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Research paper

Development of an immunochromatographic test based on monoclonal antibodies against surface antigen 3 (TgSAG3) for rapid detection of *Toxoplasma gondii*

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ABSTRACT

The immunochromatographic test (ICT) is a convenient and low-cost method that can rapidly obtain results (10 min) under normal conditions. In this study, we established an ICT assay with two monoclonal antibodies: TgSAG3-3A7 and TgSAG3-4D5 based on the conserved protein of TgSAG3 that can be expressed in all the infective stages of *T. gondii*. 20 nm gold particles were prepared and conjugated with TgSAG3-3A7 MAb at the concentration of 12.5 μg/mL. TgSAG3-4D5 MAb were used as the capture antibody because of its higher affinity tested by ELISA. The detection limit of the ICT assay was 100 ng with visual detection under optimal conditions of analysis. Positive porcine serum of *Cryptosporidium suis*, *Mycoplasma suis*, *Streptococcus suis*, *Salmonella choleraesuis*, *Cysticercus cellulosae*, *Isospora suis*, and *Trichinella spiralis* were used to evaluate the specificity of this ICT and no cross-reactivity was observed. 310 porcine serum samples obtained from pig farms in Zhejiang Province, China were detected by this ICT and ELISA kit, 23 positive samples were found by the developed strip with the rate of 7.42% comparing with 22 positive samples detected by the commercially ELISA kit which the positive rate was 7.1%, the relative sensitivity and specificity of this ICT are 100% and 99.65%. Therefore, the ICT established in this study is proved effective, simple, specific and sensitive of *T. gondii* detection.

1. Introduction

Toxoplasmosis is a parasitic zoonosis widely spread in the world caused by the opportunistic intracellular parasite, *Toxoplasma gondii* (*T. gondii*) (Dubey and Urban, 1990). This kind of parasite is an apicomplexan protozoan that can infect a wide range of hosts, including humans (Sang-Eun et al., 2014). Humans can become infected by ingesting raw or undercooked meat containing tissue cysts, which can persist for more than two years in pork (Tenter et al., 2000). The parasite is responsible for approximately 24% of all estimated deaths attributed to foodborne pathogens in the United States (Guo et al., 2015). Toxoplasmosis has been linked to a major economic impact on the livestock industry. As it results in abortions, stillbirths and neonatal deaths, especially in sheep and goats (Dubey and Jones, 2008). While toxoplasmosis is also prevalent in pork, the prevalence of *T. gondii* infection in fatting pigs had been reported to vary from 24.5% in central (Tao et al., 2011) to 58.1% in southern China (Zhou et al., 2010). Thus the parasite is of great significance in medical and veterinary.

Several methods can be used for serological survey of toxoplasmosis. The latex agglutination test (LAT) is considered to be the main method for analysis of *T. gondii in livestock* at present. However, this method was reported with poor specificity (Holliman et al., 1990; Mazumder et al., 1988). Enzyme-linked immunosorbent assay (ELISA) is another option for epidemiological investigation, and is considered the most important way for detection of toxoplasmosis in humans (Aubert et al., 2000; Gamble et al., 2000). But, these methods require expensive equipments and well-trained personnels, and can only be used in laboratories. The immunochromatographic test (ICT) had been

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considered an ideal field detection as it characterized by easy, rapid and convenient to use (Rosypal et al., 2014). This method is significant for clinical analysis. Several previous researches established ICTs based on the major surface antigen 2 (TgSAG2) (Huang et al., 2004) and dense granule antigen protein 7 (TgGRA7) of *T. gondii* (Terkawi et al., 2013) for antibody detection.

In this study, we used *T. gondii* major surface antigen 3 (TgSAG3) to target for the detection of this pathogen. According to previous researches, surface antigens (SAGs) are highly conserved in isolated *T. gondii* strains and, therefore, qualify as potential candidates for serological survey of the parasite (Pietkiewicz et al., 2004). As a member of SAGs family, although the expression level of TgSAG3 is lower than TgSAG1 and TgSAG2, the high expression level in tachyzoite, bradyzoite and sporozoite of TgSAG3 and it can be detected in both acute and chronic stages of the infection, what' more, almost there was no ICT investigation of *T. gondii* using TgSAG3 antigen (Khanaliha et al., 2014). Herein, we have prepared monoclonal antibodies against TgSAG3 and designed a novel ICT by double antibody sandwich to detect the *T. gondii*. The accuracy of the ICT was further compared with a standard ELISA kit, which was used for clinical porcine serum samples detection.

