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The Significance of Oral Fluid Antigen Rapid Test in the Time of the COVID-19 Epidemic

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Abstract

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

Methods: Compare the sensitivity, specificity and the accuracy of different manufacturers' COVID-19 antibody and antigen rapid tests. Different onset time samples were tested with RT-PCR and COVID-19 antibody and antigen rapid tests. The sample used for rapid detection of COVID-19 antigen was oral fluid. 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 was increased with the increase of the onset time of patients, while the detection rate of antigen was decreased gradually, which was corresponding to the nature of antigen and antibody in the human body.

Conclusion: Different detection methods have their own advantages in different application fields. In antigen and antibody detection, we through products (Abbott, Roche, Healgen, AllTest, Citest, Siemens, Lepu, Wondfo) to predict the advantages of convenient and sample collection, high throughput, low workload, high reproducibility and low cost in practical applications.

Keywords: COVID-19; Rapid test; Antigen; Antibody, Oral fluid

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Introduction

Currently, the COVID-19 is occurring in many areas of the world. COVID-19 is a disease that is caused by infection with the coronavirus known as SARS-CoV-2, it can cause severe complications including acute respiratory distress syndrome, acute myocardial injury and metabolic acidosis, which cause irreversible damage, even lead to death. This virus is transmitted between humankind and has spread rapidly [1]. As of July 16, 2021, more than 188 million confirmed cases have been reported in countries and regions around the world, and more than 4.065 million patients have died. The spread is still ongoing. The estimated value of the fatality rate of this disease varies greatly among countries in the world. As of February 8, 2021, the observed fatality rate of this disease in most countries is between 0.5% and 5.0%, and the global preliminary revised case fatality rate is about 2.9%. The impact of COVID-19 on human health may vary greatly. Some infected people have no obvious symptoms and may not have adverse reaction. Other infected patients have mild or severe symptoms and may be life-threatening. Therefore, there is giant demand for the COVID-19 rapid tests.

There are three main types of COVID-19 diagnostic or screening tests: nucleic acid amplification tests, antigen tests and antibody tests. Virus antigen detection not only has all the advantages of antibody detection, but also has most advantages of nucleic acid detection [2]. The England Public Health Bureau has evaluated some advanced antigen rapid test kit with NCV mutation samples [3]. 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 measurements of different manufacturers had been compared and analyzed.

Diagnostic Methods

PCR

The most common method for detecting the specific sequence of the novel coronavirus is fluorescent quantitative PCR (polymerase chain reaction). It has the characteristics of early diagnosis, high sensitivity and specificity [4], and is the "gold standard" for diagnosing novel coronavirus. Currently, the most widely used method is Real-time fluorescent quantitative PCR technology [1]. Generally, the two targets located on the ORF1ab and N genes of the virus are detected. The same sample must meet the double target positive or the repeated test as the single target positive, or the two samples must meet the single target at the same time to confirm the positive of the SARS-CoV-2 virus nucleic acid. The unique gene sequence of the virus is used as the detection target

[5]. Through PCR amplification, the target DNA sequence we choose increases exponentially. Each amplified DNA sequence can be combined with a fluorescent-labeled probe that we added in advance, produce fluorescent signal, the more target genes amplified, the stronger the accumulated fluorescent signal display [5]. In samples without infected, since there is no target gene amplification, no increase in fluorescence signal would be detected. Therefore, nucleic acid detection is actually to determine whether there is novel coronavirus nucleic acid in the sample by detecting the accumulation of fluorescent signals.

Antigen detection

The novel coronavirus antigen test can directly detect whether the human sample contains the novel coronavirus, and its diagnosis is efficient, accurate, and requires less equipment and personnel. Antigens such as the N protein, E protein and S protein of the novel coronavirus can be used as immunogens to stimulate plasma cells to produce specific antibodies after the virus infects the human body. According to the principle of double-antibody sandwich ELISA, using two antigen-specific antibodies to recognize and bind to different epitopes of a target antigen can greatly reduce the probability of cross-reaction, thereby effectively improving its specificity [1]. The type of antigen test sample is generally a sample from the site of infection, such as oropharyngeal swabs, nasopharyngeal swabs, sputum, etc [6]. The SARS-CoV-2 Antigen Rapid Test (Oral Fluid) is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigens in Oral Fluid specimen from individuals with suspected SARS-CoV-2 infection in conjunction with clinical presentations and the results of other laboratory tests. Results are for the detection of SARS-CoV-2 Antigens. An antigen is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient clinic history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude SARS -CoV-2 infections and should not be used as the sole basis for treatment or patient management decisions. Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary for patient management. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19. In areas with widespread transmission, rapid antigen testing can be used to detect the virus early and isolate positive cases. It is widely used in health facilities, COVID-19 testing centers/sites, nursing homes, prisons, schools, frontline and health care workers [6]. Oral Fluid Rapid antigen detection is easy operating, high precision, convenient sample collection, and quick results. This allows medical personnel to perform testing with less training and explanation of the principles, and to provide patients with rapid and novel coronavirus testing services, which can greatly reduce the overload of the medical system.

