Research Article

Development an Immune Colloidal Gold Filtration Assay for Detecting Antibody of *S. agalactiae* in Tilapia

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Abstract

A quasi-colloidal gold staining assay was carried out by preparing colloidal gold labeled $2C_{10}$ by preparing monoclonal antibody $2C_{10}$ with anti-tilapia immunoglobulin IgM. by Preparation of monoclonal antibody $2C_{10}$ against tilapia immunoglobulin IgM and marking the $2C_{10}$ with colloidal gold for detecting antibody of *Streptococcus agalactiae* (*S. agalactiae*) infection in tilapia. After the experimental test, the rapid immunogold colloidal gold filter detection method, the sensitivity and conventional ELISA equivalent to 1.2×10^{-6} g/mL protein; specificity is good, not with the common bacteria *Aeromonas hydrophila*, *Pseudomonas fluorescens* reaction; $4°C$ to preserve the shelf life of more than 3 months. Immune colloidal gold infiltration method Immunity colloidal gold infiltration method has the advantages of rapid and easy operation. The method takes only 5 minutes to detect the sample, only 3μL is needed to detect the sample. The detection of the method does not require special equipment and equipment, And can be detected on site. The establishment of the law provides a new method for the monitoring of tilapia-free *Streptococcus* disease, which is of great practical significance in controlling the risk of *Streptococcus agalactiae*.

Introduction

Streptococcus agalactiae is a common animal and animal disease, endangering a variety of important animal bacteria [1], in China, its main harm to tilapia aquaculture. According to the World Food and Agriculture Organization (FAO) statistics, in 2008 China tilapia farming amounted to 706,585 tons, accounting for 50% of the world's total output, tilapia became a truly "Waterfowl" [2,3]. According to statistics, after 1980, freshwater fish, marine fish in the *Streptococcus* infection in a number of countries have been reported in the outbreak of *Streptococcus aureus* in the world began widespread [4,5]. To date, the epidemic area of the disease has covered freshwater fish and marine fish in 15 countries from six continents in Europe, Africa, Australia, Asia and North Africa,

South Africa [6]. Since 2008, China's southern tilapia farming area began a wide range of outbreak of *Streptococcus agalactiae* disease, the mortality rate as high as 50% to 70%, 2009 Guangdong Province, breeding areas 7 to 11 months 4 months of *Streptococcus agalactiae* Infection caused more than 10,000 tons of tilapia died; in 2011, Guangxi breeding areas 5 months to 6 months in the absence of *Streptococcus agalactiae* infection caused more than 7,000 tons of tilapia deaths, economic losses of up to 8,000 million [7-11]. In recent years, Guangxi, Guangdong and other regions due to the harm of tilapia without *Streptococcus* has had a lot to reduce the tilapia farming, which caused the Chinese tilapia industry hit.

Streptococcus agalactiae is seriously harmful and highly contagious. At present, there is no commercial vaccine against the

prevention and treatment of tilapia streptococcal disease in China. It can only rely on strict production management and antibiotic treatment. Therefore, the establishment of rapid and effective disease Early diagnosis methods and monitoring of the epidemic, timely warning and forecasting are still the key technical problems to be solved at present. In order to provide effective diagnostic and monitoring techniques for the production of tilapia-free *Streptococcus agalactiae* in production and to control the harm of tilapia without *Streptococcus agalactiae* in a timely manner, we have previously established an immunogold gold for the detection of tilapia-free *Streptococcus agalactiae* Rapid diagnosis method [12], this study we use the preparation of monoclonal antibody and colloidal gold technology to establish a detection of tilapia anti-*Streptococcus mutans* antibody detection method, that is, tilapia anti-*Streptococcus* free antibody colloid gold infiltration Filtration, the establishment of the law will provide a new method for on-site monitoring of tilapia-free *Streptococcus mutans*.

Materials and Methods

Materials and Reagents

S. agalactiae and other encounter bacteria, experimental animals and reagents. [12]. All chemicals were of grade, and used as received.