2. Materials and methods

2.1. Preparation of the recombinant proteins

Total RNA extracted from the RH strain of *T. gondii* with TRIZOL according to the manufacturer's instructions (Invitrogen, USA). cDNA was amplified by RT-PCR using ReverTra Ace-α- (Toyobo, Japan). The cDNAs encoding TgSAG3 without the hydrophobic signal peptide (corresponding to amino acids 35–352, GenBank accession number AF340227) were amplified using PCR with specific primers sets designed from the available sequences in GenBank, as follows: 5′ TTA**G AATTC**ATGGCGATCTTGGGAACCGG 3′ includes an EcoRIrestriction enzyme site and 5′ TAT**AAGCTT**TTAGGCAGCCACATGCACAAG 3′ includes a Hind III restriction enzyme site for TgSAG3 with the glutathione S-transferase (GST), and 5′ CGC**GGATCC**ATGGCGATCTTGGG AACCGG 3′ includes a BamH I restriction enzyme site and 5′ CCG**GA ATTC**TTAGGCAGCCACATGCACAAG 3′ includes an EcoRI restriction enzyme site for TgSAG3 with N-terminal histidine-tag (His). PCR products were digested with the appropriate restriction enzymes and then constructed into the Escherichia coli expression vectors pGEX-4-T-1 (GE Healthcare, USA) and pET-30a (Novagen, China) respectively.

Recombinant proteins of TgSAG3-GST and TgSAG3-His were expressed in the E. coli BL21 (DE3) strain (TaKaRa Bio, Inc., Japan). After ultrasound pyrolysis processing and centrifugation, recombinant protein of TgSAG3-GST were obtained from the supernatant and TgSAG3-His were obtained from the precipitation. Then, TgSAG3-GST and TgSAG3-His fusion proteins were purified by Glutatione Sepharose 4 B and Ni-NTA Agarose (GE, USA). The purity and quantity of the recombinant proteins of TgSAG3-GST and TgSAG3-His were confirmed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (WB).

2.2. Preparation of the monoclonal antibodies against TgSAG3

Spleen cells of BALB/c mice (female, 6 weeks old, Hangzhou, Zhejiang, China), immunized with purified TgSAG3-His because of its smaller tag protein and better immunogenicity, were fused with SP 2/0 myeloma cells. Briefly, 10 μg of TgSAG3-His was mixed 1:1 with complete Freund's adjuvant for the first immunization and mixed with incomplete Freund's adjuvant for the second and third immunizations every 2 weeks, for 6 weeks. A week after the third injection, mice were boosted with 10 μg of the TgSAG3-His without adjuvant by intraperitoneal injection. Three days after the final booster immunization, spleen cells were harvested from immunized mice and fused with SP 2/0 myeloma cells using polyethylene glycol.

A subsequent ELISA was performed to screen which hybridomas were producing MAbs against TgSAG3 with the TgSAG3-GST being the coating proteins. Positive hybridomas were selected and subcloned 3 times from a single cell by limiting dilution. Then, ascites were produced with BALB/c mice and purified by saturated ammonium sulfate [$(NH4)_2SO_4$]. SDS-PAGE and western blot were performed to confirm the specificities of the MAbs.

Mice used as experimental animals, were treated in strict accordance with the recommendations in the Guide for the regulation for the Administration of Affairs concerning Experimental Animal of the People's Republic of China. Animal experiments were approved by Zhejiang University Experimental Animal Ethics Committee (Permit Number: ZJU201308-1-10-072).

