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Determination of *Salmonella pullorum* with Nanoparticles Immune Based Lateral Flow Strip Assay

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Abstract

The isolation and culture of conventional detection method of *salmonella* can not meet the testing requirements of quick and easy detection at the grassroots level. In this study, we prepare the fluorescent nanoparticles as a marker, covalently conjugate with monoclonal antibodies of *Salmonella pullorum*. The whole *Salmonella pullorum* antigen and goat anti-mouse antibody sprayed on the nitrocellulose membranes are used as test line and control line. The fluorescence nanoparticles immune based lateral flow strips are made according to the principle of antigen-antibody immune response. The test strips may interpret results within 30 min. The results of the *salmonella* A, *S. agona*, *S. chester* and *S. arechavaleta* are positive, including, *S. agona* for weakly positive. After analysis, it is found that in addition to the *salmonella* of group A, the other positive *salmonella* are in group B. But it is negative of *S. derby*, *S. rissen*, and other 6 kinds of *salmonella*, with good specificity. The fluorescence nanoparticles immune based lateral flow strips are a little of sample can be detected fast, easily, inexpensive, easy to universal without professional technical personnel detection method. It provides a new detection method for the detections of *Salmonella pullorum*.

Keywords

Monoclonal Antibody, Nanoparticles, Fluorescence Nanoparticles Immune Based Lateral Flow Strips, *Salmonella pullorum*

1. Introduction

Fowl Pullorosis (Pullorum Disease) also known as pullorum or pullorum disease, is an infectious disease of

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poultry disease caused by *Salmonella pullorum*. In addition to the disease level of broadcasting, mainly through the egg transfer, is a vicious spiral type throughout the period of disease of chicken. The disease can cause the fertilization rate and hatching rate and laying rate decreased, causing significant harm to the poultry industry. The prevalence of chicks to *Salmonella pullorum* for features, often manifested as acute symptoms of septicemia. OIE will be classified as class B disease, China's Ministry of agriculture listed as the two kind of disease [1]. *Salmonella* enterobacteriaceae genus has at least 2300 serotype. Based on the difference of antigen by bacteria, *salmonella* can be divided into A, B, C, D, E, F seven groups [2]. One of the most common pathogenic is group B, Pigs cholera in group C and *pullorum* and *Salmonella* enteritidis in group D [3]. Conventional detection methods of *Salmonella pullorum* by the disadvantages of long cycle and complicated operation has been far can not meet the requirements of current detection, not suitable for field detection method of port, so there is an urgent need for a rapid and accurate detection of *Salmonella pullorum*. Nano fluorescent strip is currently on a fast screening method for model [4], can be used for rapid diagnosis of some infectious diseases, for suspected infection in chicken flocks in the early disease receive timely treatment [5].

2. Materials and Methods

2.1. Test Materials

(3-Aminopropyl) trimethoxylsilane (APTMS), tetraethyl orthosilicate (TEOS), TritonX-1004, 4-bis,1,1,2,2,3,3-heptafluoro-4,6-hexanedion-6-yl) chlorosulfo-*o*-terphenyl (BHHCT) and Sodium Cyanoborohydride are bought in Sigma. The nitrocellulose membrane is bought in Millipore. *S. pullorum* monoclonal antibody is bought in Biodesign. *S. pullorum* and other reference strains of inactivated strain are from the city center for disease control and prevention. The other reagents were domestic analytical reagent.

2.2. Test Instrument

JEM 2100 HC electron microscope; Perkin Elmer-LS-55 fluorescence spectrophotometer; Ultraviolet imager (UVP company, USA); Canon Powershot A610 digital camera.

2.3. Test Method

2.3.1. The Preparation of Fluorescent Nanoparticles

The preparation of fluorescent nanoparticles [6] [7] process is shown in **Figure 1**.

2.3.2. The Antibody Markers

The antibody markers [8] flow diagram, as shown in **Figure 2**.

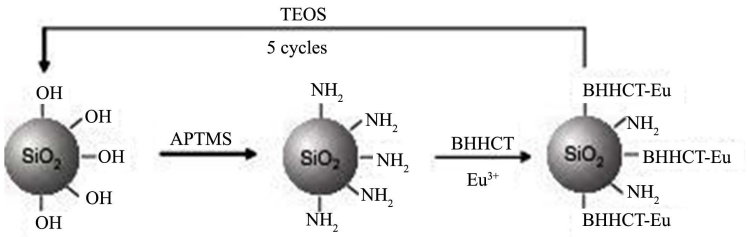


Figure 1. The flow diagram of the preparation of fluorescent nanoparticles.

