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Fluorescence Immunoassay System Plays an Important Role in Timely Diagnosis of COVID-19 Infection

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Abstract

Objective: Mainly compare the differences between PCR; antibody, antigen tests with Fluorescence Immunoassay Analyzer for COVID-19.

Methods: Compare the sensitivity, specificity and the accuracy of different manufacturers' COVID-19 antibody and antigen tests with Fluorescence Immunoassay Tests. Different onset time samples were tested with RT-PCR and fluorescence immunoassay COVID-19 antibody and antigen tests. Total 365 specimens were used in clinical study at different locations (Spain, Slovenia and United States). The sample used for fast detection of COVID-19 antigen was nasopharyngeal swab. Three kinds of sample used in the detection of novel coronavirus antibodies were venipuncture/ finger stick whole blood and serum. The fluorescence immunoassay analyzer detects the fluorescence signal value of a specific area and calculates the result of the *SARS-CoV*-2 Antigen and Antibody in the sample according to the algorithm on the ID card.

Results: The emergence of various COVID-19 fluorescence immunoassay test enables us to complete the preliminary screening. 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 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. We through products (FIATEST, SOFIA, BINAXNOW) in antigen detection and products (FIATEST, SD BIOSENSOR, BODITECH) in antibody detection to predict the advantages of convenient and sample collection, high throughput, low workload, high reproducibility and low cost in practical applications.

Keywords: COVID-19; Fluorescence immunoassay; Antigen; Antibody

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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, it can cause severe complications including acute respiratory distress syndrome, acute myocardial injury and metabolic acidosis, which cause irreversible damage, even lead to death. The most recently discovered coronavirus in 2019 causes coronavirus disease, which is called 2019-nCoV or *SARS-COV-2*. This virus is transmitted between humankind and has spread rapidly [1]. As of 2 September 2021, more than 218 million confirmed cases have been reported in countries and regions around the world, and more than 4.526 million patients have died. However, the spread is still ongoing, the estimated value of the fatality rate of this disease varies greatly among countries in the world. Some infected people have no obvious symptoms and may not have adverse reaction and other infected patients have mild or severe symptoms and may be life-threatening. The clinical presentation of infection includes respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Therefore, there is giant demand for the COVID-19 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

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detection [2]. The England Public Health Bureau has evaluated some advanced antigen test kit with NCV mutation samples [3]. In order to further clarify the inspection performance of different products, the measurements of different manufacturers had been compared and analyzed.

Diagnostic approach

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 can 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 fluorescence immunoassay antigen test can directly detect whether the human sample contains the novel coronavirus, it is efficient, accurate. 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 method, 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 nasopharyngeal swabs, sputum, etc [6]. The *SARS-CoV-2* Antigen Test Cassette (Nasopharyngeal Swab) is based on fluorescence immunoassay for the qualitative detection of *SARS-CoV-2* antigens in nasopharyngeal swab specimens from individuals with suspected *SARS-CoV-2* infection in conjunction with clinical presentation 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 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 rule out *SARS-CoV-2* infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. 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, and confirmed with a molecular assay, if necessary for patient management. The COVID-19 Antigen Test Cassette is intended for use by trained clinical laboratory personnel. In areas with widespread transmission, fast antigen testing with Fluorescence Immunoassay Analyzer 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]. Fast antigen detection is easy operating, high precision, convenient sample collection, strong stability, and quick results. With Fluorescence Immunoassay Analyzer, this allows medical personnel to provide patients with efficient COVID-19 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 COVID-19 Infection, 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. A Fluorescence Immunoassay qualitative detection of IgG and IgM antibodies to *SARS-CoV-2* in human whole blood, serum or plasma with the use of Fluorescence Immunoassay Analyzer as an aid in the diagnosis of COVID-19 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 other detection method (like antigen or antibody fluorescence immunoassay test), 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 were compared with the data of products (FIATEST, SOFIA, BINAXNOW, SD BIOSENSOR, BODITECH) to conduct Different Methods of COVID-19 Detection. 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 nasopharyngeal swab. Three kinds of sample used in the detection of novel coronavirus antibodies were venipuncture/finger stick whole blood, serum and plasma.

