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Review Article

A Review of Test Strips in Rapid Detection of Food Safety

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Abstract

As a simple, efficient and low-cost detecting instrument, test strips provide an effective guarantee for the rapid detection of food safety. In this review we discuss the development of test strips from the perspective of rapid detection technology, including paper chromatography, chemical colorimetric, enzyme inhibition, immunoassay, biochemistry and molecular biological technology. Then, the innovation of the typical test strips is briefly analyzed, such as paper-based microfluidic analytical devices, nano-materials markers and paper analyzer. Finally, the prospects of test strips are predicted.

Keywords: The test strip; Food safety; Rapid detection

Introduction

In recent years, major food safety incidents are constantly happening around the world. To solve these food safety issues, we need to monitor and control the multi-steps 'from farm to table', including the production, processing, distribution and marketing of food [1]. Therefore, a large number of fast, accurate, sensitive and inexpensive in situ analytical techniques are badly needed for food safety detection. Under such background, test strip has attracted much attention as a great analytical technique with its superior features, including low costs for manufacturing and using, biodegradable and biocompatible, easy coating, and porous fibrous structure. Besides, it works under capillary force and no external power is needed. And its common white background can better demonstrate chemical colorimetric and fluorescent detection [2]. With the continuous development of the test strip method in the past decades, accompanied by a variety of detecting technologies, test strips can now be applied in the detection of all kinds of typical food contaminants. Therefore, test strip has raised more and more attention in the detection area.

Test strip is a quick detection method based on visual observation or quantitative analysis of the results shown in color, fluorescence or magnetic change, after the specially prepared test strip reacts with the substance to be tested [2]. The processing of test strips is relatively simple. A strip is usually coated or impregnated with a prepared liquid, and dried in an appropriate manner. It is also very easy to use, simply dropping the test substance onto the strip or immersing the strip in the test solution. Therefore, operators could easily master the testing without any training. As is seen, test strip method as a rapid, in situ detection method, has multiple advantages: easy to produce and use, low in price, high sensitivity and specificity, good stability, rapid reaction, no equipment or maintenance needed, immediate display of results, disposable, etc. The method has shown great potential in the field of food safety testing and is expected to become the cheapest detection technique.

Development and current situation of test strip method

Currently, test strip method is usually accompanied by detection techniques such as paper chromatography, chemical colorimetric techniques, enzyme inhibition technology, and immunoassay, biochemistry and molecular biology techniques.

Paper chromatography: Paper chromatography technique, also known as filter paper chromatography, was invented as early as the 1940s and had shown the original idea of test strip method. The development of paper chromatography saw a great breakthrough in 1952 when its two inventors, Martine and Synge, were awarded the "Nobel Prize" [3]. In about twenty years between the late 20th century and the early 21st century, paper chromatography was widely applied in the detection and analysis field, from organic matter testing to inorganic matter testing, and from qualitative testing to quantitative testing [4].

Paper chromatography takes filter paper as the reaction carrier, on which the solution to be tested is dropped by a sample applicator or a capillary tube and is considered as a stationary phase. Under the force of paper chromatography, the organic solvent moves as a developing agent, while the test solution as the mobile agent will also start to move, and the components of the test solution will dissolve and re-distribute into different groups, which will appear as separated spots on the paper. Qualitative detection compares the distance traveled by those groups of components (Rf value) with moving distance of the known sample; while quantitative detection collects the spots from the paper, dissolute and separate the components out, and use colorimetric or spectrophotometric methods to complete the quantitative analysis [4]. Paper chromatography is currently applied in the rapid detection of organophosphorus pesticides [5], metal ions [6], amino acids [7], Sudan red [8] and other substances in food.

Chemical colorimetric technique: Chemical colorimetric technique refers to a qualitative or semi-quantitative detection method, in which the test substance in food reacts with a specific chemical reagent and causes color changes, which will be compared with the standard color cards and analysis will be made based on the color comparison [5]. Chemical colorimetric technique has the advantages of low costs, short production cycle, simple operation process, rapid color display, direct result, and so on. The downside is that the technique is low in sensitivity and therefore it can hardly be used in the detection of trace substances. Moreover, since such methods highly depend on the chemical reactions of the test substances, the results are more likely to be disturbed by external interference in the testing process [4]. Currently, chemical colorimetric test strip is the most widely used and the most mature

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rapid detection method and is applied in the detection of illegally added substances and hazardous substances in agricultural products. For example, chemical colorimetric test strips are used in the rapid detection of chloride, nitrite, urea [9] and other substances that might exist in food.

