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Different Methods of COVID-19 Detection

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Abstract

Objective: Explore the performance of different test methods in detecting COVID-19 antibodies and antigens.

Methods: Compare the results of different test methods on samples from novel coronavirus pneumonia patients, and randomly select some of the confirmed samples after gradient dilution to detect the sensitivity of the test products. The sample used for rapid detection of COVID-19 antigen was nasal swab. Three kinds of sample used in the detection of novel coronavirus antibodies were venipuncture/fingerstick whole blood, serum and plasma.

Results: The detection rate of covid-19 antibody increased with the increase of the onset time of patients, while the detection rate of antigen decreased gradually, which was in line with the nature of antigen and antibody in the human body.

Conclusion: In view of the advantages of antibody and antigen detection, such as convenient and sample collection, high throughput, low workload, high reproducibility and low cost, we hope to lay a scientific foundation for the horizontal comparison of the data of products (Abbott, Roche, Healgen, AllTest, Citest, Siemens, Lepu, Wondfo) obtained in the research and practical application of epidemic prevention and control of COVID-19.

Keywords: COVID-19; Rapid test; PCR

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The Current Situation of the COVID-19

Novel coronavirus pneumonia, also known as coronavirus disease 2019 (COVID-19), is an acute respiratory infectious disease caused by 2019 Novel Coronavirus. The novel coronavirus pneumonia is a highly regarded infectious disease worldwide [1]. By the end of June 3, 2020, the total number of confirmed cases had exceeded 6.4 million. No specific drug for novel coronavirus pneumonia had been found yet, and vaccine research was still in progress. Novel Coronavirus pneumonia is mainly caused by lung diseases due to severe acute respiratory syndrome coronavirus type 2 infection. It can cause severe complications including acute respiratory distress syndrome, acute myocardial injury and metabolic acidosis, which cause irreversible damage, even lead to death [2]. Novel coronavirus pneumonia is highly infectious. Most of the patients have respiratory symptoms, and a few patients are asymptomatic. Asymptomatic infection brings great difficulties to clinical diagnosis and epidemic prevention and control. At present, the global epidemic is becoming increasingly severe. As of July 11, 2020, novel coronavirus pneumonia has spread to over 210 countries and regions with over 12.1 million confirmed cases worldwide and more than 550 thousand cases died. And there is cluster infection and outbreak infection in multiple countries [3]. Convalescent plasma therapy is currently a limited treatment for patients with acute, severe and critical illness. Convalescent plasma therapy is a clinical treatment method to treat patients with corresponding diseases by extracting plasma from cured patients, but not all convalescent plasma has good therapeutic effect, and the concentration of specific protective antibody is an important factor affecting the therapeutic effect [4]. Virus antigen detection not only has all the advantages of antibody detection, but also has all the advantages of nucleic acid detection. The England Public Health Bureau has evaluated some advanced antigen rapid test kit with NCV mutation samples [5]. Abbott, Healgen, Roche, AllTest and other companies have developed Novel coronavirus antigen and antibody rapid test kit. In order to further clarify the inspection performance of different products, the data of different manufacturers had been compared and analyzed.

Different Covid-19 Detection Methods

The lateral flow method is a simple, reliable and rapid analysis platform. The combination of isothermal amplification technology and lateral flow method provides a rapid, economical and highly sensitive solution for nucleic acid analysis [6]. The whole experiment can be completed in 30 minutes. Combined with microfluidic technology, the basic steps of sample analysis, such as preparation, separation, reaction and detection, can be

integrated into a micro scale chip, which has the advantages of miniaturization, small sample size, rapid detection and portability.

