Research on the Application Immunochromatography in POCT

Zhang Dandan

Baicheng Normal University, Baicheng, 137000, China

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Abstract: Immunochromatography is an immunoassay, which based on chromatographic techniques and antigen-antibody-specific immune responses. Owing to its simplicity, convenience, ease of operation, rapidity, and relatively low price, Immunochromatography has been widely used in Point-of-Care Testing (POCT). With the increase of POCT detection items and the improvement of the requirements of quantitative, sensitivity and specificity of existing items, this paper reviews the latest developments and applications of immunochromatography.

1. Introduction

Immunochromatography (ICA) is a rapid detection technique developed at the end of the 20th century. It is based on the principle of antigen-antibody specific binding and is applied to nitrocellulose membrane by capillary chromatography under the action of antigen and antibody. Chromatography using colloidal gold as a marker, as a rapid test strip, is currently widely used in many fields. With the development of nanotechnology, various markers have appeared one after another, and immunochromatography has been greatly developed. From the previous qualitative detection to the current semi-quantitative or quantitative detection, the sensitivity of the detection is improved as well as the result. The rate of false positives has also been greatly reduced. The development and application of immunochromatography technology has broad prospects in the future research.

2. Related Basic Concept

2.1 Introduction to Immunochromatography

Immunochromatography is a rapid detection and analysis method that combines immunology and chromatographic techniques to detect serum proteins. It uses colloidal gold, colloidal carbon, magnetic nanomaterials, rare earth nanomaterials, quantum dots, etc.[1] At the time of chromatography, the complex of the label and the analysts is captured by the corresponding ligand to concentrate the detection line on the nitrocellulose membrane, and the presence or absence of color bands on the fiber membrane is used. And reflected light to be qualitative or quantitative, and is widely used in immediate detection (POCT).[2] The immunochromatographic test strip consists of a sample pad, a bonding pad, a nitrocellulose (NC) film, a test line (T line), a quality control line (C line), an absorbent pad, a polyvinyl chloride (PVC) bottom plate, and the like. [3] According to the size of the analysts and the way the antigen and antibody bind, it is divided into a double antibody sandwich method and a competition method.

2.2 POCT

“POCT” is an abbreviation for Point-of-Care Testing in English. Because some different names are made in the English literature Use, such as Near-Patient Testing, On-Site Testing, Bed Side Testing, Home Use Testing, Extra Laboratory Testing, etc., thus causing certain difficulties for its Chinese name and its accurate definition. [4] However, from the current situation, “bedside inspection”has been accepted by most people. The general definition of POCT mainly refers to some simple operations (non-professional inspectors can operate with simple training), and can be outside the central laboratory, such as: ward, patient residence, doctor's office, emergency department, operating room, ambulance Inspection techniques carried out in any place such as a car,

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a battlefield, or even a school or factory. [5] Because POCT has the advantages of simple operation, rapidity, high efficiency, low cost, stable reagents and easy storage and carrying, and the test results are comparable, it is showing good development momentum. Like central laboratories, POCT relies on the support of modern analytical techniques such as chemistry, enzymes, enzyme immunoassays, immunochromatography, immunolabeling, electrodes, chromatography, spectroscopy, biosensors, and optoelectronic analysis.

3. The Application of immunochromatography in POCT

The key to immunochromatography is the immunolabeling material that relies on labeled antibodies, and nanomaterials are favored for their excellent sensitivity and specificity due to superior signal amplification. The following are mainly introduced from colloidal carbon, quantum dots, rare earth nanomaterials, upconversion luminescence technology, superparamagnetic nanomaterials and aptamers.

3.1 Colloidal carbon

Compared with colloidal gold, the advantages of colloidal carbon are quiet obvious, which include higher color strength, higher sensitivity and labeling efficiency, wider kinetic detection range, lower cost, simple manufacturing process, large-scale production and more environmentally friendly. However, due to its long marking and sealing time, it does not reflect the absolute advantage of colloidal gold, so its commercialization is low. Colloidal carbon test strips made from carbon nanoparticles are used to detect Plasmodium and can be quantified by scanning for grayscale values. Using a new colloidal carbon material, graphene oxide, as a marker, a test strip for the detection of aflatoxin B1 was successfully produced, and the minimum detection limit of the naked eye was 0.3 ng/mL. Advances in materials science can facilitate advances in detection, such as carbon quantum dots and graphene quantum dots.Materials are also marker materials that can be utilized in immunochromatographic studies.

