Scholar Publishing Group
Academic Journal of Environmental Biology
https://doi.org/10.38007/AJEB.2021.020404
ISSN 2789-8407 Vol. 2, Issue 4: 28-35

Visualization of Salmonella Based on Nanobiosensing Technology

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Keywords: Nano Biotechnology, Sensing Technology, Salmonella Testing, Visualization Technology

Abstract: The establishment of an efficient, rapid and specific method for the detection of foodborne salmonella plays a crucial role in ensuring food safety and citizen health. The purpose of this paper is to study salmonella visualization based on nanobiosensing technology. In this study, a new salmonella biosensing detection method is proposed by combining the previously constructed solid-phase enrichment method based on swab fiber material and nano-fluorescent bioprobe detection technology. The targeted bacteria can be efficiently localized and amplified on the solid-phase carrier fiber, and at the same time, the purpose of in situ isolation of the targeted bacteria can be realized by fluorescence visualization. Through verification, a visual immunosensing detection technology for salmonella based on the improved solid-phase carrier culture method and water-soluble quantum dot bioprobes was successfully established. Due to its high specificity, high efficiency and high sensitivity, this technology is very suitable for large-sample salmonella screening work in disease control departments.

1. Introduction

Food contamination and the resulting diseases have become the most common health problems in the world, which not only affects the environment, causing hidden health hazards to the public, but also affects the stability and development of the social economy, and has become a social problem related to the national economy and people's livelihood [1]. Over the past decade, the incidence of food-borne diseases has been increasing, and there have been serious outbreaks. In some developing countries with relatively poor sanitary conditions and large populations, the problems caused by food-borne diseases are particularly serious. Salmonella can infect a variety of foods. With the rapid development of social science and technology, the detection methods and identification technologies of food-borne pathogens are also constantly being developed and

improved. Traditional detection technologies have been developed to be more sensitive, simpler and more time-saving, such as biosensors technology [2-3].

Fields such as molecular diagnostics and healthcare can greatly benefit from this new technology, which includes low-cost, rapid-response measurements that can be performed at the point of care by non-specialists [4]. Kemp n t begins with a theoretical overview of the study, including ac impedance, formation and structure of the electric double layer at the liquid-solid interface, and an equivalent circuit model for representing interfacial phenomena. Biosensing is then introduced and recent research highlights of resistive and capacitive biosensors based on nanogap electrodes are reviewed, especially those with new applications ranging from dna detection to nanoparticle capture [5]. Another optical biosensor developed by an academic achieves sub-ppt higher sensitivity; it is based on an optical planar waveguide used as a polarization interferometer (pi). This method holds promise for the development of portable, high-sensitivity, and simple-to-use biosensors suitable for point-of-care detection of mycotoxins [6]. Therefore, the establishment of biosensors that can specifically detect salmonella and can accurately distinguish different serotypes is of great significance for food safety and clinical diagnosis [7].

This paper focuses on the application of quantum dot-labeled biological probes to solid-phase carrier enrichment systems to achieve intuitive in situ separation under fluorescence imaging. Although fiber swabs have been applied to sample collection and transport, there are no reports on immunological detection, let alone quantum dot immunofluorescence detection technology. In addition, a stereo microscope observer that integrates blue light-emitting diode artificial light sources and special filters suitable for the naked eye observation of fluorescence will be developed. Efficient acquisition of isolates of targeted bacteria is the greatest purpose and advantage of this technology, which will help to further carry out molecular epidemiological analysis and clinical pathogenic confirmation in the field of public surveillance (such as food poisoning and water pollution). Drug susceptibility test.

2. Research on Visualization of Salmonella Based on Nano-Biosensing Technology

2.1. Biological Characteristics of Salmonella

Salmonella is a gram-negative, aerobic and facultative anaerobic bacterium of the enterobacteriaceae family and is a spore-free, non-enveloped food-borne virus. Most in flagella and cilia, cells are oval in shape, and they have three complex antigenic structures: flagellar antigens, surface antigens (cap or envelope antigens), and bacterial antigens [8-9]. Among many foodborne pathogens, salmonella serotypes are the most common bacteria causing foodborne gastroenteritis and are potential bioterrorist microorganisms. There are more than 2500 serotypes of salmonella, all of which are potential pathogens. Therefore, salmonella is considered a zero-tolerance microorganism in food, and microbiological testing of food samples is mandatory [10-11].