PCR

After the outbreak of COVID-19, bioinformatics analysis of the virus genome further found that the SARS associated coronaviruses have only 40% identity in amino acid sequence, and the orf3b of the virus encodes a new short protein [7]. The RNA extracted from bronchoalveolar lavage fluid (BALF) was used to identify SARS-CoV-2 by metagenomic sequencing. Phylogenetic analysis showed that SARS-CoV-2 was highly homologous with bat-slcovzc45 and bat-sl-covzxc21. In addition, a detection method based on nanopore targeted sequencing [8] was innovatively developed. This method combines the advantages of virus targeted amplification, long reading and real-time nanopore sequencing, and can simultaneously detect SARS-CoV-2 and other 10 types of respiratory viruses within 6 ~ 10 hours. The detection range includes 12 SARS-CoV-2 genomic sites, covering a region of nearly 10 KB [9]. Although sequencing technology has a high degree of accuracy and sensitivity, and can fully reflect the genetic information of pathogens, it is difficult to be applied to large-scale clinical detection because it needs expensive instruments and equipment, and the sequencing results rely on professional analysis and interpretation [10]. By the end of March 2020, the State Drug Administration had approved more than 20 SARS-CoV-2 detection products, most of which are based on RT-PCR. Because it involves multiple manual operation steps, relies on complex thermal cycle process, and has defects such as long detection time, the application potential of this kind of technology is limited in rapid detection. In order to solve the problem of complex thermal cycle in PCR, thermostatic amplification technology based on recombinase polymerase amplification has been gradually applied to nucleic acid detection [11]. At present, a variety of new technologies for on-site rapid nucleic acid detection have been developed, which break through the key technical problems of rapid nucleic acid extraction, thermostatic amplification, portable real-time fluorescence detection, and can complete the process from sample pretreatment to detection within 1 hour. Now there are mainly the following detection methods.

Principles, methods and advantages of SARS-COV-2 antigen detection

The novel coronavirus gene encodes many structural proteins, such as N protein, E protein and S protein. These proteins contain multiple epitopes, and the principle of antigen binding with antibodies can be applied to detect the presence of antigens by antibodies. The applicable sample type of antigen detection rapid test is usually the sample of swab.

Rapid antigen test can play an important role in guiding patient management, public health prevention and control decision-making, and COVID-19 surveillance, especially for grass-root areas where clinical diagnosis, treatment, prevention and control may be hindered due to lack of nucleic acid testing capabilities or long testing time. A rapid antigen test should be performed within 7 days after the onset of symptoms. According to WHO recommendations, based on the high specificity of the antigen test, a positive result indicates a novel coronavirus infection. In areas with widespread community spread, rapid antigen test can be used to detect and isolate positive cases early in health facilities, COVID-19 testing centers/sites, nursing homes, prisons, schools, frontline and health care workers, and to trace contacts. For antigen-negative persons, repeat antigen test or preferably confirmatory nucleic acid test should be performed as much as possible, especially in symptomatic patients. In this case, even if rapid antigen test is not specifically authorized to test contacts of asymptomatic cases, it can be used as primary screening.The advent of rapid antigen detection technology marks that the detection of novel coronavirus has entered the 3.0 era. Novel coronavirus detection will be easy to enter into a broader market, and people can even detect and manage novel coronavirus infection at home (subject to local regulations). The advantages of antigen detection, such as being fast, accurate and easy to operate, will benefit many industries, including customs (personnel and import and export goods), hotels, airlines, restaurants, conferences, live concerts, sports events, etc. In addition, the operation of rapid antigen detection is simple. This makes it possible for more than just professional medical technicians to easily grasp and provide patients with rapid novel coronavirus detection service, which can greatly reduce the overload of the medical system. The advent of novel coronavirus rapid detection products is conducive to reducing the risk of virus transmission to the minimum and promoting the complete prevention and control of the epidemic in countries around the world.

Principles, methods and advantages of SARS-COV-2 antibody detection

Plasma therapy is essentially an antibody drug, which uses neutralizing antibodies in the blood of recovered patients to help others kill pathogens. Antibodies are proteins produced by the body's immune system to resist the virus after the virus invades the human body. IgM and IgG are two common immunoglobulins in human blood, and are the most commonly used infectious disease antibody markers. The first to appear is the IgM antibody for early defense. The antibody maintains for a short time and disappears quickly. It remains in the blood for several days to several weeks. The next thing that appears is IgG antibody, which remains for a long time in the blood, several months or even years. In this epidemic, research on patients with novel coronavirus found that after the virus infects the human body, it takes about 5-7 days to produce IgM antibodies and 7-12 days to produce IgG antibodies. In general, IgM antibody is produced early. Once a person is infected, it produced quickly, maintained for a short time, and then it disappears quickly. Positive detection of IgM in blood can be used as an indicator of early infection. IgG antibody is produced later, maintained for a long time and it disappears slowly. Positive detection of IgG in blood can be used as an indicator of infection and previous infection. Antibody drugs are generally safe and effective, and are currently being used more and more to treat various diseases. Screening and verification of neutralizing antibodies, and large-scale production require long-term trials, making it difficult to function in the critical window of the early virus outbreak.

