

Research status of colloidal gold detection technology in medicine

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Abstract. The application of antigen testing is widely used in the diagnosis of COVID-19 and antigen tests have also been simplified for everyday use due to their convenience and operational capabilities. Colloidal gold nanoparticles are an important component in most antigen tests. This paper summarizes the principles and characteristics of colloidal gold nanoparticles mainly by reviewing a large amount of literature and also performs antigen testing and observes the development process, thus validating the theoretical aspects. In this paper, it was found that colloidal gold nanoparticles can react with viruses and produce visible results on immunochromatographic test paper. However, when the concentration of the virus is insufficient, visualization effect may not be discernible to the naked eye. This is the reason why antigen testing is not as accurate as nucleic acid testing. Therefore, antigen testing is more suitable for home-based daily testing. This article has helped to understand the role of colloidal gold nanoparticles in immunochromatographic test strips, which in turn aids in improving the accuracy of these test strips.

Keywords: Colloidal Gold Nanoparticles, Immunochromatographic Test Strips, COVID-19, Nucleic Acid.

1. Introduction

At the end of 2019, an unknown new virus emerged and soon spread rapidly around the globe, which attracted great attention from the World Health Organisation. High-throughput sequencing eventually verified a novel coronavirus—currently known as severe acute respiratory syndrome coronavirus (SARS-CoV-2)—to be the germ responsible for the outbreak [1]. The virus typically presents clinically with fever, cough, myalgia, shortness of breath etc. And real-time polymerase chain reaction (RT-PCR) is a nuclear-derived method, which is mainly used for standard laboratory virus detection. The sample is collected from patients' noses or throats, and then separates the ribonucleic acid (RNA) from other components including protein and lipids. If the virus is present, its RNA is mixed with the individual's genetic material in its retrieved RNA. Therefore, professional medical staff and sophisticated instrument are required to do the test, which costs a long period and lower the efficiency. In order to improve efficiency and speediness, the colloidal gold immunochromatographic test strip is highly frequently used due to its simple operation, low cost and fast result feedback. It is a technology that uses in vitro detection

to perform qualitative, semi-quantitative, and quantitative detection of detecting chemicals. It combines single (multiple) immunoassay technology, chromatography analysis technology, colloidal gold labeling technology, and a solid-phase labeling detection method that combines the use of cloned antibodies with other methods. In practical detection, it utilizes colloidal gold-labeled antigens or antibodies as tracers and takes nitrocellulose membrane (NC membrane) as reaction carriers which is based on the capillary chromatography principle of microporous membranes. It will bind to certain antigens and antibodies at the detection line to generate a color that is visible to the naked eye, collecting the test liquid that contains free colloidal gold particles on the capture region of the nitrocellulose membrane [2]. And colloidal gold nanoparticles have been widely utilized in a variety of fields, such as detecting biomarkers in the diagnosis of diseases and cancers, lateral flow immunoassays, etc.

In this paper, we classify it into 3 types of analysis, which are Immunochromatographic Assay (ICA), Enzyme-Linked Immunosorbent Assay (ELISA) and transcription polymerase chain reaction (RT-qPCR) respectively. Additionally, we also divided its basic principles into 2 categories for discussion: competitive and non-competitive. Also, the paper compares the colloidal gold immunochromatographic test strip with RT-PCR nucleic acid test from their sensitivity, stability, color rendering, etc. to sum up the benefits and shortcomings of the test strip. Furthermore, some suggestions are provided to promote the practicality of it. For instance, urging businesses to routinely validate the most recent viral mutations is one method to lessen the detrimental effects of false negatives and false positives. And, manufacturers must pay attention to the raw material, such as the quality of bovine serum albumin (BSA), particularly their pH level, solubility, etc.

2. Analysis of the Role of Colloidal Gold Nanoparticles in Immunochromatographic Test Strips

2.1. Classification and applications of colloidal gold nanoparticles

Under the impact of the COVID-19 pandemic, various testing methods have emerged in the field of medicine, such as nucleic acid testing, blood testing, and antigen testing. Different testing methods require different conditions and testing times, and antigen testing, due to its affordability, accuracy [3], and simplicity of operation has significant value in the diagnosis of COVID-19 and is widely applied.

Traditional antigens can be classified into four categories based on detection methods. The main types of antigens include Immunochromatographic Assay (ICA), Enzyme-Linked Immunosorbent Assay (ELISA) and transcription polymerase chain reaction (RT-qPCR) [2]. Although RT-PCR was initially widely used due to its high accuracy, it has high requirements for sample transportation and a long waiting time for results [4]. The method of ICA is detected by binding specific antigens or antibodies with the sample, forming visible color lines or spots on a test strip, which is the most mainstream method.

