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DEVELOPMENT OF A PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS COLLOIDAL GOLD DETECTION STRIP

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important causative agents to the worldwide swine industry, caused significant economic losses annually. Here, we aims to prepare a colloidal gold cards for PRRSV early screening.

A double-antibody sandwich colloidal gold cards were optimized and prepared using recombinant PRRSV antigen and its paired monoclonal antibody. The test performance of the colloidal gold cards were evaluated using positive and negative samples.

We successfully prepared the colloidal gold cards for fast screening of PRRSV. The colloidal gold cards has no cross reaction with various serum samples, showed high specificity. The detection sensitivity reached 1:800. The colloidal gold cards can be stably stored at 37°C for 7 days without any decrease in sensitivity. At 1:800 dilution, the repeatability was still good.

The prepared colloidal gold cards has high specificity, sensitivity, stability and repeatability, it provided an effective monitoring of PRRSV infection.

Keywords: porcine reproductive and respiratory syndrome virus, colloidal gold, card, nucleocapsid N protein.

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РАЗРАБОТКА ТЕСТ-ПОЛОСКИ С КОЛЛОИДНЫМ ЗОЛОТОМ ДЛЯ ОБНАРУЖЕНИЯ ВИРУСА РЕПРОДУКТИВНО-РЕСПИРАТОРНОГО СИНДРОМА СВИНЕЙ

Цель исследования – разработка тест-полоски с коллоидным золотом для обнаружения вируса репродуктивно-респираторного синдрома свиней (PRRSV), являющегося одним из важнейших возбудителей, наносящих значительный экономический ущерб мировому свиноводству.

Тест-полоски с коллоидным золотом, содержащие двойные антитела, были оптимизированы и приготовлены с использованием рекомбинантного антигена вируса PRRSV и его парных моноклональных антител. Тестовые характеристики полосок оценивали путем применения положительных и отрицательных образцов.

В результате разработаны тест-полоски с коллоидным золотом для скрининга вируса PRRSV. Они не имели перекрестной реакции с различными образцами сыворотки и показали высокую специфичность. Даже при разведении сыворотки 1:800 наблюдалась чувствительность обнаружения. Тест-полоски в течение 7 сут при температуре 37°C сохраняли стабильность без снижения чувствительности, и повторяемость анализов была хорошей при разведении 1:800.

Разработанные тест-полоски с коллоидным золотом обладают высокой специфичностью, чувствительностью, стабильностью и повторяемостью, обеспечивают эффективный мониторинг инфекции PRRSV.

Ключевые слова: вирус репродуктивно-респираторного синдрома свиней, коллоидное золото, тест-полоска, белок нуклеокапсида N.

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Introduction. Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-stranded RNA virus belonging to the family Arteriviridae Synthesis, which is one of the most important animal pathogens of whole world [1]. PRRSV has caused huge economic losses in the global swine industry, and some natural outbreaks in wild pigs, but its control is still unsatisfactory, no adequate control measures are yet available to eliminate their infection [2, 3]. Molecular methods are still the main methods for PRRSV detection [4]. Currently, some rapid detection methods are being developed, e.g. a method that combines reverse transcription recombinase polymerase amplification with a lateral flow dipstick for detecting North American PRRSV (PRRSV-2), has high specificity and accuracy [5]. In a word, the rapid and sensitive detection method for PRRSV is critically important for diagnosing of PRRS.

The common strains of PRRSV are the European and North American strains, which have distinct antigenic types. Since 1996, the North American type PRRSV has spread throughout in China. From the viewpoint of diagnostics and control, it is crucial to determine the conserved proteins of multiple PRRSV strains with different genetic backgrounds in the swine. PRRSV has 9 open reading frames (ORFs), the ORF7 encodes the nucleocapsid protein (N), the major structural protein and the most abundant viral protein in virus-infected cells and the most immunodominant antigen in the pig immune response to PRRSV [6–8]. 20 years ago, ORF7 has been used for ELISA assay of PRRSV antibodies [9, 10]. Recently, it has been confirmed again that ORF7 is a promising tool for diagnostics and epidemiological survey of PRRSV [11]. Therefore, ORF7 is a highly valuable PRRSV structural protein for serological detection and diagnosis. In this study, we prepared a colloidal gold cards for PRRSV early screening using recombinant nucleocapsid N protein antigen and its paired monoclonal antibody, which is important for developing a successful strategy for the prevention and control of PRRS.

Main part. Recombinant nucleocapsid N protein antigen and its paired monoclonal antibody (mAb, capture and detection mAb) were obtained from Guangzhou Youdi Bio-technology Co., Ltd. (Guangzhou, China). Chloroauric acid ($HAuCl_4$), polyvinyl chloride (PVC) soleplate, nitrocellulose (NC) membrane, absorbent paper, binding pad and sample pad were purchased from Goldbio (Shanghai, China). 110 negative samples and 24 positive samples if PRRSV, the positive serum sample of swine acute diarra syndrome coronavirus (SADS CoV, 5 cases), transmissible gastroenteritis virus (TGEV, 4 cases), porcine respiratory coronavirus (PRCV, 3 cases), porcine hemagglutinating encephalomyelitis virus (PHEV, 6 cases), porcine delta coronavirus (PDCoV, 1case) (positive samples confirmed by nucleic acid method) were obtained from South China Agricultural University.