Antibody tests look for variations in the above antibodies as individual or combined antibody measurements. Venous blood samples are usually used for antibody test in laboratory settings using enzyme-linked immunosorbent assay or chemiluminescence immunoassay (CLIA). Disposable devices for lateral flow measurement using finger prick blood are also available. At present, the main antibody tests used in the UK include Abbott SARS-CoV-2 assay which detects IgG, Roche Elecsys assay which detects IgM and IgG. Both require CLIA determination of venous blood. A Cochrane review of SARS-CoV-2 antibody test included 57 publications on 54 cohort studies with 15 976 samples, of which 8526 were from cases of confirmed SARS-CoV-2 infection. Measures of diagnostic accuracy varied depending on the timing of the tests. Antibody is an essential component of the adaptive immune response, providing specificity and memory against future infection. This is achieved through neutralisation by binding pathogens, activation of complement to destroy cells by lysis, presentation or opsonization to immune cells to facilitate phagocytosis, degranulation, and antibody dependent cell mediated cytotoxicity. Lastly, antibodies have the ability to provide long term immunity but non-neutralising antibodies can also be produced, and a phenomenon known as antibody enhancement can occur where antibodies facilitate a secondary infection that can be more severe than the primary infection. This has been reported with other 15 corona viruses, but not to date with SARS-CoV-2.

Pathogen detection

Pathogen detection aims at the detection of pathogen itself, including virus isolation, culture and identification, as well as electron microscope observation. It can detect whether there is virus in human body in the early stage of infectious disease outbreak, so as to provide the most direct evidence for the diagnosis of virus infection [12]. The real morphology of NCV after inactivation was observed by freezing electron microscope: the virus particles were generally spherical or moderately polymorphous; the spike outward was nail like; and the virus body was embedded in the envelope. At the same time, the results captured the important intermediate state that the virus was about to fuse with cells, which provides an important basis for the identification and clinical research of novel coronavirus [13]. The bronchoalveolar lavage samples from 3 patients with severe pneumonia from unknown causes were studied by cell culture and transmission electron microscopy. The novel coronavirus causing severe pneumonia was isolated from clinical specimens. In vitro, the effect of SARS-CoV-2 on the surface of human respiratory epithelial cells was observed 96 hours after inoculation, while the effect of SARS-CoV-2 on veroe6 and Huh-7 cells was observed 6 days after inoculation [14]. Compared with other detection methods, the experimental environment for pathogen isolation and identification is harsh, the operation is cumbersome and time-consuming, so it is difficult to meet the needs of virus detection in large-scale outbreaks **(Figure 1).**

COVID-19 Antigen Rapid Tests Comparison Data

The performance of different COVID-19 Antigen Rapid Tests have been compared and the data are shown in the **Table 1** below.

The Binax NOW and ID NOW COVID-19 test developed by Abbott can provide results in 15 minutes or less. Tests that do not require equipment can be performed at home (providing services with almost guided services) or outside of traditional healthcare settings, driving clinics, schools, workplaces, and standing community testing sites. Abbott's rapid test is one of the most widely used tests in the United States. Since April 2020, it has been rapidly tested more than 200 million times. The clinical performance of this rapid test was determined by testing 60 SARS-ClV-2 antigen (Ag) positive specimens and 181 SARS-ClV-2 antigen (Ag) negative specimens confirmed with FDA EUA recommended RT-PCR reference method. And the sensitivity is 93.33% (95% Cl: 83.8-98.2%), and the specificity is 99.45% (95% Cl: 97.0-100%).

The SARS-CoV-2 rapid antigen detection developed by Roche is a rapid chromatographic immunoassay method for the qualitative detection of SARS-CoV-2 specific antigens present in the human nasopharyngeal. The test is designed to detect SARS-CoV-2 virus antigens in individuals suspected of being infected with COVID-19. This product is strictly used for professional use in laboratories and Point of Care environments. This test can provide results within 3 and a half hours, and at most 4128 results can be produced within 24 hours. SARS-CoV-2 rapid antigen detection showed sensitivity of 96.52% and specificity of 99.68%.