The colloidal gold immunochromatographic test strip has the characteristics of high specificity, rapid reaction, simple operation, and low cost. It consists of five parts: sample pad, colloidal gold pad, nitrocellulose membrane (NC membrane), absorbent filter paper, and PVC bottom plate. The colloidal gold solution used for detection is a well-dispersed solution of gold nanoparticles formed by reducing chloroauric acid to gold atoms. The outer ion layer of colloidal gold particles carries a positive charge, while the inner ion layer carries a negative charge. The internal ion layer exhibits zeta potential. Colloidal gold solutions with a zeta potential greater than $\pm 30\text{mV}$ have good stability [5], which helps to maintain repulsion between colloidal gold particles and keeps them dispersed and stable without easy aggregation.

Colloidal gold immunochromatographic test strip technology has been widely used in the field of virus detection due to its high selectivity, repeatability, and stability. Colloidal gold immunochromatographic test strips can be used for the detection of animal viruses. They can detect various diseases in pigs and quickly identify the target pathogens within a short time, with a detection speed of more than 20 times faster than fluorescent PCR. They are also capable of detecting infectious diseases transmitted between sheep, cattle, and humans, with an accuracy rate as high as 99%. This is beneficial for the rapid detection of infected targets. Colloidal gold test strips are widely used in the field

of human virus detection. For example, they can accurately detect the presence of IgM and IgG antibodies against the novel coronavirus (SARS-CoV-2). DNA-modified colloidal gold immunochromatographic test strips can quickly detect infectious plant viruses with high sensitivity and accuracy. They can detect low concentrations of protein gene fragments, achieving a lower detection limit. Infectious diseases in aquatic organisms pose challenges in detection due to the influence of testing conditions. However, colloidal gold immunochromatographic test strips can facilitate small-scale testing, protect the organisms from harm, and demonstrate higher sensitivity and specificity compared to fluorescent PCR detection methods. This enables more accurate and rapid detection.

2.2. Principle of Colloidal Gold Immunoassay

Immunochromatographic strip detection technology is a technology that uses the amplification effect to amplify the photoelectric signal of markers on biomolecules to transform microscopic immune responses into macroscopic visualization [6]. Colloidal gold is a sol substance, which is less stable than a solution and more stable than a turbid solution, its atomic structure is a gold nucleus surrounded by two ion layers, the inner ion layer is the zeta potential, and the outer ion layer is dispersed in the solution, both of which ensure that the colloidal gold solution is more stable. On the other hand, colloidal gold molecules have smaller size and larger surface area, therefore extremely easy to produce precipitation particle collision and aggregation of the phenomenon, which is not conducive to stability

There are two main categories of qualitative detection of colloidal gold immunochromatographic test strips in antigen detection: competition and non-competition methods. Meanwhile, non-competitive law also includes the sandwich method and indirect method [7]. When detecting small molecules substances, experimenters usually use the competition method [1]. When the colloidal gold is labeled with the corresponding antibody, the target antigen will participate in the competition for the labeled antibody, to achieve the qualitative detection of the target antigen, in this method, a small amount of antibody is coupled with colloidal gold, and then the coupling product is coated with antigen when it reaches the detection line.

Throughout the process, if the sample tested contains the target antigen, the antigen in a sample will preferentially combine with the antibody (which is coupled to the colloidal gold), because the number of antibodies labeled with colloidal gold is much less than the number of antigens, the antigen at the detection line will compete with the target antigen in the sample for the limited number of colloidal gold-labeled antibodies. During the whole reaction, the limited antibodies labeled with colloidal gold will all bind to the antigen in the sample and can no longer bind to the antigen on the detection line, thus crossing the first detection line and accumulating and developing color at the second detection line. When qualitatively detecting macromolecular substances (like bacteria and viruses), most of the time will use Sandwich method. The Sandwich method [8] in the non-competition method also called the Double Antibody Sandwich method, is characterized that both the colloidal gold conjugate on the test strip and the coating at the test line are specific antibodies, if the tested substance is present in the sample, tested substance will first binds to antibodies labeled with colloidal gold, then, under the action of capillary action, it gradually moves to the detection line and binds to the antibody at the detection line, a double sandwich antibody with the structure such as 'colloidal gold labeled antibody-detector-antibody at the detection line' is formed. The testing sample of this method will bind with the antibody on the test paper at two different locations.

When serum antibodies need to be detected, indirect methods [9] are usually used, which are generally used to detect specific pathogens in animals to achieve the purpose of indirect detection, it works by using a colloidal gold conjugate as an anti-antibody and using a specific antigen at the detection line, the IgG type antibody and IgM type antibody in the sample will first bound to the anti-antibody labeled with colloidal gold, then moved to the detection line by capillary action and bound to specific antigen, the structure of 'the antigen the test line - sample - colloidal gold conjugated anti- antibody' is formed, the colloidal gold particles will gather on the detection line and develop color because of indirect effect.