Enzyme inhibition technique: Enzyme inhibition technology is a rapid detection method mainly applied to the detection of heavy metals and pesticide residues. This technique is based on the inhibitory effects on enzyme by the test substances. Heavy metals are commonly detected with urease [10], and pesticide residues with plant esterase, animal esterase or cholinesterase [11]. The mechanism is to allow the test substance (serving as the substrate) to combine with the active center of a corresponding enzyme, so that the nature and structure of the enzyme is changed and its activity reduced, which can be identified. And the test substance can therefore be analyzed according to the changes, most commonly, and color changes. Usually, the enzyme and the test substance are fixed onto two test strips respectively and put in contact with each other, when inhibitory effects of the test substance will take on the enzyme and catalytic reaction will occur, accompanied by color changes. Based on these changes, the substance will be detected qualitatively by visual inspection or be detected quantitatively. This method has many prominent advantages, including simple operations and low costs, and is suitable for the regulatory authorities to exercise fast testing in the farmers' markets, supermarkets and other food distribution centers. But this method has some limitations in terms of sensitivity and preservation, and has higher false-positive rate and false-negative rate. It is susceptible to interference in the detection of some agricultural products, such as ginger, onion and garlic. Therefore, this technique can only serve as a preliminary screening method in food safety testing. At present, this method has become a basic means in field-testing and pesticide residues screening in developed countries, but has not been widely applied in underdeveloped countries.

Immunoassay technology

Immunoassay technology develops from the serological detection method in medicine and depends on the high specificity of reactions between antigens and antibodies [12]. The technology contains three approaches: sandwich method, competition method and indirect method. Taking the 'sandwich' approach for example, the mechanism is to crosslink the specific antibody of the test substance to a colored material (such as colloidal gold, carbon, up-converting phosphorus, etc.) and to a test strip (detection line) respectively. After the "colored substance - antibody" combines with the antigen, the combination will then bind with the antibody on the test strip and form a sandwich-like structure. Visual qualitative detection can be conducted by observing the color changes of the detection line and the control line, while quantitative detection is based on the principle that the shades of the color on the detection line are in proportion to the amount of the substance to be examined [13].

Immunoassay-based test strip is one of the most developed methods for food safety, which is widely used to detect toxin [14] and veterinary drug residues [15], microorganisms [16] and genetically modified foods [17]. In order to improve the performance of the strip, researchers have made a lot of effort in the past two decades, such as increasing sensitivity and specificity, speeding up response time, improving the ability of multiple analysis, etc. Hongfei Gao, et al. [15] proposed a immunochromatographic strip using horseradish peroxidase-tagged antibodies for rapid and multiple detection of β_2-agonists by utilizing ractopamine (RAC) and salbutamol (SAL). The whole process can be completed within 20 min, and the detection limits of RAC and SAL were 0.20 and 0.040 ng mL^{-1} (S/N = 3), respectively. With high sensitivity and specificity, this technique is suitable for the regulatory authorities to do field screening. However, this technique can hardly be applied to a wide range, because the reaction between the antigen and antibody is highly specified, which requires to establish specialized detection reagents and conditions for different test substance. Besides, the accuracy of the test results also depends on whether the antigen component is damaged during the processing of food. Currently, numerous immunoassay test strips have been commercialized. For example, the ROSA series colloidal gold immunochromatographic test strip, produced by Charm Science Inc, has been used to detect a variety of antibiotic residues in milk [18].

Biochemical technology

Biochemical techniques, combined with test strip method, are mainly used in the testing of food borne pathogens and other microorganisms. This microbiological test strip method usually requires two layers of films; one is a layer of polypropylene film printed with a grid, and the other a polyethylene film coated with culture medium and chromogenic material. After treatment, the sample can be inoculated directly onto the microbial paper without enrichment. Cultured at suitable temperatures, specific enzymes will be produced as the growth of microorganisms and will react with the fixed chromogenic material, after which will appear colonies of different colors. Rapid detection can be conducted by simply counting the number of the colonies [19].