3.2 Quantum dot chromatography

A quantum dot is a semiconductor nanoparticle composed of IIB~ VIA or IIIA~ VA elements and capable of being excited to emit fluorescence. Among them, Cd X (X=S, Se, Te) is mainly studied. Quantum dots are considered to be the most promising immunochromatographic markers. Its main advantages include that the emission spectrum can be controlled by the size of quantum dots, the absorption spectrum is wide, the emission spectrum is narrow, the fluorescence intensity is high, the lifetime is long, and the stability is good. Better biocompatibility, combined with a reader, enables quantitative detection. An immunochromatographic test strip for the detection of microcystin in water was developed using quantum dots as a marker with a detection limit of 0.1 μg/L. However, the quantum dot itself is insoluble in water and needs to be modified into a hydrophilic substance to be used for labeling biomolecules; for a sample with a low residual amount, the detection sensitivity is not required; in actual detection, it is also susceptible to matrix and background.

3.3 Lanthanides

The lanthanide element is a general term for the 15 elements from the 57th element to the 71st element. It is represented by the symbol I, n. Time and fluorescence immunoassay chromatography (TRFICA) was formed by combining immunochromatography with immunochromatography. Compared with traditional fluorescent labels, lanthanides have unique fluorescent properties: long fluorescence lifetime, large wavelength difference (stokes shift) between excitation and emission. There is no intersection between the excitation spectrum and the emission spectrum, and the characteristic peak is very sharp. The marker is small in volume (atomic marker), and the marker does not affect the spatial stereostructure of the marker (especially the effect on the protein), ensuring the stability of the substance to be tested, and To achieve multi-site marking and other characteristics. High signal-to-noise ratio and sensitivity are achieved by effectively utilizing wavelength-resolved and time-resolved techniques to eliminate non-specific fluorescence. The

specific fluorescence is measured by time-resolved technique, and the substance to be tested is quantitatively analyzed, which solves the high background problem of non-specific fluorescence in materials and samples in common fluorescence analysis, among which Eu is most commonly used. A time-resolved fluorescent immunochromatographic test strip made of Eu3+-conjugated polystyrene microspheres as a marker to detect alpha-fetoprotein. The team used quantitative T-ray fluorescence (HT/Hc ratio) to quantify alpha-fetal Quantification of the protein at the HT/Hc ratio eliminates the inherent heterogeneity between the test strips and the matrix effect of the test specimens, resulting in better accuracy and repeatability. Finally, the panel tested the linear range of alpha-fetoprotein to 1.0-1000.0 IU / mL, the minimum detection limit reached 0.1 IU / mL, and the results of the commercial chemiluminescence kit were different. Similarly, TR-FICA can implement a test strip to detect multiple substances. There is also a competition method, one C line and three T lines are drawn on the NC membrane, and three T lines are used to detect three different β-agonists: clenbuterol, ractopamine, salbutamol. In theory, the sensitivity of TRFICA can reach 10-18 mol / L, but it can not be achieved in the existing research, so future research still needs to further develop its high sensitivity advantage.

3.4 Upconversion Luminescence Technology

The upconversion luminescence technique uses nano or submicron particles composed of rare earth metal elements (mainly lanthanides).The upconversion luminescent material can emit visible light of different wavelengths under infrared excitation light, which has strong anti-light bleaching ability, wide anti-Stokes displacement, low toxicity, small background fluorescence interference, stable luminescent characteristics, etc. Sensitivity and signal-to-noise ratio can be improved during detection, especially for the detection of complex samples. It developed an immunoassay based on up-conversion nano-fluorescence for rapid quantitative detection of procalcitonin. The minimum detection limit was 0.02ng/mL and the linear range was 0.05-44.00ng/mL. Based on the characteristics that up-conversion luminescence is particularly suitable for the detection of complex samples, a multi-detection test strip based on up-conversion luminescence can be developed.