2.2. Salmonella Detection Method

(1) traditional detection methods

Traditional testing methods are still the standard method used by many national testing agencies to test for salmonella. Traditional detection methods have many inspection procedures and cumbersome operations. During the detection, the enrichment failure is likely to occur due to the low concentration of target microorganisms, and there are defects such as low detection accuracy, slow detection speed, and insufficient specificity [12]. In the face of some sudden food safety

incidents in real life, it cannot meet the needs of rapid, accurate, sensitive and specific detection [13].

(2) immunological detection methods

Immunomagnetic separation technology (ims) is a new type of research technology that is more and more accepted by people. Its main principle is the specific immune reaction between antigen and antibody. The antibody of the target bacteria is conjugated on the surface of the magnetic beads to prepare immunomagnetic beads (imb). After the specific immune reaction between the antigen and the antibody, the immunomagnetic beads (imb) and the target bacteria carry out the immune reaction. Under the action of a magnetic field, the target bacteria can be isolated and enriched [14-15].

(3) biosensor detection method

Biosensors are immobilized biomolecules as recognition elements [16-17]. Due to the advantages of high sensitivity and high specificity, biosensors have been widely used in clinical diagnostic inspection, monitoring during treatment, food safety, fermentation industry and environmental monitoring, etc., and in food and drug analysis, environmental protection and cancer. It has a wide range of application prospects in the research of early detection, biochip and pathogen detection. At present, various biosensors such as electrochemical biosensors and surface plasmon resonance biosensors (spr) have been applied to the detection of salmonella [18].

In recent years, biosensors based on magnetic nanoparticles have received increasing attention, mainly due to their unparalleled advantages over other methods. The so-called nuclear magnetic resonance effect is that when the atom is in the external magnetic field b0, due to the different orientation of the magnetic moment of each atomic nucleus, the initial energy level will be divided into different 2i+1 energies, when its frequency v0 satisfies the following relationship:

$$h\nu_0 = \Delta E \tag{1}$$

Among them, planck's constant $h=2\pi\hbar$. Δe is expressed as the energy difference between the two energy levels in the spin system. Because:

$$\nu_0 = \frac{\omega_0}{2\pi} \qquad \Delta E = \gamma\hbar B_0 \qquad \text{and} \qquad h = 2\pi\hbar \tag{2}$$

But:

$$\omega_0 = \gamma B_0 \tag{3}$$

It means that when the angular frequency ω of the radio frequency pulse is equal to the precession larmor angular frequency ω_0 of the nuclear magnetic moment of the nucleus around the external magnetic field b0, the nuclear magnetic resonance phenomenon will occur.

(4) molecular biological technology detection methods

With the vigorous development of modern biotechnology and the continuous development of new technologies and new theories, the theory and technology of modern molecular biology continue to develop. Modern molecular biology technology has high sensitivity, accuracy and specificity, which can greatly reduce the detection time when used as a detection method. At present, both nucleic acid probe technology and polymerase chain reaction (pcr) can provide new technical support for the detection of salmonella. The basic principle of nucleic acid probe technology is based on the specific nucleic acid sequence (dna or rna) of salmonella to determine the analysis of

salmonella genus or species.

3. Investigation and Research on Visualization of Salmonella Based on Nanobiosensing Technology

3.1. Experimental Animals and Related Experimental Materials

Sixteen spf-grade big-eared experimental rabbits (eight males and eight males) tested by the experimental animal quality inspection station, all weighing about 2.5 kg, were purchased from the experimental animal center. After purchase, the experimental rabbits were directly placed under spf-grade rearing conditions and firstly adaptively reared, and immunized after 5 days. Relevant materials include sterile 5ml cryovials, 2.5ml and 1.0ml syringes.

3.2. Antibody Purification

Apply protein separation and purification column chromatography (spa-sepharosecl-4b column). The operation method of column regeneration: first, use 10 times the column volume of 0.1mol/l ph8.6 tris containing 0.5mol/1nacl solution to elute the residual impurities on the column; then use 10 times the column volume of 0.1mol/l ph4.5 sodium acetate the buffer contains nacl solution (0.5mol/l) to elute the residual impurity protein on the column; 0.1mol/l phosphate buffer at ph 8.0 is equilibrated and stored at 4 ℃.

3.3. Detection of Quantum Dot Bioprobes

The ultraviolet absorption spectrum of the quantum dot bioprobe was detected by a uv-visible spectrophotometer at room temperature, and the fluorescence spectrum was detected by a fluorescence spectrophotometer at room temperature. Use agarose gel electrophoresis (0.8% agarose gel, the voltage is 80v, electrophoresis 15min) to observe the electrophoresis results in a uv analyzer to detect whether the labeling of mab-conjugated qds is successful.