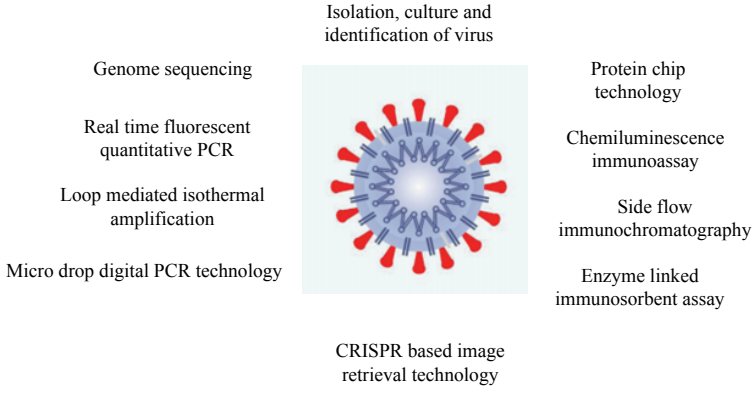

Figure 1 Main methods of COVID-19 antigen test.

Table 1 Comparison of COVID-19 antigen detection data of different Rapid Tests.

	Abbott	Roche	Healgen	AllTest	Citest	Wondfo	Lepu	Siemens
Relative sensitivity	93.33%	96.52%	98.32%	96.4%	94.8%	96.18%	92.00%	96.72%
Relative specificity	99.45%	99.68%	99.60%	99.2%	99.2%	99.72%	99.26%	99.22%
Accuracy	97.93%	98.83%	99.42%	98.0%	97.5%	97.67%	96.67%	98.74%

The kits developed by Healgen are used for qualitative detection of suspected cases of novel coronavirus. The sensitivity and specificity were 98.32% and 99.60% respectively.

AllTest's novel coronavirus antigen test aims to give a qualitative detection of SARS-CoV-2. The sensitivity and specificity of detection were 96.4% and 99.2%.

The COVID-19 antigen rapid test (Swab) developed by Citest is a qualitative detection of SARS-CoV-2 antigen. SARS-CoV-2 antibody is applied to the test line area. In the test, the sample will react with SARS-CoV-2 aniline coated particles. Then the mixture migrates upward on the membrane by capillary action, and reacts with SARS-CoV-2 antibody in the test line area. If the sample contains SARS-CoV-2 antigen, the result will appear as a colored line in the test line area. If the specimen does not contain SARS-CoV-2 antigen, no colored line appears in the test line area, which indicates a negative result. In order to be used as a program control, a colored line will always appear in the control line area, indicating that an appropriate volume of sample has been added and film wicking has occurred and also it's a valid result. Besides, the COVID-19 antigen rapid test (Swab) has been evaluated with specimens obtained from the patients.The sensitivity has been tested with 153 independent samples that have been measured as positive by real-time PCR up to the 33rd cycle, while the specificity has been tested with 251 independent samples that have been measured negative by real-time PCR. 145/153 pcs yielded correct positive results. 249/251 pcs yielded correct negative results. The sensitivity of the product is 94.8% and the specificity of the product is 99.2% based on the results.

Wondfo SARS-COV-2 ANTIGEN TEST detect the antigen of the virus, indicating the active viral infection. The antigen test showed a sensitivity of 96.18% and a specificity of 99.72%.

The rapid detection kit of SARS-CoV-2 antigen developed by Lepu is used for qualitative detection of SARS-CoV-2 antigen in clinical samples (nasal lining). The test card consists of gold standard pad (coated with monoclonal antibody against SARS-CoV-2 labeled with colloidal gold), sample pad and nitrocellulose membrane (the test area (T) is coated with monoclonal antibody against SARS-CoV-2 protein), absorbent paper and hydrophobic hard card. The quality control area (c) was coated with Goat anti-mouse antibody. The test results will be read out in 15-20 minutes. The antigen test showed a sensitivity of 92.00% and a specificity of 99.26%.

Siemens Rapid COVID-19 antigen test is an in vitro immunochromatographic assay for the qualitative detection. The sensitivity and specificity of detection were 96.72% and 99.22%.

SARS-CoV-2 Antibody Rapid Tests Comparison Data

Data comparison of rapid tests and RT-PCR system

As a control method, all samples were detected by RT-PCR system. Currently, manufacturers of SARS-CoV-2 antibody detection tests developed mainly include Abbott, Roche, Healgen, AllTest, Citest and Wondfo. To further compare the influence of sampling time on antibody detection and nucleic acid detection, the sensitivity of samples from patients with different onset time were tested in this study.

Table 2 Detection rate of two methods in different periods of onset.

