Research Progress and Application Prospects of Immunochromatography Technology in Bacterial Detection

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Abstract: Immunochromatography plays a significant role as a rapid and simple detection method in the field of bacterial detection. This article reviews the principles of immunochromatography and the progress of its application in bacterial detection. It mainly includes two types of techniques: colloidal gold immunochromatography and fluorescence immunochromatography, and elaborates on their detection principles and technical characteristics in detail. In terms of practical application, the specific application and effect of this technique in the detection of carbapenemase, Salmonella, Shigella flexneri, Brucella and other pathogens are highlighted. The study shows that immunochromatography has the advantages of simple operation, rapid detection, high sensitivity and strong specificity, and has broad application prospects in fields such as clinical diagnosis, food safety and environmental monitoring. The future development will focus on improving detection sensitivity and specificity, developing multi-target detection systems, and combining with modern sensing technology, artificial intelligence, etc. to achieve more accurate quantitative detection.

***Keywords*:** immunochromatography technique; bacterial detection; quick diagnosis; pathogenic microorganisms

1. Introduction

Immunochromatography, a new detection method developed at the end of the 20th century, combines the advantages of immunotechnology and chromatography. It is characterized by simplicity of operation and rapid response, and has been widely used in clinical diagnosis, environmental monitoring and food safety[1].

2. The principle of immunochromatography

2.1 Principles of colloidal gold immunochromatography

After the target antigen in the sample to be tested binds to the colloidal gold-labeled antibody to form a complex, it moves autonomically through the capillary action of the nitrocellulose membrane (chromatographic membrane), passing through the detection line (T line) and the quality control line (C line) in sequence. The T line is coated with another specific antibody, which captures the complex and causes it to aggregate to form a red band (indicating a positive); A C-line coated antibody that binds to a colloidal gold-labeled antibody will show color regardless of whether it contains the target antigen (to verify the validity of the test strip). Ultimately, by observing the color development of the T and C lines, one can simultaneously determine whether the sample contains the target antigen and whether the detection system is functioning properly.

2.2 Principles of Fluorescence immunochromatography technique

When the sample to be tested is added to the sample pad, the substance to be tested in the sample binds to the fluorescently labeled antibody fixed on the binding pad to form an antigen-antibody complex. As the sample solution continues to permeate, the complex is pushed forward along[2] the nitrocellulose membrane by capillary action. When the complex reaches the test line, it specifically binds to another antibody fixed on the test line, causing a large accumulation of fluorescent markers at the test line, which appear as bright fluorescent bands[2] under ultraviolet light. At the same time, the unbound fluorescently labeled antibody will continue to move forward and eventually be captured and bound by the control line, forming the control line fluorescence band. By observing the fluorescence intensity of the test line and the control line, it is possible to determine whether the target substance is present in the sample and how[2] much it is present.

3. Detection Applications

3.1 Carbapenemase detection

Carbapenemases, which hydrolyze carbapenem antibiotics and are A key mechanism[3] of resistance in Gram-negative bacteria, were classified into three groups A, B, and D by Ambler: groups A and D were serinases (such as KPC in group A

and OXA-48 in group D), and group B were zinc ion-requiring metalloenzymes (such as NDM)[4].

The research results of Meng Shuang et[3] al. show that the sensitivity and specificity of colloidal gold immunochromatography in detecting carbapenemase of 87 Enterobacteriales bacteria are 100.00%, and the detection time of colloidal gold immunochromatography is shorter than that of the mCIM combined with eCIM test. It was superior to the mCIM combined with eCIM test in identifying bacterial carbapenemase types, but the test cost was higher than that of mCIM combined with eCIM.

3.2 Salmonella detection

Zhao[5] et al. established colloidal gold immunochromatography for the detection of Salmonella paratyphoid b in meat products, with a detection limit of 9.0CFU/25g and a compliance rate of 58% with the microbiological method (false negatives are likely to occur when contamination is less than 10CFU/25g); Although not as sensitive as real-time fluorescent quantitative PCR, it is easy to operate and fast to detect, making it more suitable for on-site rapid screening.

In his review, Zhu Minglei pointed out that colloidal gold immunochromatography has shown good application prospects[6] in the detection of Salmonella. Liu Jian[7] et al. used colloidal gold test strips for detecting Salmonella O9 antigen to test egg, chicken and pork samples, with a sensitivity of 90.0% and a specificity of 98.6%, suitable for grassroots units and on-site testing.

3.3 Detection of Shigella flexneri

Based on the background that Shigella fuciformis is the dominant strain in China, a detection method[8] combining fluorescence microsphere immunochromatography test strips (FMs-ICTS) with PCR and magnetic purification was studied. The ipaH gene and resistance gene CTX-M-9 of Shigella were selected. 63 clinical samples were tested and compared with the classical method, PCR-GE and RTFQ-PCR. The results showed that the detection limit was 2.5×10^{-7}ng/μL, and the diagnostic coincidence rate was 100% with conventional PCR and RTFQ-PCR, which could correctly distinguish Shigella from non-Shigella. Magnetic purification eliminates false positivity and can be completed within 3 hours. It has the advantages of rapidity, sensitivity, specificity, non-toxicity, simplicity and low cost, and is expected to be used for clinical sample screening and monitoring.

3.4 Brucella animalis

Research on the establishment of quantum dot fluorescent microsphere immunochromatography test strips[9] for brucellosis (a serious zoonotic disease that endangers livestock and human health). Quantum dot fluorescent microspheres were covalently coupled with the Brucella outer membrane protein OMP22 as tracers, and OMP28 (detection line) and OMP22 monoclonal antibody (quality control line) were respectively coated on nitrocellulose membranes. The results showed that the method had a detection limit of 1.05ng/mL (diluted 1:512) and an overall average coefficient of variation of 8.78%; The total coincidence rate of 150 clinical samples with the tiger red plate test was 97.3% (positive coincidence rate 98.8%, negative coincidence rate 95.3%), and there was no cross-reaction with other related disease sera, with the advantages of fast detection, high sensitivity, strong specificity and low cost.

4. Prospects

One of the important directions for the future development of immunochromatography technology is to further enhance detection sensitivity and specificity. With the increasing demand for clinical diagnosis and food safety testing, the development of multi-target immunochromatography systems capable of detecting multiple pathogens simultaneously has become an inevitable trend. Simultaneous detection of multiple bacteria or bacterial toxins can be achieved by designing multiple detection lines on test strips and combining them with labeling techniques of different colors or different fluorescence wavelengths. Combining immunochromatography technology with modern sensing technology, artificial intelligence, etc. for accurate quantitative detection.

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