Added 1.0 mL of 1% $HAuCl_4$ solution to 100 mL of double distilled water to obtain a concentration of 0.01% chloroauric acid solution. After boiling, added 2 mL of 1% trisodium citrate solution, continued heating until the solution turns wine colored. After cooling, stored in a brown bottle at 2–8°C for later use.

A total of 10 μL 0.1mol/L of K_2CO_3 solution was added into 1 mL of colloidal gold solution, mixed fully and added 10 μL mAb, gently shak and incubated for 15 min. After that, added 50 μL 10% BSA solution for 10 min (blocking). Centrifuged at 4°C at 10,000 rpm for 30 min, dissolved the precipitate in reconstitution buffer to the original volume, and obtained the AuNPs-mAb complex. Stored it at 2–8°C for later use. The reconstitution buffer contained 0.02 mol/L Tris, 0.1% Casein, 0.3% PVP40, and 3% trehalose, pH 8.2.

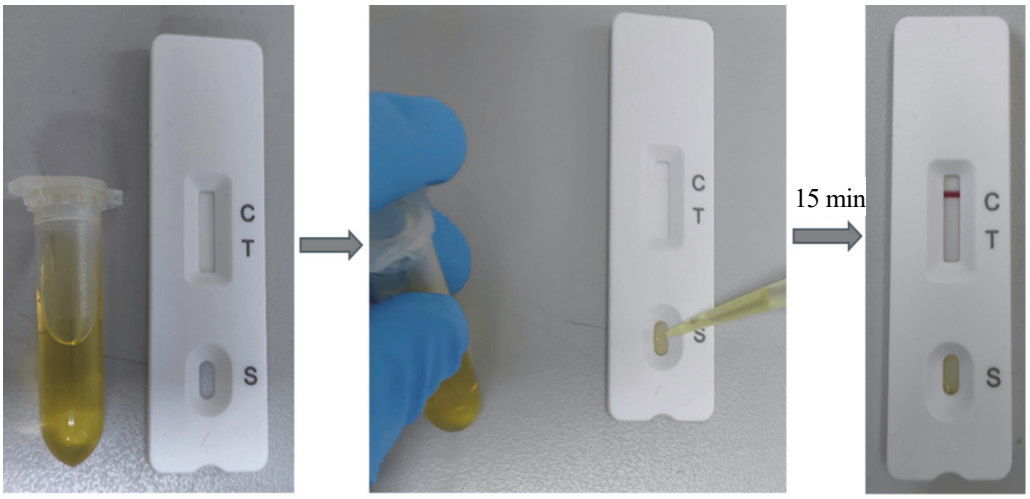
Sprayed the AuNPs-mAb complex onto the binding pad using a XYZ3060 3D spraying platform, with a spray volume of 2–4 μL/cm. Placed the sprayed binding pad in a drying room (humidity < 30%) for 12 h, then sealed it for later use.

Soaked the sample pads into the sample pad pretreatment solution for 30 min, drain and placed them in a drying room (humidity < 30%) for 12 h. Then, sealed it for later use. The sample pad pre-treatment solution contained 0.02 mol/L Tris, 0.5% Tween 20, 0.1% trehalose, pH 7.0.

Diluted recombinant N antigen with 0.01 mol/L PBS (pH 7.4) to 1.0 mg/mL for C line coating; Diluted the capture mAb with PBS to 1.0 mg/mL for T line coating and sprayed the above solution onto the NC membrane to obtain the C line and T line, respectively. The interval between C line and T line was 8 mm, with a spray volume of 2.0 μL/cm, using a XYZ3060 3D spraying platform. After drying the NC membrane in a drying chamber (humidity < 30%) for 12 h, then sealed it for later use.

The sample pad, binding pad, NC membrane and absorbent paper are sequentially fixed on the PVC board. After fixing, the PVC board was cut into 3 mm wide strips and loaded into a plastic cards. Then, the cards were sealed and stored in a dry place.

Sample was added into sample diluent buffer, mixed well. 80 μL of the above liquid was dropped into the detection well of colloidal gold card. After 10–15 min of horizontal placement, observed the color development of the T line and C to determine the negative (N) or positive (P) (Figure). When red band appears on the C line and the T line, regardless of the color intensity, it is judged as positive; when red band appears on the C line but not on the T line, it is judged as negative; when no red band appears on the C line, regardless of whether a red band appears on the T line, the test is considered invalid and should be repeated



Schematic diagram of test procedure

The positive serum of PRRSV, SADS CoV, TGEV, PRCV, PHEV, PDCoV, and the negative serum of PRRSV were detected using these colloidal gold cards. Repeated the test three times for each sample, and recorded analyzed test results.