The biggest advantage of this biochemical technique is that it saves people from the heavy preparation and finishing work, shortens detection time and helps to improve the efficiency of microbial testing. In addition, the mechanism of this technique is based on living cell technology, in line with the standards of food safety and hygiene quality regulations, and is therefore easier to be received by the public [20]. The downside is that the present color system is relatively simple; and the irregular distribution of culture medium or chromogenic substance on test strip may lead to the non-uniform growth or distribution of microorganism or uneven color display. Currently, microbial test strips have been commercialized. For example, the Perrifilm TMPlate series microbiological testing piece, developed by the U.S. company 3M, has become a very mature product, and can accurately detect bacterial count, coliform count, molds and yeasts, etc [21].

Molecular biology techniques

The combination of molecular biology techniques and test strip method allows target at molecular level to be tested. In recent years, nucleic acid paper chromatography and microarray have become hot research topics. Nucleic acid paper chromatography is a technique combining the nucleic acid amplification technology in molecular biology and the chromatography paper method. It inherits the high sensitivity from nucleic acid amplification technology, and the simple, inexpensive features from chromatography paper method [22].

Microarray is a technology derived from the continuous development of gene technology and material science, which can quickly detects a gene sequence and the information carried by it. The technical mechanism is to hybridize a known sequence with an unknown sequence, analyze the hybridization signals, and then deduce the unknown sequence information [23]. The advantages of using test strips at the nucleic acid molecule level include its low detection limit, short cycle, high efficiency, multi-detection capability, and that it makes it possible to detect deep processed products. Its disadvantages are that it costs high and requires the preparation of a large quantity of pre-sequenced DNA fragments. Molecular biology techniques, combined with test strip method, have shown great prospects and have been applied to the detection of pathogens [24], viruses [22], MicroRNA [25] and genetically modified organisms [26] in food.

Innovations in test strip method

In recent years, innovations of the test strip method have mainly focused on improvement of carrier material, selection of marker and quantitative detection of target.

Carrier innovation: paper-based microfluidic chip: Since Swiss scientist Manz proposed the concept of Micro Total Analysis in the 1990s [27], microfluidic chip as the core technology of the concept has gradually developed into one of the world's most advanced science. The idea of paper microfluidic chip was first proposed in 2007 by Martinez [28], and has seen rapid development recently. The microfluidic chip uses filter paper as carrier, employing a series of production technologies as lithography, ink-jet printing technology, wax printing technology, PDMS printing and plasma oxidation. Its finished products can be used to detect and analyze samples with high efficiency. The manufacturing cost of paper microfluidic chips is low and the preparation without complicated equipments is simple. And since it is comparatively miniaturized, integrated and multi-functional, it is very suitable for use in resource-poor conditions [29].

Existing paper-based microfluidic chips are mainly divided into three categories: two-dimensional paper-based microfluidic chip [29], two-dimensional paper-based ELISA plate [30] and three-dimensional paper-based microfluidic chip [31]. Whitesides' team uses hydrophobic polymer to photolithographs lines on hydrophilic paper and forms a two-dimensional paper-based microfluidic channel, making it possible to simultaneously detect proteins and glucose on the same platform [28]. And by replacing with appropriate detection reagents, it can be further applied to the determination of other substances. Later on, the research team developed a two-dimensional paper-based ELISA plate, which could replace the expensive traditional ELISA plate [30], and is easier to store, more biocompatible with plastic plate's experimental methods and the test results can be archived directly. For example, paper-microzone plates are thin (180 μm), require small volumes of sample (5 μL per zone), and can be manufactured from inexpensive materials ($0.05 per plate). The research team went further and developed a novel three-dimensional paper-based microfluidic chip [31], using double-sided adhesive tape to stick together multiple pieces of wax printing paper. The device has four independent channels which do not interfere with each other, and can be used to analyze multiple compounds simultaneously on a single paper-based chip. It has shown strong practicality and superiority in multi-analyses in situ rapid detection tasks. Liu and Crooks innovated from this and developed a three-dimensional origami-based microfluidic chip, simplifying several steps of Whitesides' process [32]. And Lewis employed the Batik technique to produce a high-throughput three-dimensional paper-based microfluidic chip. Hundreds of such chips can be produced within one hour [33]. As we can see, paper-based microfluidic chip has many advantages including low producing costs, short producing cycle and high detection speed. In addition, it is portable, disposable and capable of multiple detections; it also requires very small sample volume. It has therefore attracted more and more attention and research efforts, and has shown great prospects for future development.