Antibody detection

Testing for past infections is also known as antibody testing. It analyzes a sample of human blood to see if there are antibodies to SARS-CoV-2. Antibodies are a protein that the immune system makes to help identify and defend against pathogens like viruses [7]. It generally takes several weeks for some antibodies to develop, so these tests are usually not effective for detecting active infections. Seven days after the onset of novel coronavirus, serum-specific antibodies were gradually produced [7]. The immunoglobulin IgM antibodies appeared first, and then IgG antibodies appeared. In general, IgM antibody is produced earlier. Once a person is infected, it produced quickly, maintained for a short time, and then it disappears. 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 [8]. Therefore, an increase of IgM antibody indicates a recent acute infection, and an increase of IgG antibody indicates a previous infection. The biggest advantage of serological testing that is convenient and fast, the testing time is short. It can effectively break through the limitations of existing testing technology on personnel and places, and shorten the testing time. If the suspected case is positive for specific IgM and IgG antibodies, therefore the IgG antibodies will change from negative to positive or the recovery period is 4 times or higher than the acute period, it can be diagnosed as infected with the novel coronavirus [8]. At present, the main antibody tests used in 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. The accuracy of detection depends on the time of the measurement. It is an important component of the reaction, with long-term immunity and hypothesis memory for the future [9]. The United States has made a great progress in antibody detection methods. The detection time ranges from the earliest two hours to the last five minutes, and the detection accuracy rate exceeds 90%. For example, the American Abbott Pharmaceuticals Co., Ltd. announced a novel coronavirus detection method that can determine whether someone is infected in just 5 minutes. The test kit is small in size, easy to carry, and can be used in almost any medical environment. The United States has also adopted a serum test kit that can detect whether there are antibodies to the new coronavirus in the subject within two minutes, greatly shortening the test time. South Korea uses a method where multiple samples are mixed together for testing. Once the result is positive, the sample would be tested separately. Using this method can greatly improve the detection efficiency, and to a certain extent alleviate the shortage of detection reagents and other problems. This test is mainly used for virus screening for asymptomatic people, so that potential infections can be quickly found in the community. This method will not be used for confirmatory testing for the time being.

Pathogen detection

Pathogen examination is the detection of the pathogen itself, which includes virus isolation, culture and identification, and electron microscope observation. It can detect the presence

of virus in the human body in the early stage of the COVID-19 outbreak, providing the most immediate for the diagnosis of virus infection [10]. However, compared with another detection method, the pathogen isolation and identification experimental environment is harsh, the operation is cumbersome and time-consuming, and it is difficult to meet the demand of virus detection in a large-scale epidemic, so it won't be compared with the other three detection methods in this report.

Sampling techniques

Now, in view of the advantages of antibody and antigen detection, for example easy sample collection, high throughput, low workload, high reproducibility and low cost, we compare with the data of products (AllTest, Norman Biotech, Clongene Biotech) to conduct COVID-19 antigen rapid test. 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 testing products. The sample used for rapid detection of COVID-19 antigen was oral fluid. Three kinds of sample used in the detection of novel coronavirus antibodies were venipuncture/ fingerstick whole blood, serum and plasma.

Ethics:

Ethical approval is of great significance to the whole research. Without ethical approval, it means that if a participant make a claim regarding the research, the researcher will assume personal responsibility [11]. This research will respect the autonomy of participants, which includes four parts. Firstly, to provide sufficient information for the research participants. For example, the significance of research and learning objectives. Make participants to make an informed decision as to whether to take part in research. Secondly, ensuring that participants are not subject to coercion to take part or not taking part [11], the survey is entirely dependent on the wishes of the participants. Thirdly, ensuring that participants are free to withdraw from the research at any time and that there can be no reason. Fourthly, all samples are anonymous, research will strictly confidential and respect personal information by participants provided, and that information will not be disclosed to the outside world. In addition, this research paper has an obligation to conduct research with sincere and impartial attitude, ensuring that research maximize to get the useful results and appropriate and effective dissemination. However, research will not have practical or potential interests and will be honest and transparent in the whole process. When rejecting any participants, this research paper will give a reasonable ethical interpretation in the application, and that research does not discriminate against certain individuals or groups.