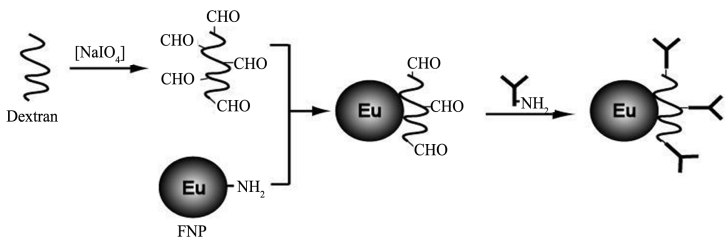


Figure 2. The antibodies labeled flow diagram.

2.3.3. The Immune Based Lateral Flow Strips [9]

1) The labeled antibody into solid phase

The good marker particles of preparation with 1% casein diluent makes up of 10 mM Tris 7.8 buffer according to 1:1000 diluted; Then the glass fiber as markers mat immersing among them, will be subject to soak, and then freeze-dried for later use.

2) Package capture antibody

The membrane dispenser system will be for 2 mg/mL antibodies into linear or strip on the nitrocellulose membrane, the package volume is 0.7 μL/cm as detection line; 0.5 cm from the detection line on the membrane is packaged by 2 mg/mL sheep against mouse IgG to draw a quality control line, dry reserve at 37˚C for 2 h.

3) Nanoparticles marker combination mat processing and related material of choice.

4) The structure diagram of the strip is shown in **Figure 3**.

5) Samples detection

Add 60 μL sample which to be tested on the strip mat. After 20 min, detecting the strip under UV lamp observations or measuring degree by fluorescence detector. If the sample target content is low, it will need to extend the time. The positive result presents two red belts, and the negative result shows only a red belt in 30 min. In this experiment, we use Canon Powershot A610 digital camera for auxiliary observation and record the results.

3. Test Results

3.1. The Preparation and Characterization of Fluorescent Nanoparticles

From the **Figure 4**, we can see the morphology of the blank silica nanoparticles made by reverse micelle method is in good condition. The size is between (60 ± 5 nm), The surface of the particle is smooth.

3.2. Sensitivity Test

The strain used in this test, are all inactivated strain after boiled. We used the real-time fluorescence PCR to detect *S. pullorum*, and then it will be diluted to different concentrations to examine the sensitivity of the detection system. The result is shown in **Figure 5**, the detection sensitivity of the strips can reach 5×10^3 copies/mL. The two highest detection concentrations showed decline fluorescence phenomenon in **Figure 5**.

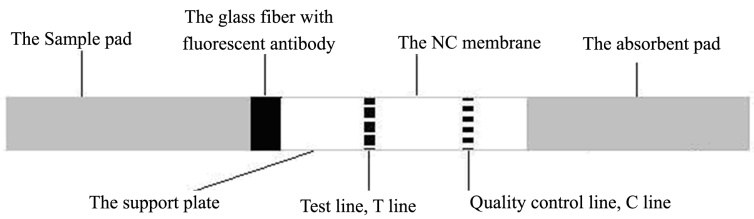


Figure 3. The structure diagram of the strip.

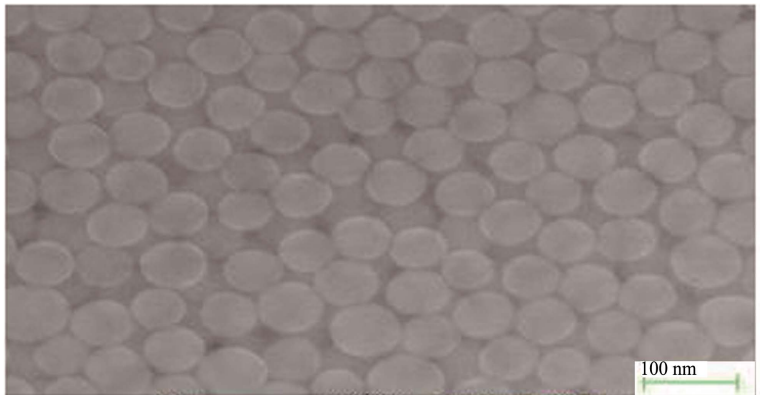


Figure 4. The SEM photo of silicon nanoparticles.

3.3. Specific Test

First, we use the 16 collected strains of not *salmonella* to investigate the specificity of the detection system. They are: *Escherichia coli*, *E. coli* 8099, *EPEC* (*pathogenic E. coli*), *EPEC O*125:*K*70, *EPEC O*128:*K*67, *EPEC* O44:K74, *EIEC* (*enteroinvasive E. coli*) *O*164, *EIEC O*28:*K*73, *E. coli O*157:*H*7, *Sh. flexneri* 2*a*, *Vibrio parahaemolyticus*, *VP*, *Vibrio cholerae O*1, *Vibrio cholerae O*139, *Staphylococcus aureus*, *Listeria monocytogenes and Yersinia enterocolitica*. As shown in **Figure 6**, the detection system of the 16 strains of not *salmonella* without nonspecific reaction.