Onset time (d)	Positive rate (%)	
	COVID-19 Ab	nucleicacid
1-7	50.00(6/12)	75.00(9/12)
8-14	77.27(17/22)	31.82(7/22）
15-21	100.00(23/23)	8.70 (2/23)
＞22	90.91(10/11)	0.00(0/21)

It can be seen from the above **Table 2** that the detection rate of COVID-19 antibody increases with the onset time of patients, while the detection rate of nucleic acid gradually decreases, which is in line with the production rule of nucleic acid and antibody in patients with infectious diseases. The results report should give a clear and complete description of the overall design and key points of the research, and should give a clear description of the implementation process of the research, including the necessary basic data and statistical analysis methods.

Comparison of Different SARS-CoV-2 Antibody Rapid Tests

At present, the manufacturers of SARS-CoV-2 antibody detection tests mainly include Abbott, Roche, Healgen, AllTest, Citest and Wondfo. The sensitivity of these tests was detected with samples of serum from patients with different onset time. The comparison results are shown in **Table 3.**

Table 3 Comparison of different SARS-CoV-2 antibody Rapid Test.

	Abbott	Roche	Healgen	AllTest	Citest	Wondfo
Relative sensitivity	89.34%	86.49%	100.00%	96.9%	96.9%	86.43%
Relative specificity	99.63%	99.80%	97.50%	96.3%	96.3%	99.57%
Accuracy	98.57%	99.01%	98.18%	96.4%	96.4%	91.61%

WHO's antibody performance data comparison table

Sensitivity and specificity are the two indexs used to evaluate the accuracy of a test. As shown below:

• Those testing positive who have the disease are called "true positives"(A);

• Those testing positive who do not have the disease are called "false positives"(B);

• Those testing negative who have the disease are called "false negatives"(C);

• Those testing negative who do not have the disease are called "true negatives"(D).

Sensitivity = percent of those who have the disease and are so indicated by the test

• Sensitivity (in percent) = (A/(A+C)) x 100

Specificity = percent of those who do not have the disease and are so indicated by the test.

• Specificity (in percent) = (D/(B+D)) x 100

For all COVID-19 tests, sensitivity and specificity are both important, as they determine the extent to which the test results can be used to draw clinical and epidemiological conclusions, and to understand other evidence that might be needed. According to the data in Table 1, Healgen has the highest relative sensitivity (100.00%). Roche had the highest relative specificity(99.80%).

Conclusions

The global situation of COVID-19 remains grave. Different detection methods have their own advantages in different application fields.

PCR method

Initially, PCR was used for the qualitative and quantitative diagnosis of novel coronavirus, with high accuracy, even for the early infected patients can be diagnosed well. However, PCR typically require well-resourced laboratory facilities, multiple reagents, sample referral systems and skilled personnel. Many settings lack the sophisticated infrastructure required to provide widespread molecular testing for COVID-19, particularly in LMICs. Long transport distances for referral and slow turnaround times can limit the clinical and public health impact of molecular testing for COVID-19, where timely detection is critical. Supply shortages of essential and compatible reagents are further complicating the scale-up of molecular testing for COVID-19 in certain settings. The high cost of molecular testing also limits the testing coverage that can be achieved within countries' diagnostic funding envelopes. Similarly, access and price remain substantial barriers to point-of-care molecular testing in many settings.

Rapid test

At present, the emergence of various COVID-19 rapid tests enables us to complete the preliminary screening. With its' short result time, lower cost and simple operation, the application range of rapid test is wider.There are two types of SARS-CoV-2 rapid diagnostic tests: antigen (Ag) tests that directly detect the SARS-CoV-2 virus antigen(s), and antibody (Ab) tests that detect one or more types of antibodies produced by the host immune response against the virus[15]. Antigen (Ag) tests can detects active SARS-CoV-2 infection and also be used at the point of care. Antibody (Ab) tests can be used to detect previous infection with SARS-CoV-2. Both tests are easy-to-use, rapid tests that can be used at or near the point of care, without the need for laboratory infrastructure or expensive equipment.

Some people are being asked to self-test for COVID-19 to help them stay safe and stop the spread of the virus. Thus, many countries, like Germany and the UK, are now encouraging citizens to complete self-testing for COVID-19. The COVID-19 Rapid Tests provide strong support for this new service and also reduce the burden on government health institutions and expanding the testing base for subsequent control of COVID-19.

Only better combination and use of different detection methods can we better detect COVID-19.

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