3.4. Detection of Analytical Sensitivity

In this study, mixed bacterial liquid samples were prepared, each tube containing 101cellsml-1 salmonella manhattan and 105cellsml-1 or 106cellsml-1 or 107cellsml-1 non-salmonella (escherichia coli, proteus, citrobacter), 10 samples as a group. Swabs inoculated with sterile distilled water were blank controls. The relevant evaluation criteria are the occurrence rate of dark spots, the identification rate of naked eye fluorescence and the identification rate of confocal microscopy. All isolates were confirmed by traditional standard identification methods (including serological and biochemical identification).

4. Analysis and Research of Salmonella Visualization Based On Nanobiosensing Technology

4.1. Related Detection of Immune Preparation and Purification of Antiserum and Antibodies

Determination of antibody concentration by protein separation and purification column chromatography: the detection value and the standard value of bradford method after doubling the dilution of the extracted antibody are shown in table 1 and figure 1.

Table 1. od values and concentrations

Serial number	Od value	Concentration (ug/ml)
A	0.67	820
B	0.55	680
C	0.57	716
D	0.54	593
E	0.48	460
F	0.54	613

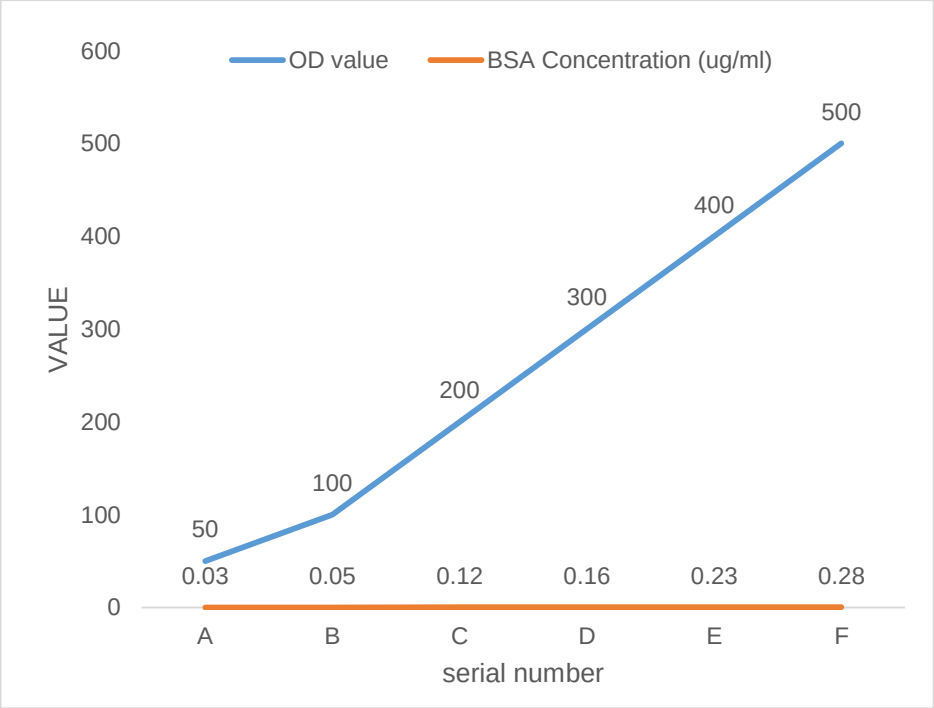

Figure 1. antibody concentration and sds-page detection chart

Characterization and evaluation of labeled probes: after agarose gel electrophoresis test, the monoclonal antibody-conjugated quantum dot solution is in lane a, while the pre-labeled quantum dots are in lane b 21, and the movement of quantum dots coupled with monoclonal antibodies is significantly higher than quantum dots are slow. The results showed that the maximum wavelength of the emission spectrum of the quantum dots before labeling was 615 nm, while the maximum wavelength of the emission spectrum of the probes after labeling was 621 nm.

4.2. Visual Observation with Naked Eyes

As shown in figure 2, (a) are arranged from left to right as strong positive, positive, weak positive and negative in the preliminary screening of hydrogen sulfide production reaction. The designed sampling hook has a shank length of 15 cm and a hook length of 2 mm. Visualization of fluorescence magnified under a stereoscope: (a1, b1) strong fluorescence reaction (plaque) on positive swabs. (c) fluorescence reaction (filamentous) on positive swab.