Then, we make use of the collected 12 strains of *salmonella* and different species to inspect the interspecific specificity of detection system. They include: *S. pullorum*, the *Salmonella D*, *S. agona*, *the Salmonella A*, the *Salmonella E*, *S. eastborne*, *S. rissen*, *S. weherreden*, *S. chester*, *S. anatum*, *S. chailey and S. panama*. The result is shown in **Figure 7**, besides the result of *S. pullorum* is positive, the results of the *salmonella D*, *S. eastborne and S. panama* are positive, too. After analysis, it is found that in addition to the *salmonella* of group D, the other positive *salmonella* are in group D. *Salmonella* group is classified according to the difference of LPS, and chooses the monoclonal antibody of *S. pullorum* resistance loci for LPS, so the D group with *S. eastborne* and *S. panama* are also cross reaction.

3.4. Selective Test

This experiment mainly inspects to detect the *S. pullorum* from other strains of higher interference environment infected in chickens artificially. The background of *E. coli O*157:*H*7, keep the total concentration of 5×10^8/mL unchanged in this experiment. The proportion of the content of the *S. pullorum* is changed from 50% to 0.001%, investigating the sensitivity. From the **Figure 8** you can see, the detection sensitivity of the system can still achieve 5×10^3 copies/mL. Below diagram is about two groups of parallel test.

4. Test Conclusions

This paper established "fluorescent nanoparticles" detection technology platform based on *S. pullorum* detec-

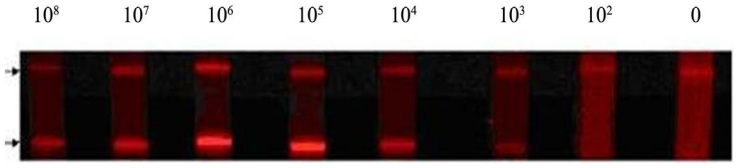
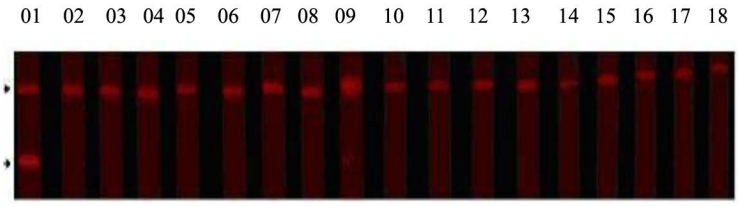
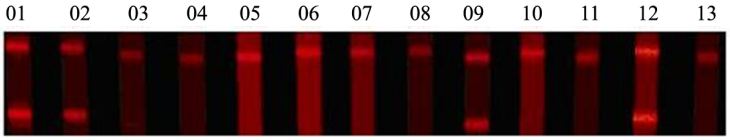


Figure 5. The sensitivity test.



01 S. pullorum
02 Escherichia coli
03 EPEC
04 EPEC O128 : K67
05 EIEC O164
06 E.coli O157 : H7
07 Vibrio parahaemolyticus, VP
08 Vibrio cholerae O139
09 Listeria monocytogenes
10 Yersinia enterocolitica
11 E. coli 8099
12 EPEC O125 : K70
13 EPEC O44 : K74
14 EIEC O28 : K73
15 Sh. flexneri 2a
16 Vibrio cholerae O1
17 Staphylococcus aureus
18 Negative control

Figure 6. The specificity test.



01 *S. pullorum*
02 *the Salmonella D*
03 *the Salmonella E*
04 *S.rissen*
05 *S.chester*
06 *S.chailey*
07 *the Salmonella A*
08 *S.agona*
09 *S.eastbourne*
10 *S.Weherreden*
11 *S.anatum*
12 *S.panama*
13 *Negative control*

Figure 7. The *salmonella* specificity test between species.

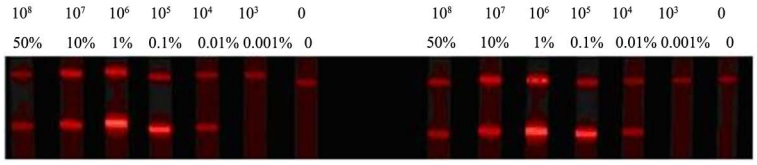


Figure 8. The selective test of detection system.

tion object. Its related principle and technology can be applied to other harmful microbial detection. And with the further development of research in the future, it can be achieved through synthesis of multicolor fluorescence nanoparticles on a variety of harmful microbial detection at the same time, in order to achieve rapid, sensitive and high throughput detection. In this paper, it is made the nano gold test strips for *S. pullorum*. And the experiments of detection sensitivity, repeatability and specificity and stability of the strips obtained good results.

This paper established a technology platform versatility. Comparing with the existing products, the nanoparticles immune based lateral flow strips have obvious competitive. The product can be widely used in healthy and epidemic prevention, food, agriculture, animal husbandry, import and export inspection and quarantine, and other fields.

Fund Project

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