Data quality assurance

Comparison of different SARS-CoV-2 antigen rapid test data:

The COVID-19 antigen rapid test is a convenient method to qualitatively detect SARS-CoV-2 nucleocapsid protein antigens present in human oral fluid. SARS-CoV-2 antibody is coated in the test line region. During testing, the specimen reacts with SARS-CoV-2 antibody-coated particles in the test. The mixture then migrates upward on the membrane by capillary action and reacts with the SARS-CoV-2antibody in test line region. If the specimen contains SARS-CoV-2 Antigens, a colored line will appear in test line region as a result of this. If the specimen does not contain antigens to SARS-CoV-2, no colored "T" line will appear in the test line region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

From the table 1 below, the performance of the products from different companies are compared by sensitivity and specification. The novel coronavirus oral fluid antigen rapid test for self-test developed by AllTest aims to qualitatively detects SARS-CoV-2. The sensitivity and specificity of the detection were 90.1% and 99.3% respectively. The novel Coronavirus Antigen Testing Kit from Nanjing Norman Biotech Company is a similar product. The sensitivity of this product is 91.13%, and the specificity is 99.09%. Besides, the COVID-19 Antigen Rapid Test Cassette from Hangzhou Clongene Biotech is a qualitative detection of SARS-CoV-2 antigen. The sensitive of this product is 91.3% and the specification is 99.6% **(Table 1).**

SARS-CoV-2 antibody rapid tests comparison data:

Data comparison of rapid tests and RT-PCR system

RT-PCR system is used as a reference method for many SARS-CoV-2 antibody detection rapid test products as most of whom has specified this in their instruction for use. 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.

It can be seen from the above Table 2 that the detection rate of COVID-19 antibody increases with the increase of 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 **(Table 2).**

Comparison of different SARS-CoV-2 antibody rapid tests data:

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 1 products of antigen diagnostics by saliva from different companies.

	AllTest	Norman Biotech	Clongene Biotech
Sensitivity	90.1%	91.13%	91.3%
Specification	99.3%	99.09%	99.6%

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)

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%

Calculation method and principle using at this report for sensitivity and specificity are shown as below:

• Those testing positives who have the disease are called “true positives” (A);

• Those testing positives 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

Sensitivity and specificity is important for COVID-19 detection, cause they determine the 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%).

Conclusion

Different detection methods have their own advantages in different application fields.

PCR method:

Initially, PCR is used for the qualitative and quantitative diagnosis of the new coronavirus, because it directly detects the viral nucleic acid in the specimens we collected, so it has strong specificity and relatively high sensitivity, even the early infected patients can be diagnosed well. However, the source of the sample is difficult to standardize. Samples include pharynx, nasopharynx secretions, sputum, bronchus, lavage fluid, lung biopsy, conjunctiva, stool, etc. [12]. The copy number of the virus in an individual and in different parts is not the same, so it is hard to standardize the sample. The testing condition is high. The laboratory needs a high levels hospital, P3 level protection, and a certified gene amplification laboratory. It requires PCR-certified personnel to operate, and the steps are cumbersome, during which it requires multiple centrifugation, repeated operations such as opening the lid and adding samples, and the whole process takes 5 to 8 hours, and it takes a long time to complete a batch of tests. It is difficult to avoid aerosol pollution. The storage conditions of nucleic acid samples are harsh, and RNA is easily lysed. It can only be stored for 24 hours at 4°C, but samples for antibody detection such as serum can be stored for 72 hours [12]. In the case of gene sequencing, although the accuracy is higher, it needs to be interpreted by professionals, and the expenditure is obvious. Timely testing is crucial, and long distances and slow turnaround times will limit the clinical and public health impact of COVID-19 molecular testing. So this method is not suitable for large-area screening.

Rapid test:

So far, the emergence of various COVID-19 rapid tests enables us to complete the preliminary screening. Sensitivity and specificity are the two indexs used to evaluate the accuracy of a test. They are important parameter for rapid test product, 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. There are two types of SARS-CoV-2 rapid diagnostic tests: antigen (Ag) tests that directly detect the SARS-CoV-2 virus antigen, and antibody (Ab) tests that detect one or more types of antibodies produced by the host immune response against the virus. The antibody detection method is simple and the detection time is short, but the false negative rate is high. In the early stage of the infection, the human body has not yet produced antibodies, which cannot be detected by this method. Moreover, there are now some asymptomatic carriers of the COVID-19, whose antibodies are usually negative, which cannot be detected by this method [13]. Therefore, antibody testing can be used to assist in the diagnosis of cases with negative nucleic acid tests, to detect previous SARS-CoV-2 infection, and to screen the cases. Rapid antigen detection (especially oral fluid) is easy to operate, high precision, convenient sample collection, and quick results. This allows medical personnel to perform testing with less training and explanation of the principles, and to provide patients with rapid and novel coronavirus testing services, which can greatly reduce the overload of the medical system. 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. Only a better portfolio and by using suitable detection methods in different situations can we be more effective in preventing the COVID-19 virus.

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