Marker innovation: nano-particle markers: Nano-particles, also known as ultrafine particles or nano dust, refer to particles measuring at nanometers (typically between 1-100 nm). These particles are different from normal particles in terms of light, heat and magnetic susceptibility features; and they have large specific surface area. Nano particles have important scientific value and application prospects [34]. Several types of nano particle markers have already been applied in test strip method, including organic nanoparticle nanoparticles, colloidal gold, lanthanides, quantum dots, magnetic nanoparticles and carbon nanotubes.

Organic nanoparticles, such as fluorescein isothiocyanate (FITC) nanoparticles, have good optical properties and were one of the earliest markers applied in test strip method. The FITC nanoparticles provide the primary amines of proteins to form the desired dye-protein conjugate [35]. The detection of the protein can be achieved by simply examining the amount of fluorescein in the compound. But this method has low sensitivity and poor photochemical stability; it overly depends on the chemiluminescent group of the particle itself, and do not have the size effects of inorganic nano particles which could control wavelength. Therefore, nanoparticles are gradually being replaced by new markers.

Colloidal gold, also known as gold sol is a stable multiphase uneven system in water, formed by the electrostatic repulsion among gold particles [36]. Due to its high electron density, the system can absorb biological macromolecules without affecting the biological activity of the molecules, and meanwhile it will take on different bright colors ranging from orange, red to purple, so that it can be used as markers to pinpoint a variety of macromolecules, including proteins, polysaccharides, nucleic acids and hormones. Test strip with colloidal gold as marker is the earliest and most widely studied technique, and has been frequently reported. It is applied in the detection of aflatoxin [37], vibrio parahaemolyticus [38], Sudan red [39], MicroRNA [40] and other substances, many of which have been commercialized on a very large scale, such as gold marker test strips for the detection of veterinary drug residues and pesticides. Jia Wanga et al. [39] reported a colloidal gold-based immuno-dip strip applied to the rapid detection of Sudan red I residue in tomato sauce and chili powder samples with a LOD of 10ng/g.

Lanthanide elements refer to a group of transitional elements in the periodic table whose atomic numbers range from 57 to 71. Select two different lanthanide ions, use them as "light absorber" and "emitter" respectively, and incorporate them into ceramic particles serving as the "main substrates"; we can get a group of fluorescent up-

conversion phosphor particles [41]. Hong W et al. [42] developed a kind of test strip, using up-conversion phosphor particles as markers, could be stable for 10 days at 37°C with an average CV of 10.3%. Its sensitivity and quantitative results are comparable to the classic immunology experiment - enzyme-linked immunosorbent assay (ELISA), and its linearity fitting coefficient of determination (R^2) for different antibody detection is between 0.93 and 0.99. Therefore, employing lanthanide elements in test strips brings ideal detection limit and stability, so that it has seen rapid development in application.

Quantum dot, also called fluorescence semiconductor nanoparticles, includes main groups of II-IV (e.g. CdSe) and III-V (e.g. InP), sub-group compounds and nanoparticles composed by Si and the like elements. The diameter of these particles is about 1-10 nm. They look like tiny dots and are therefore named after Quantum dot. The current most commonly used marker is a core-shell structured quantum dot, which not only has good photochemical stability, but also has a high luminescence quantum yield (30% -50%) [43]. At present, quantum dot as test strip marker is still in the researching stage, and has been reported several times at home and abroad. Petryayeva E et al. [44] was reported to succeed in completing the quantitative detection of protease within 5 min, with a minimum test line of 1-2 nm.

Nano-magnetic particles, also known as superparamagnetic particles, are newly emerged nanomaterials in recent years. They have the dual advantages of both magnetic particles and nanomaterials, including their superparamagnetic property, large specific surface area and small particle size. Typical markers are magnetic materials (e.g. iron oxide), which serve as a solid phase carrier. When active groups are introduced onto the surface of them, coupled reaction will happen between the magnetic materials and biological molecules, such as enzymes and antibodies. In this way, test substance can be detected quickly and quantitatively [45]. M. Fisher et al. [46] provided a immunomagnetic lateral flow device enabled detection of B. anthracis spores at concentration of ~5×10^5 CFU ml^{-1} in 10 ml diary samples(n = 38), resulting in an improvement of 60-fold in sensitivity, compared with the traditional strip methods. However, since magnetic particles are prone to aggregation in the chromatographic process, reports about using nano-magnetic particles as test strip markers are still rare.