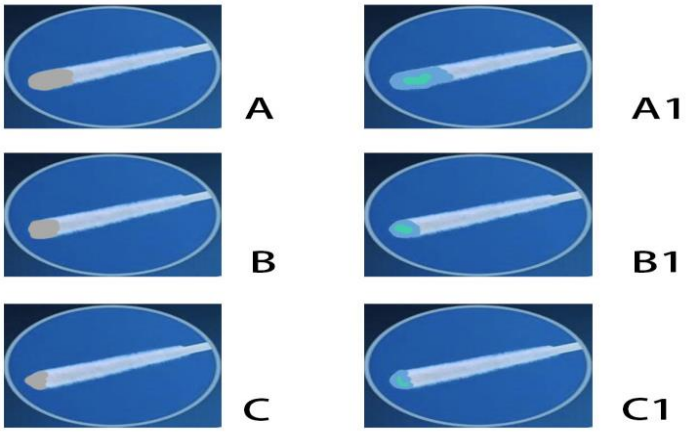

Figure 2. Visual observation of fluorescence under the action of hydrogen sulfide reaction color development and artificial fluorescence excitation light source

4.3. Analytical Sensitivity

The prepared mock samples contained 101cellsml-1 salmonella manhattan and 105cellsml-1, 106cellsml-1 or 107cellsml-1 non-salmonella (including escherichia coli, proteus, citrobacter), a total of 6 groups of 10 samples in sterile distilled water. Synchronized blank controls. Dark spots, naked eyes or fluorescent signals recognizable under the lscm microscope are the indicators of positive detection, and they are confirmed according to the standard identification process after separation. The sensitivity test results are shown in table 2.

Table 2. Determination of minimum detection limits in mock samples

Sample	Positive detection rate (%)	Ratio of identifiable fluorescent signals (%)
Escherichia coli	94	60
Proteus	96	62
Citrobacter	99	65

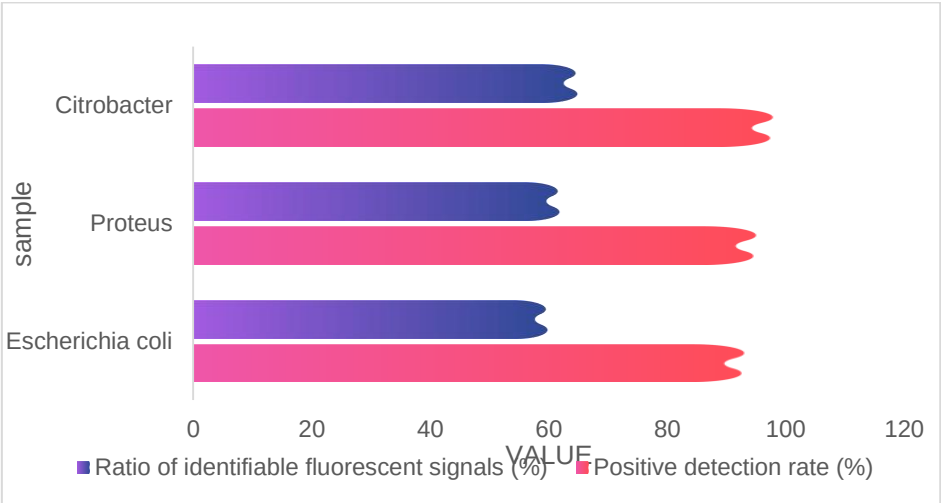

Figure 3. sensitivity test results in 3 groups of samples

As shown in figure 3, the results show that in the three groups of samples containing 101cellsml-1 salmonella and 105cellsml-1 non-salmonella (including escherichia coli, proteus, and citrobacter), the final positive detection rates were 94% and 96%, respectively. And 99%; the ratios of the fluorescence signal identifiable to the naked eye were 60%, 62% and 65%, respectively. More importantly, all negative mock samples were screened out, which significantly reduced the follow-up workload. The positive detection rates of the three groups of samples containing 101cellsml-1 salmonella manhattan and 106cellsml-1, 107cellsml-1 non-salmonella (including escherichia coli, proteus, and citrobacter) were all lower than 50% (within 24 hours of culture).

5. Conclusion

Salmonella is one of the food-borne enteric pathogens that endanger public health and affect the development of national economy. The feces of people and animals infected with salmonella are an important source of infection, and once they contaminate food, they can cause food poisoning and cause severe symptoms such as nausea, headache, vomiting and diarrhea. This paper will study the new technology of salmonella detection, realize rapid and visual detection, develop a simple and easy-to-operate detection method with visual results, provide technical support for the detection of salmonella in food, and provide a strong guarantee for food safety supervision.

Funding

This article is not supported by any foundation.

Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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