and antigen, among which 76 samples (11.0%) tested positive for SARS-CoV-2 by RT–qPCR and 45 samples (6.5%) tested positive by Standard Q COVID-19 AGT.[39] Stratified by C_t values, the sensitivity of the SARS-COV-2 antigen assay was 100.0%, 94.4% and 81.1% for $C_t \le 20$ ($n = 18$), $C_t \le 25$ ($n = 36$) and $C_t \le 30$ ($n = 53$), respectively. Samples with C_t values ≥ 30 ($n = 23$) could not be detected.[39] The overall specificity of the COVID-19 antigen reagent was 99.7%.[39] The sensitivity of the Panbio™ COVID-19 assay is even worse for the diagnosis of COVID-19 due to vaccination breakthrough Omicron infection compared with Delta (the sensitivity is 36.1% vs. 67.7%).[40]

In summary, the SARS-CoV-2 mutant reduced the virus detection rate of the SARS-CoV-2 antigen reagent, especially the SARS-CoV-2 mutant that escaped from the vaccines.

1.3 | Laboratory standards for lifting COVID-19 patients from isolation

On March 15, 2022, China released the latest version of the COVID-19 pneumonia diagnosis and treatment protocol (9th edition). Compared with the previous version, one of the biggest changes is that the standard value of discharge and removal from isolation dropped from 40 to 35 in the original Ct value of SARS-CoV-2 nucleic acid detection. Even so, there is still controversy about what CT value representing viral RNA load is disease-producing and/or infectious.[41] Diagnostic methods to certify the noninfectious status of patients who have recovered from symptoms and discontinued isolation are not unanimously recognized.

In fact, the presence of culturable SARS-CoV-2 particles in respiratory tract samples can be regarded as a marker of infectivity, but virus culture is not widely used in routine diagnosis. To date, the correlation between SARS-CoV-2 nucleic acid results and virus culture and infectivity is still rarely studied.[42] Some studies believe that Ct ≥ 35 is considered to be a weakly positive sample for SARS-CoV-2 because its ability to create cytopathic effects on the medium and isolate the virus is less than 3%.[43,44] It is impossible to grow the virus in specimens with a viral load < 10^6 copies/ml.[45,46] In clinical practice, we have found that SARS-CoV-2 nucleic acid results can remain positive for a long time after COVID-19 patients have recovered. Therefore, molecular detection of viral RNA means that many patients are hospitalized or isolated for much longer than

necessary, placing a heavy and unnecessary burden on patient management, psychology, society, and the economy.[47–50] Considering the issues described above, it is essential to provide accurate, rapid, and simple laboratory tests to indicate whether COVID-19 patients have recovered and to evaluate the infectivity of COVID-19 patients.

In a study, the Panbio™ COVID-19 Ag Rapid Test Device for the diagnosis of COVID-19 in symptomatic patients ($n = 412$) attending primary healthcare centers was evaluated. This study showed that SARS-CoV-2 could not be cultured from specimens yielding RT-PCR positive and SARS-CoV-2 antigen assay negative results, and the data suggested that patients with RT-PCR-proven COVID-19 testing negative by SARS-CoV-2 antigen assay are unlikely to be infectious.[34] Other studies have also confirmed that there is a certain relationship between the amount of SARS-CoV-2 antigen and the infection status and virus shedding.[51–55] However, this specific connection needs to be explored continuously.

1.4 | Future development direction of SARS-CoV-2 antigen reagent

In addition to traditional SARS-CoV-2 antigen reagents, researchers have developed a number of promising SARS-CoV-2 antigen reagents based on new detection technologies and detection principles. For a product based on microfluidic technology, the whole detection process takes only 15 s, the linear detection range is 10^{-5} to 10^{-2} ng/ml, and high selectivity of 6369:1 is achieved.[56] After application and evaluation in different environments, body fluid matrices have been proven to be label-free, real-time, and easy to operate and can be used for the screening and diagnosis of SARS-CoV-2.[56] Laura Fabiani et al. developed a novel paper-based immunoassay using magnetic beads to support the immunological chain. The color can be read on a 96-hole stencil board with a Spotxel free-charge app on a smartphone to interpret the result.[57] The linear range of this product for detecting SARS-CoV-2 in saliva is 0.1 μg/ml^{-10}. The consistency between the detection results and real-time fluorescence quantitative PCR was 100%.[57] SARS-CoV-2 S1 spike protein antibodies immobilized on graphene material can capture 60 copies/mL of the virus by electrical transduction in midturbinate swabs and exhaled breath aerosol samples.[58] The B.1.1.7 mutant can be detected at 400–8000 copies/ml of the virus.[58] Furthermore, the detection reagent can be reused after elution by NaCl solution or heat treatment above 40°C.[58] Amazing, Carbon nanotube field-effect transistor (CNT-FET)-based biosensor for rapid detection of SARS-CoV-2 surface spike protein S1 with a limit of detection (LOD) of 4.12 fg/ml.[59] Even more surprising, integrating PCR-free amplification and synergistic sensing for ultrasensitive and rapid CRISPR/Cas12a-based SARS-CoV-2 antigen assays can detect SARS-CoV-2 at the single-virus level.[60] As technology continues to advance, our ability to detect pathogens will continue to improve.

2 | CONCLUSION

SARS-CoV-2 antigen detection provides a trade-off between clinical performance and speed and accessibility. The sensitivity and specificity of the SARS-CoV-2 antigen assay are ideal in the high prevalence areas of COVID-19. However, at the same time, we must understand that due to the limitation of detection-based sensitivity, there is still a certain percentage of false-negative results. When screening asymptomatic individuals in settings known to have a low prevalence, such as school, the workplace, and large gatherings, SARS-CoV-2 antigen reagent with a high negative predictive value can be safely used to eliminate the infection.[61] However, the probability of false positives is high in a low prevalence environment, and the positive results need to be confirmed by SARS-CoV-2 nucleic acid reagent.[61] To date, SARS-CoV-2 nucleic acid detection remains the standard laboratory method for diagnosing COVID-19.

AUTHOR CONTRIBUTIONS

Qing Ye led the manuscript writing. Qing Ye and WXS developed the initial concept and framework for the manuscript and oversaw the drafting. All authors contributed to the content, drafting, and critical review of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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