Carbon nanotube, or Buckytube, is a quantum material with a topological structure, which can be viewed as a curved hexagonal grid structure of graphite. It has the quantum effects of ordinary nanoparticles and large specific surface area, high conductivity and high mechanical strength. Its distinct black color is more conducive to be spotted by naked eyes in qualitative or semi-quantitative detection. Martina et al. [47] presented a new nucleic acid lateral flow for the assessment of listeria contamination with the lowest visually detectable amount was 0.1 ng of labeled amplicon. The PCR solution is directly added to the strip and the appearance of a grey/black line mediated by using carbon nanoparticles is indicative of the presence of specific amplicons (max 15 min). However, technically, it is difficult to remove the carbon graphite and amorphous carbon debris mixed in the carbon nanotubes. Relevant literature is also very limited [48], showing that the research is still in its early stages.

Quantitative innovation - strip analyzer: At the beginning of the test strip study, only qualitative testing is available, that is, only "negative / positive" conclusions can be made. With the continuous development of the study, especially after the introduction of standard colorimetric cards, test strip method can manage both visual qualitative detection and semi-quantitative analysis [2]. After the supportive micro-measuring reading instruments were invented, the accuracy of the quantitative results has been greatly improved. And test strip method has advanced into an analytical technique that can accomplish quantitative detection and analysis directly as needed [49]. These supportive analyzers have very high sensitivity and can meet the general requirements for detection. It also has the advantages of small, light, portable, fast, low-cost, capable of independent data processing, less demanding on staff, etc., and have broad prospects in markets and for further development.

Existing test strip analyzers can be divided into many types, which are focused respectively on optical signals, fluorescent signals, magnetic signals, chemiluminescent signals, electroconductive signals, electrochemical signals, etc [50]. The great majority of the analyzers are optical signal analyzers, which can be further categorized into three kinds according to their mechanisms: a) using photosensitive resistance to measure the light intensity of the test strip and pick up signals of reactions; b) using reflective optical fiber sensor to obtain signals of reactions; and c) using image sensors to capture the strip images and analyze the intensity of the reaction signals [51]. German company Merck produced a test strip analyzer, which uses the photosensitive resistance mechanism as mentioned above. On the side of the instrument's reflector, a small door is designed to insert a test strip and a small window which allows light to go through. When light is exposed on the test strip, a portion of it is absorbed and the other portion is reflected to CDS photocell. The amount of the flowing current will be detected by a microampere meter, and then converted to the corresponding amount of the test substance and showed directly on the screen. In this way, a quick and visualized quantitative detection is conducted. For instance, Yinli Zhao et al. [52] used a strip analyzer with a lateral flow colloidal gold strip for the quantitative detection of enrofloxacin residues, which indicated that the detection limit was as low as 0.138μg/kg, showing a reliable quantitative detection capabilitie.

With the rapid development of electronic information technology, people can have access to information almost anytime, anywhere and in a variety of ways, among which, smart phone is one of the most commonly used tool. Many research teams [53,54] are studying the possibility of using smart phones, cameras or scanners to record the colored area of a strip, and using Adobe Photoshop to analyze the colors, in order to achieve the rapid completion of data acquisition, signal processing, data analysis and drawing conclusion steps. This method is to obtain strip's image by image sensors, and use software to capture the color features of strips, based on the measurement principle. Then use statistical data analysis or image interpretation to draw quantitative results. Although the resolution of image sensors has some impact on the accuracy of strip image analysis, this method is still receiving growing attention because of its low costs, simplicity, rapidity, portability and other advantages.

Conclusion

Test strip method, as a rapid and sensitive detection method,

has demonstrated great application prospects in the field of food safety testing. In the past two decades, many research teams around the world have paid great efforts to improve the performance of test strips, such as increasing sensitivity and specificity, speeding up response time, improving analytical capabilities and reducing costs [29,55]. However, existing test strips are very limited in types, and their properties are far from meeting the requirements for in situ testing. For example, they have poor stability and therefore demand very strict preservation conditions; their storage time is short and cannot be applied in extreme environments; their sensitivity is low and cannot accomplish trace detection; they have poor specificity and are prone to cross-reactions. Therefore, future researches on test strips should focus on its superior material properties, multivariate testing substances and forms, the new chromogenic reagent and colorimetric systems, precise quantitative methods and instrumentation, etc. Researchers should pay more efforts to expand test strips' detection range, improve the detection performance of test strips to provide broader space for the further development of using test strips in the rapid detection for food safety. In addition, to effectively prevent hazards from going further beyond food's production and circulation process, and to improve the public's confidence in food safety, test strip method should be promoted and applied to food production and processing, regulatory inspection and even people's daily life.

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