Preparation of Monoclonal Antibodies Against Tilapia Immunoglobulin IgM

The preparation procedure of monoclonal antibodies against tilapia immunoglobulin IgM is shown (Figure 1) below:
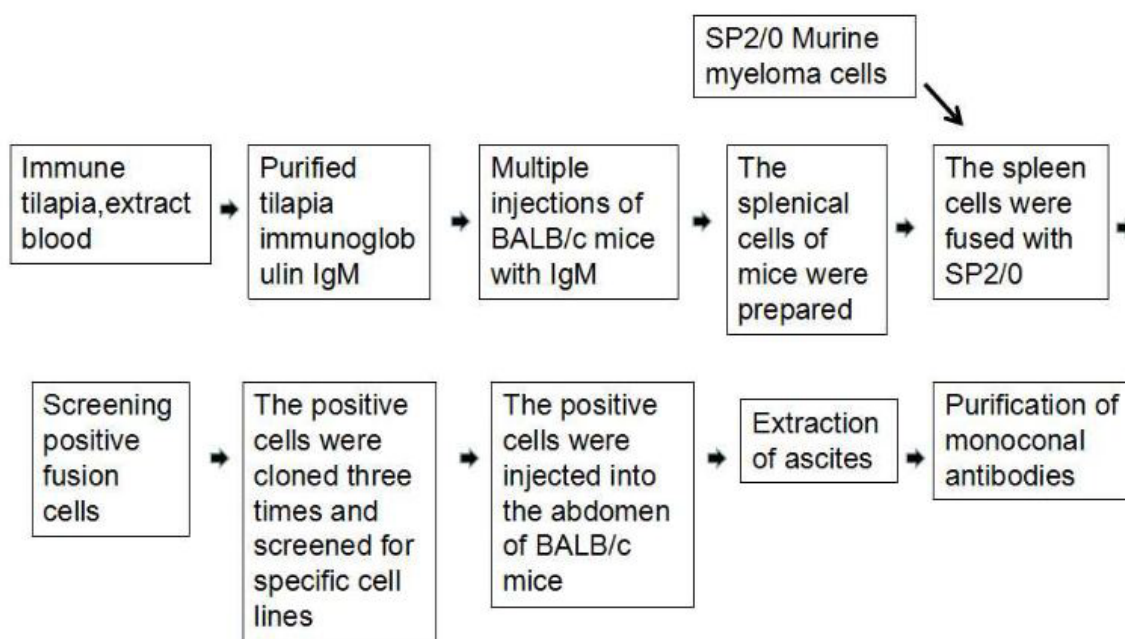
Figure 1: The preparation procedure of monoclonal antibodies.

A monoclonal antibody against tilapia immunoglobulin IgM was prepared by conventional methods: tilapia was immunized with inactivated *Streptococcus agalactiae* and then immunized tilapia immunoglobulin IgM was extracted, precipitated with saturated ammonium sulfate and purified by Protein-A column [12,13]. The purified tilapia immunoglobulin IgM was used to immunize Balb / C mice. After three times of immunization and potency, the mice were sacrificed with high titer of mouse spleen cells. A monoclonal antibody hybridoma cell line with specificity for tilapia immunoglobulin IgM was selected by semi-solid culture. Anti-tilapia immunoglobulin IgM monoclonal antibody was prepared by conventional mouse ascites method.

Preparation of Colloidal Gold Suspension

[12,14] prepared by the Frens method of Nano-colloidal gold, prepared by the colloidal gold solution in the UV-visible scanning, identification of the preparation of colloidal gold quality and size.

## Preparation of the Colloidal Gold-Mab Conjugate

Anti-tilapia immunoglobulin IgM monoclonal antibody labeled with prepared colloidal gold: Determine the optimum pH and minimum protein content by a series of optimized tests. Take 10 mL of colloidal gold solution, add the optimal amount of K_2CO_3, add a $2K_{10}$ monoclonal antibody to the magnetic stirrer while stirring, and add 20% $2C_{10}$ monoclonal antibody on the basis of optimization. Stir for 15 min, add the newly prepared 10% BSA 1 mL, continue stirring for 15 min. Dispense, centrifugation, 12000 rpm, 4°C centrifugation 1 h. After centrifugation, remove the centrifuge tube, carefully discard the supernatant, add 1% BSA PB buffer, wash, and then centrifuged once. Finally, discard the supernatant, add the suspension, according to every 1 mL of gold alloy gold solution by adding 100 μL heavy suspension, that is concentrated 10 times. The activity of the labeled antibody was examined by ELIA or direct spotting. Colloidal gold labeled monoclonal antibody placed 4°C preservation, cannot be frozen.

Optimization of the Optimum Amount of Coating and Gold Standard Solution

[12,15], take nitrocellulose membrane (NC film), perforation, diameter 3 mm, inactivated *Streptococcus agalactiae*, bacteria concentration adjusted to 1.5×10^9-1.5×10^4 CFU, Hole, 2 μL per hole. After drying at room temperature, wash with PBST 3 times and dry. With 5% BSA, closed for 15 min; then PBST washed 3 times, dry, put 4°C save. Test, add positive negative serum 2μL / hole, 1 min, with PBST wash, and then add 2 μL / well labeled monoclonal antibody, 1 min, with PSBT washing, positive that color, negative color. After optimization, the labeled monoclonal antibody solution diluted with different times to detect, optimize the amount.