Diluted 10 positive serum samples of PRRSV at 1:100, 1:200, 1:400, 1:800 and 1:1600, as the samples, performed these colloidal gold cards test to evaluate the sensitivity.

The colloidal gold cards were stored in dark for 7 days at 37°C. Utilizing the high, medium and low concentration positive serum sample (1:100, 1:200, 1:800 dilution) for daily testing, recorded and analyzed test results.

5 positive serum samples were diluted at 1:800, as the samples were tested using one batch and three different batches colloidal gold cards. Repeated the test three times for each sample, recorded and analyzed test results.

Table 1 showed that 10 PRRSV positive serums were all positive, 108 out of 110 negative samples were negative, and 2 were false positive. 5 SADS CoV samples, 4 TGEV samples, 3 PRCV samples, 6 PHEV samples, 1 PDCoV sample were all negative, indicating the colloidal gold cards has high specificity to PRRSV.

Table 1

Specificity results

Samples	*n*	Results
Positive PRRSV	24	All P
Negative PRRSV	110	108 N
SADS-CoV	5	All N
TGEV	4	All N
PRCV	3	All N
PHEV	6	All N
PDCoV	1	All N

Tested 10 positive serum samples at the dilution of 1:100, 1:200, 1:400, 1:800 and 1:1600, respectively.

Results showed that when the serum dilution reached 1:800, the test result still showed positive, when the serum dilution reached 1:1600, some test results showed negative (Table 2), indicating the colloidal gold cards has high sensitivity.

Table 2

Sensitivity results

No.	Serum dilution				
	1:100	1:200	1:400	1:800	1:1600
1	P	P	P	P	N
2	P	P	P	P	N
3	P	P	P	P	P
4	P	P	P	P	N
5	P	P	P	P	N
6	P	P	P	P	N
7	P	P	P	P	P
8	P	P	P	P	P
9	P	P	P	P	N
10	P	P	P	P	N

After 1, 2, 3, 4, 5, 6, 7 days of 37°C storage, the positive samples at the 1:100, 1:200, 1:800 dilution were all positive (Table 3), indicating that the stability of the colloidal gold cards was good.

Table 3

Stability results

Dilution	1 day	2 days	3 days	4 days	5 days	6 days	7 days
1:100	P	P	P	P	P	P	P
1:200	P	P	P	P	P	P	P
1:800	P	P	P	P	P	P	P

The results showed that the 5 positive serum samples with 1:800 dilution, the results were all positive in intra-batch. And, the 5 positive serum samples with 1:800 dilution, the results were all positive in inter-batches (Table 4). The results indicated the repeatability of colloidal gold cards was good.

Table 4

Repeatability results

No.	Intra-batch	Inter-batches
1	All P	All P
2	All P	All P
3	All P	All P
4	All P	All P
5	All P	All P

Since it first emerged in 1987, PRRSV has spread widely throughout the world, imposing a considerable economic burden on the swine industry [12]. Screening and control of PRRSV is a challenging task. Although the PCR method is the gold standard for various virus, more diagnostic method is also necessary for the fast diagnosis of PRRSV [13, 14]. In this study, we developed a rapid and convenient colloidal gold detection method for the diagnosing of North American type PRRSV popularly in China using recombinant nucleocapsid N protein antigen and its paired monoclonal antibody, its test procedure is simple and fast, without the need for professional technician, truly achieving self inspection at home.

When designing molecular or serological detection methods for PRRSV diagnosis, the high genetic variability of PRRSV should be taken into consideration. ORF7 is a conserved gene due to its sequence stability relative to other structural genes, there was a common linear epitope conserved among different isolates of European and North American origin in the amino acid segment 50–66 of ORF7, and several antigenic domains mapped onto N [15, 16]. PCR is a widely used method for detecting the PRRSV, with the primers and TaqMan probes usually targeting ORF7 gene [17, 18]. More study has conformed ORF7 gene and its encoded N protein are good targets for PRRS screening and diagnosis [9–11]. Therefore, we established a colloidal gold method for PRRS diagnosis using recombinant nucleocapsid N protein antigen and its paired monoclonal antibody. The prepared colloidal gold cards has high specificity, sensitivity, stability and repeatability. There was no cross reaction with various serum samples, the detection sensitivity reached 1:800, can be stably stored at 37°C for 7 days, the repeatability was good at 1:800 dilution. We believe that the method can be readily adapted by routine diagnostic laboratories and will be very useful during PRRS control. Inadequately, we do not have the positive samples of European strain, so we cannot predict the detection ability of these cards for European strain.

Conclusion. A rapid and convenient detection method for the diagnosing of PRRSV has been developed, and the prepared colloidal gold cards has high specificity, sensitivity, stability and repeatability. The colloidal gold cards provides a new alternative for simple and reliable detection of PRRSV with great potential for application in swine industry.

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