2.3. Advantages and Disadvantages of Colloidal Gold Immunoassay Strips

The nucleic acid test (NAT) was commonly used in the early stage of detecting SARS-CoV-2. Later, a more efficient and rapid method appeared. It is the colloidal gold immunochromatographic test strip. Although it requires no professional skills and large instrument and a short detection time, there are still some drawbacks. Firstly, the target of antigen detection, site of sample collection, sample quality and the concentration of viruses in the patient have effects on the results of colloidal gold immunochromatographic test strip. At the same time, the load and concentration of the virus are different in patients' bodies during different infection periods. And the instruction of the test strip demonstrates that the minimum detection limit is 70TCID₅₀/ml after serial dilution of the positive samples of SARS-CoV-2. Since there is no amplification of the target, the rate of detecting positive samples with a low load of SARS-CoV-2 is relatively low. Therefore, negative results cannot be evidence of ruling out infection [8]. SARS-CoV-2 typically takes 1–14 days to incubate, and most infected individuals take 3–7 days on average, according to epidemiological statistics. The infected person may have discomfort after the incubation period is over. The window period for antigen detection is 5 to 12 days after the onset of symptoms due to individual variability. The 10 patients in Table 1 took 3.1 days on average to detect positive samples by antigen after seeing the doctor, and the analysis of the patients may be taking place during the window. The antigens remained negative even though the nucleic acid test was positive [9].

Table 1. Comparison of 10 results between antigen detection and reverse transcription - polymerase chain reaction (RT-PCR)nucleic acid test [10].

No. of patient	Result of antigen	Result of antibody	Result of NAT		No. of days that antigen becomes positive after visit
			lab	N	
1	Negative	Negative	19.2	18.25	4
2	Negative	Negative	17.2	16.17	3
3	Negative	IgG+	20.62	19.16	Never tested positive
4	Negative	Negative	18.24	17.67	3
5	Negative	Negative	20.09	18.5	2
6	Negative	Negative	21.47	20.61	4
7	Negative	Negative	25.08	24.11	3
8	Negative	Negative	15.61	15.27	4
9	Negative	Negative	18.41	16.6	5
10	Negative	Negative	16.45	15.35	3
Average	Negative	Negative	19.29	18.17	3.1

Nitrocellulose membrane (NC membrane) block effect is a factor that influences the sensitivity of the colloidal gold immunochromatographic test strip. Non-specific adsorption has negative impacts on the sensitivity, specificity and reproducibility of biosensors [1]. Bovine serum albumin (BSA) could be blocked on the nitrocellulose membrane to undermine non-specific adsorption (NSA). In the experiment, normal human sera were tested on different strips blocked by four concentrations of BSA, which were 0mg/ml, 0.5mg/ml, 1.0mg/ml and 2.0mg/ml respectively. There are signals shown on the testing line (T line) on the 1.0mg/ml and 2.0mg/ml strips. However, there were false positives shown on the other two strips. In conclusion, an excessive amount of BSA will reduce the sensitivity of gold nanoparticles rather than offset the effect of non-specific binding. Therefore, it is most suitable to select 1.0mg/ml BSA or a concentration lower than 1.0mg/ml BSA to block nitrocellulose membrane [8].

Colloidal gold nanoparticles have a wide range of applications across various fields, such as Biomedical Research, Diagnostics, Drug Delivery, Catalysis, Electronics and Optics, and Surface-Enhanced Raman Spectroscopy (SERS). Therefore, colloidal gold nanoparticles have significant value in many fields. In the field of diagnostics, colloidal gold nanoparticles are widely used for their unique optical properties. They can be functionalized with specific biomolecules such as antibodies or DNA probes to create biosensors for detecting various diseases or pathogens. The interaction between the target analyte and the functionalized gold nanoparticles can be easily visualized through color changes or fluorescence signals, enabling rapid and sensitive detection. In addition to diagnostics, colloidal gold nanoparticles also find applications in therapeutics. Their small size and high surface area-to-volume ratio allow for efficient drug loading and delivery. By modifying the surface of the nanoparticles with targeting ligands, such as peptides or antibodies, they can specifically accumulate at the site of disease, improving drug efficacy and minimizing systemic toxicity. Moreover, colloidal gold nanoparticles have shown great potential in imaging and photothermal therapy. Due to their strong light absorption and efficient heat conversion properties, they can be used for photoacoustic imaging, where laser pulses generate ultrasound signals that can be used to visualize tissues with high resolution. Furthermore, when exposed to near-infrared light, colloidal gold nanoparticles can produce localized heat, which can be utilized to destroy cancer cells or bacteria through photothermal ablation. Beyond biomedicine, colloidal gold nanoparticles have applications in catalysis, electronics, and environmental sensing. Their unique surface chemistry and catalytic properties make them ideal catalysts for various reactions. They are also used in electronic devices such as sensors, displays, and memory devices due to their excellent electrical conductivity and stability. Additionally, they are employed in environmental sensing for detecting heavy metals, pollutants, and contaminants in water and soil. Overall, the versatility and unique properties of colloidal gold nanoparticles make them indispensable in numerous fields, holding great promise for advancements in healthcare, technology, and environmental sustainability.