Immuno-Colloidal Gold Infiltration Test Plate Assembly and Testing

The detection mechanism of immuno-colloidal gold infiltration test plate is shown in (Figure 2) below.
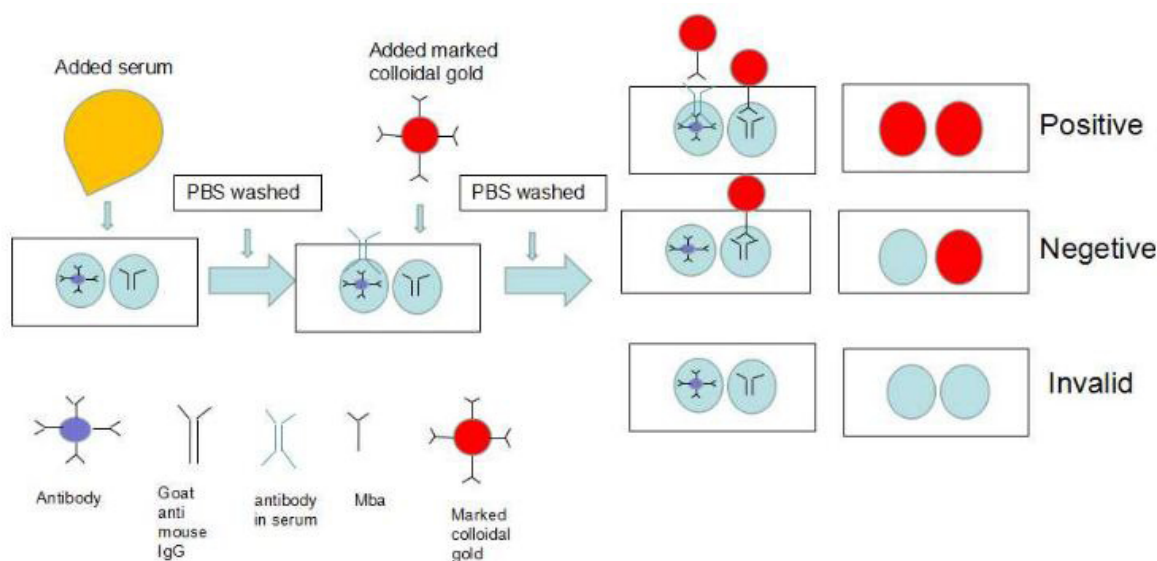
Figure 2: The detection mechanism of immuno-colloidal gold infiltration test plate.

According to the above optimal method in the NC film spot treatment, while the anti-mouse IgG anti-mouse point, as the control point. The treated NC film is loaded into 2.5cm × 3cm. When assembling, the absorbent paper is loaded on the bottom of the round plate, and then the NC film is attached and the plate can be closed. A package consists mainly of a plate, a bottle of gold standard liquid, a bottle of washing liquid composition.

Detection, the spot to be added to the serum 2μL, serum quickly diafied in the past, and then add the washing solution, the filtrate after diafiltration, add the gold standard solution, the gold standard solution after dosing and then add the washing liquid.

5 min if positive, there are two spots, if only a negative spot; blank control group does not appear spots, the results sentenced to invalid.

Sensitivity of the Immune Colloidal Gold Filtration Assay

Take a positive serum for serial dilution, with the establishment of colloidal gold infiltration detection method to detect the final can detect the dilution. The same positive serum was precipitated by saturated ammonium sulfate precipitation method. The immunoglobulin was extracted and its protein content

was measured. Then, serial dilutions were carried out. The final dilution was detected by the established colloidal gold infiltration test.

Specificity of the Immune Colloidal Gold Filtration Assay

The strains of Pseudomonas aeruginosa and Pseudomonas aeruginosa were tested as specific test strains, the immunoassay was used to detect the specificity of immune colloid gold infiltration by cross-reaction with immunized serum of various bacteria.

Colloidal Gold Diafiltration Assay (stability of the immune colloidal gold filtration assay)

The prepared colloidal gold leachate test strip was placed at 4°C and the test was taken weekly.

Comparison of Colloidal Gold Infiltration Detection with ELISA Method

The sera of tilapia were collected and the OD values of these sera were measured by ELISA. A total of 20 samples were tested by ELISA. The results were compared with the colloidal gold infiltration method. The results were compared with the methods of medical statistics [16], including sensitivity, specificity, total consistency, etc. Related indicators.

Results and Discussion

Production of mAb Against Tilapia Immunoglobulin IgM

A number of hybridoma cell lines secreting anti-tilapia immunoglobulin IgM-specific monoclonal antibody were obtained by monoclonal antibody preparation. After identification of the biological characteristics of the antibody secreted by these hybridoma cell lines, two high titer hybridoma cell lines were obtained, named $2C_{10}$ and $4G_6$, respectively. The preparation of anti-tilapia immunoglobulin IgM-specific monoclonal antibody lays the foundation for further establishment of standardized detection methods.