3.5 Magnetic Nanospheres

In traditional immunochromatography methods, quantification relies mainly on the tester to read visible light or fluorescence, but the disadvantage of this method is that only visible or fluorescent light on the surface of the film and below 10μm can be detected. However, in general, the thickness of the NC film is generally several hundred micrometers, and the analyte has a distribution of indicating substances from the surface to the bottom of the film during chromatography, thus causing up to 90% or more of the indicator substance to be invisible or optical. As measured by the instrument, this is a great loss for the sensitivity of the test. The immunochromatography method based on superparamagnetic nanoparticles (MNPs) as a marker can realize the complete reading of the signal on the membrane by using the magnetic signal analyzer (MAR), without considering the thickness of the membrane, so that the sensitivity can be It is increased by 10 to 1000 times, and the magnetic signal does not have obvious loss after the test strip is placed for a long time. There is a rare magnetic signal in the biological material, so the interference to the test strip is small, so such a method can be achieved. Based on immunochromatography of superparamagnetic nanoparticles, there are two quantitative detection modes: the first mode is to use a giant magnetoresistive sensor to detect the magnetization saturation intensity of all magnetic nanoparticles on the T line in an oscillating magnetic field; The second mode is to use a magnetic signal detector to scan various parts of the NC film in a magnetic field to obtain magnetic flux. However, the detection instruments used in the two modes are not suitable. GONG et al. also used magnetic microspheres to establish a method for simultaneous detection of cardiac troponin I and creatine kinase isoenzyme (CK-MB) with a minimum detection limit of 0.049ng/mL and 0.085ng/mL. This also provides a certain guide for the method of establishing immunochromatography using magnetic microspheres in the future, and can use the smart devices such as mobile phones as the detection devices to achieve more efficient and rapid detection.

3.6 Aptamer

Conventional immunochromatography uses antibodies as markers and captures, and is now being screened out with the use of index-rich ligand-based systems (SELEX). Compared with traditional antibodies, aptamers have many advantages: short screening period, high affinity and stability, high specificity, good repeatability, easy synthesis and modification, low cost, easy storage, no immunogenicity and Toxicity, compatible with targets such as cells, heavy metals, proteins, bacteria and viruses. AHMAD RASTON et al. used SELEX to screen serine protease inhibitor homologous aptamers in combination with colloidal gold technology to detect serine protease inhibitors, and the detection limits in solution and serum reached 0.137 nmol and 0.105 nmol, respectively. For the first time, WU et al. used aptamers to detect zearalenone with a detection limit of 20ng/mL. Due to the many advantages of aptamers and the gradual maturity of SELEX technology, it is a good direction to use aptamers instead of antibody detection on immunochromatography.

4. Conclusion

Immunochromatography is widely used in many fields due to its advantages of simplicity, speed, and ease of operation. However, this technology still has some problems in the rapid detection process and has a lot of room for improvement. The emergence of photoelectric detection instruments and the application of new marking materials have made a huge breakthrough in quantitative detection of chromatographic techniques, but most of the current detection results are still qualitative or semi-quantitative. The popularization of quantitative detection is still the immunochromatographic test paper technology. In terms of sensitivity, the emergence of some new markers (such as rare earth elements or QDs) and signal amplification systems (such as magnetic separation technology, biosensors) has made the sensitivity of immunochromatographic test strips somewhat improved. In addition, simultaneous detection of multiple antibodies can be achieved using multi-membrane complexes or single-membrane multi-labeled acceptors using fluorescent labels (fluorescent microspheres, quantum dots or lanthanides). With the development of biotechnology, nanotechnology, and optoelectronic technology, more and more new types of markers, antibodies, and quantitative detection instruments will appear. The performance of immunochromatography technology will be greatly improved. I believe this is simple. Fast detection methods must be applied to a wider field.

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