There are some benefits. Firstly, each test strip only consumes 10–20 μL of a sample, whereas the RT-PCR typically requires more than 100 μL for the nucleic acid extraction process. So that there are fewer sample uses. Second, it is more convenient for operators to collect samples, observe results and clean up the wastes. Because it requires no professional skills and sophisticated instruments. And test strips are portable so that operators can use them in any place. Furthermore, it can prevent the collection of respiratory tract samples from patients, greatly lowering the risk of infection for medical personnel [9].

2.4. Suggestions

In order to solve these problems, there is some advice. The most important component of the colloidal gold immunochromatographic test strip is the NC membrane. NC membranes vary from batch to batch, and from roll to roll. Therefore, the key to guaranteeing the quality of test strips is choosing trustworthy suppliers for NC membrane raw materials and setting up suitable inspection stations. Furthermore, the issues of false positives and false negatives should be the primary considerations in the design and development of the test strips. False positive results are typically caused by sample components that can bind to the detection antibody non-specifically, such as heterophile antibodies(HA), rheumatoid factors(RF), lysosomes, and so on. The manufacturer should thoroughly investigate the product's potential for false positive results before attempting to add it to the sample pad. To stop these compounds from interacting with the detection antibody, antibodies of the same species as the detection antibody binds to them beforehand. In order to prevent them from reacting with the detection antibody, the industry should thoroughly investigate the false positive of the product and attempt to include antibodies from the same species as the detection antibody in the sample pad. The sensitivity is fairly low as a result of its methodology's properties. As a result, when the test strip being tested has a very low concentration, some false negative findings will unavoidably be produced. The virus's mutation, which prevents it from combining with the antibody and causes the kit to fail, could possibly be to blame. To prevent false negatives, businesses can create several cloning sites and frequently check the most recent virus mutant strains [10].

3. Conclusion

At present, the impact of the COVID-19 epidemic on society has not diminished, and most parts of the world are still under the threat of the epidemic. The COVID-19 has caused irreversible damage and even death to the infected people. Therefore, the detection of viruses is particularly important. In this study, the authors carried out in-depth research on the colloidal gold immunochromatographic test strip detection technology. In the application of colloidal gold immunochromatographic test strip detection technology, the authors found that it has the characteristics of high specificity, fast reaction speed, simple operation and low cost. As a macromolecular marker, colloidal gold is more stable than other alternative substances. The outer electrons of its atoms are positively charged and the inner electrons are negatively charged, which helps to maintain the repulsion between colloidal gold particles and is equivalent to maintaining its stability. In most cases, colloidal gold immunochromatographic test strips can be used to detect some low concentrations of protein fragments, but because of its relatively simple detection method, it is more likely to be affected by the environment, but also because of its convenience, it is still widely used. Colloidal gold immunochromatographic test strips are mainly divided into two types, namely competitive method and non-competitive method, and the non-competitive method is also divided into two types, namely sandwich method and indirect method. One thing that all methods have in common is that they use colloidal gold solutions as markers. They are composed of a sample pad, colloidal gold pad, colloidal membrane (NC membrane), absorbent filter paper, and PVC backing plate. In the detection of colloidal gold immunochromatographic test strips, there are some very obvious shortcomings. One is that it has high requirements for the location and quantity of sample sampling. If not, it will cause very significant errors in the test results, such as false positive or false negative, so that patients cannot understand their situation in time. Nonspecific adsorption has a negative impact on the sensitivity, specificity, and repeatability of biosensors. An excessive amount of BSA can also reduce the sensitivity of gold nanoparticles rather than offset the effect of non-specific binding. In summary, this paper studied the application, principle and characteristics of colloidal gold immunochromatographic test strip detection technology. In this paper, we can better understand the role and significance of colloidal gold immunochromatographic test strip technology. Due to the limitation of hardware conditions, it is impossible to carry out actual experiments, so some contents of the article cannot be tested (the materials of this article are from the network literature search). In the future, the authors believe that colloidal gold immunochromatographic test strips will be applied and popularized in more fields.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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