In addition, Wang Weifang et al. [17] immunized with Bovine Serum Albumin (BSA) to identify the pathogens, and use rabbit anti-turbot (*Scophthalmus maximus*) multi-labeled colloidal gold, in the use of colloidal gold immunochromatographic detection of fish antibody reported, the specific anti-BSA antibody was detected in the sera of the turbot, and the sensitivity was similar to that of the ELISA method. Our method is to prepare this monoclonal antibody with colloidal gold by preparing anti-tilapia immunoglobulin IgM-specific monoclonal antibody. Because the production of monoclonal antibodies with hybridoma cells is stable and adaptable to scale and standardization, it can provide a better basis for the popularization and application of immunogolds.

Immuno-Colloidal Gold Infiltration Test

The positive and negative sera were detected by the established immunogold gold infiltration method according to the method described in 3.6 The results are shown in (Figure 3).

Figure 1: The composition of the test board with the test results.

The result is positive appearance of two spots, negative only a spot, blank control spots do not appear, sentenced to invalid. Immune colloidal gold infiltration method compared with the conventional ELISA method, this method is very fast detection, in a few minutes to determine the test results, but also on-site testing, this field of rapid detection method is important for the producers. The application value and urgent needs.

Sensitivity, Specificity and Stability of the Immune Colloidal Gold Filtration Assay

The results showed that the highest dilution ratio of serum was 256 times. Protein, immune filtration method can detect the dilution factor is 64 times. The amount of immunoglobulin extracted with the same amount of serum was white 0.8 mg. The minimum detection limit of immune filtration was 1.2×10^{-6} g / mL, which indicated that the sensitivity of the antibody was similar to that of ELISA. Immunity colloidal gold infiltration method and the commonly used ELISA method is also similar to the detection principle, the difference is only the antibody markers are different, ELISA method is to use enzyme labeled antibody, and immunogold gold infiltration method is to use colloidal gold labeled antibody, so its Detection of antibody sensitivity and ELISA method is similar, but the ELISA method requires a microplate reader to read the data, and immune colloidal gold infiltration method does not need to detect the instrument, according to the reaction color to directly determine the test results. As the method is simple, the producers

do not need to have the expertise to use, only need a simple test training can be used, the development and application of this technology will be able to complex laboratory and professional testing process directly into a simple scene Detection. As tilapia lactobacilli disease in the epidemic season is usually a trend, the incidence of acute, so rapid on-site diagnostic monitoring, for the early control of the disease to win valuable time. Therefore, the establishment of immune colloidal gold infiltration method will provide a strong technical support for the monitoring and control of *Streptococcus agalactiae*.

The immune spheric gold permeation test plate was prepared by using N-*Streptococcus*, *Streptococcus davidianus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and the like as coated bacteria. The results showed that the sera of *Aeromonas hydrophila* and *Pseudomonas fluorescens* did not react with *Streptococcus agalactiae* and did not react with *Streptococcus lactis* sera. But found that, in turn, with dolphin *Streptococcus* serum and *Streptococcus agalactiae* detection when there is a certain response. Indicating that the establishment of the immune colloidal gold infiltration method has been well tested for specificity, but also needs to be further optimized to improve the specificity of detection with dolphins. The results showed that the immunogold gold permeation test plate could store at least 3 months at 4°C for 4 months.

1.1. Comparison with the ELISA Test Results

Colloidal gold leachate detection method and ELISA method comparison test results in (Table 1).

Defiltration	ELISA Law	
	Positive	negative
Positive	5(A)	1(B)
Negative	1(C)	13(D)

Table 1: Comparison of two methods of detection results.

According to the data in the table, refer to the method of medical statistics [16] calculated:

Specificity = D / (B + D) × 100% = 92.8%; Sensitivity = A / (A + C) × 100% = 83.3%; Positive Consistency = A / (A + C) × 100% = 83.3% (B + D) × 100% = 7.6%; false negative = C / (A + C) × 100% = false negative = 16.6%. Total rate = (A + D) / (A + B + C + D) × 100% = 90%

The experimental results show that the coincidence rate of the immune colloid gold infiltration method and the conventional ELIA method is 90%, the reliability is very high, and it has practical application value and deserves further optimization and development.

Conclusions

An immuno-colloidal gold infiltration assay for the detection of tilapia-free *Streptococcus agalactiae* antibodies has been developed. This new method can be used for the production and clinical monitoring of tilapia-free *Streptococcus agalactiae*. The results show that the method has high sensitivity and high specificity, and it has the advantages of fast, simple operation and field use compared with traditional detection methods such as ELISA method. More optimization and testing work is still in progress.

Acknowledgments

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