Ethics: Ethical approval is of great significance to the whole research. Without ethical approval, it means that if participants 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 test data: The COVID-19 Antigen Test Cassette (Nasopharyngeal Swab) is a qualitative membrane-based fluorescence immunoassay for the detection of *SARS-CoV-2* antigen in human nasopharyngeal swab specimen. *SARS-CoV-2* antibody is coated in test line region. During testing, *SARS-CoV-2* antigen in the specimen reacts with *SARS-CoV-2* antibody-coated by fluorescent microspheres in the test; the mixture then migrates upward on the membrane by capillary action and reacts with the *SARS-CoV-2* antibody in test line region. The fluorescence immunoassay analyzer detects the fluorescence signal value of a specific area and calculates the result of the *SARS-CoV-2* Antigen in the sample according to the algorithm on the ID card.

From the table 1 below, the performance of the products from different companies are compared by sensitivity and specificity. This study was designed for COVID-19 Antigen Test Cassette performance with clinical specimen. Total 365 specimens (244 COVID-19 positive specimens and 141 COVID-19 negative specimens confirmed by RT-PCR) were used in clinical study at different locations (Spain, Slovenia and United States). Commercial PCR served as the reference method for the COVID-19 Antigen Test Cassette (Nasopharyngeal Swab). The result shows the COVID-19 Antigen Test Cassette has a high relative sensitivity and high relative specificity. The sensitivity and specificity of the detection were 95.6% and 98.4% respectively. The novel Coronavirus Antigen Testing Kit from SOFIA Company is a similar product. The sensitivity of this product is 95.1%, and the specificity is 99.09%. Besides, the COVID-19 Antigen Test Cassette from BINAXNOW is a qualitative detection of *SARS-CoV-2* antigen. The sensitive of this product is 97.1% and the specification is 98.5% **(Table 1).**

Comparison of different *SARS-CoV-2* antibody tests data

The COVID-19 IgG/IgM Test Cassette (Whole Blood/Serum/Plasma) is a qualitative membrane-based fluorescence immunoassay for the detection of IgG and IgM antibodies to *SARS-CoV-2* in whole blood, serum and plasma specimen. During testing, the specimen which contain IgG or/and IgM antibodies to *SARS-CoV-2* reacts with *SARS-CoV-2* antigens conjugated with fluorescence particles in the label pad of test cassette. Then the mixture migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG or/and

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Table 1 Products of Antigen Diagnostics by Nasopharyngeal Swab from different companies.

Test	Fiatest	Sofia	Binaxnow
Sensitivity	95.6%	95.1%	97.1%
Specificity	98.4%	99.09%	98.5%

Table 2 Comparison of different SARS-CoV-2 Antibody Test.

Test	Fiatest	Sofia	Binaxnow
Sensitivity	99.3%	97.7%	90.0%
Specificity	98.3%	95.3%	100.0%

IgM in IgG or/and IgM test line region of NC membrane. The concentration of IgG or/and IgM to *SARS-CoV-2* in the specimen correlates with the fluorescence signal intensity captured on the Test line, which can be scanned by Fluorescence Immunoassay Analyzer. The testing result of COVID-19 IgG and IgM will display on the Fluorescence Immunoassay Analyzer screen.

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. At present, the manufacturers of SARS-CoV-2 antibody detection tests mainly include FIATEST, SD BIOSENSOR, BODITECH. The sensitivity of these tests was detected with samples of serum from patients with different onset time. The comparison results are shown in **Table 2.** Sensitivity and specificity are the two indexes used to evaluate the accuracy of a test.

They are important parameter for the 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. According to the data in Table 2, FIATEST has the highest relative sensitivity (99.3%). BODITECH had the highest relative specificity (100.0%).

Calculation method and principle using at this report for sensitivity and specificity are shown as 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)) × 100

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

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

Conclusion

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.

Fluorescence immunoassay test

So far, the emergence of various COVID-19 fluorescence immunoassay test enables us to complete the preliminary screening. Sensitivity and specificity are the two indexes used to evaluate the accuracy of a test. They are important parameter for fluorescence immunoassay 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* 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 with Fluorescence Immunoassay Analyzer 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.

Antigen fluorescence immunoassay test (especially nasopharyngeal swab) with Fluorescence Immunoassay Analyzer 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 efficient novel coronavirus testing services, which can greatly reduce the overload of the medical system. Both tests are easy-to-use, fluorescence immunoassay test 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 be more effective in preventing the COVID-19 virus.

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