2.3. Identification of hypo types and epitopes' specificities of the MAbs

Isotyping of the MAbs was carried out using a mouse monoclonal antibody isotyping kit (Biodragon, China). ELISA overlap tests was performed to identify the epitopes' specificities of the MAbs (Friguet et al., 1983). Briefly, constant amounts of TgSAG3-GST were coated in wells, then various concentrations of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb were incubated in wells respectively until the saturated MAbs' concentrations were measured by ELISA. Then the mixture of saturated concentrations of TgSAG3-4D5 and TgSAG3-3A7 were incubated as primary antibodies measured by ELISA. Other steps carried out conventional ELISA procedures. Additivity indexes (A.I) of different MAbs in combination are calculated in accordance with this formula:

$$A.\ I = \frac{A(1+2) - \frac{A1+A2}{2}}{A1 + A2 - \frac{A1+A2}{2}} \times 100\%$$

A1, A2, and A (1 + 2) are the measured OD values of the first MAb, second MAb, and the first and second MAb in combination tested from the ELISA, respectively. When any combination of two MAb bind to different epitopes, A (1 + 2) = A1 + A2 and A.I = 100%. When the two MAb recognize the same epitopes, A (1 + 2) = (A1 + A2)/2, and A.I = 0%. Taking technical error into account, the recognized antigenicity domains are considered to be different when A.I > 40%.

Noncompetitive ELISA tests was performed to compare the affinity between the two MAbs(Beatty et al., 1987). TgSAG3-GST were serial diluted 1:1、1:2、1:4、1:8 and coated in wells, then serial concentrations of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb were incubated in wells respectively until the saturated MAbs' concentrations were measured by ELISA. Then the amount of MAb adherent to TgSAG3-GST on the plate was reflected by the values of OD. The use of serial dilutions of MAb resulted in a sigmoid curve of OD *versus* logarithm of total MAb added to the well. Then we could get the OD at the upper plateau (OD-100) and the MAbs' concentration at OD-50 (50% of OD-100) in different concentrations of TgSAG3-GST. The affinity constant (Ka) could be calculated in accordance with this formula: $Ka = \frac{n-1}{2(n[Ab']t - [Ab]t)}$, n is the dilution ratio, $[Ab']t$ and $[Ab]t$ are the concentrations of MAbs with two different concentrations of TgSAG3-GST in the coating solutions.

2.4. Preparation of immunoassay materials

All glassware used was siliconized with sigmacote, cleaned in aqua regia [HNO_3/HCL (v/3 v)] before use. Briefly, 100 mL of 0.01% $HAuCl_4$ solution in a beaker was boiled, and 2.5 mL of 1% trisodium citrate solution was then added under continuous stirring. When the color of the solution changed from blue to dark red in 2 min, the solution was boiled for another 10 min. Subsequently, the colloidal gold solution was continuously stirred for another 5 min. The obtained gold colloid supplemented with 0.01% (m/v) sodium azide (NaN3) was stored in a dark bottle at 4 °C. The gold colloids were identified by transmission electron microscopy.

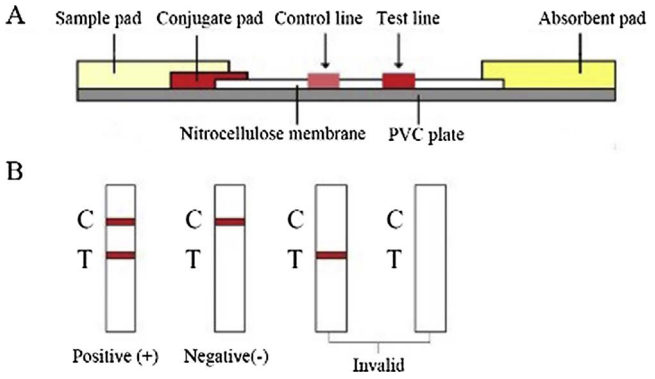

Fig 1. The composition of the immunochromatographic test strip. (A) The Schematic Description of the Immunochromatographic Device. (B) The Illustration of Immunochromatographic Test Results.

TgSAG3-3A7 MAb was selected for use as an antigen detector and conjugated with the colloidal gold, TgSAG3-4D5 MAb was used as the capture MAb. After a preliminary experiment, 0.05 mL of purified TgSAG3-3A7 MAb (5.0 mg/mL) was added to 20 mL colloidal gold solution (pH 8.2) with stirring gently. The admixture was mixed gently for 15 min, blocked by 2 mL of 10% BSA solution for 60 min, and then centrifuged at 13,000 rpm for 30 min. After centrifugation, the colloidal gold–MAb conjugate was suspended in 2 mL dilution buffer [0.002 mol/L sodium carbonate solution (pH 8.5) containing 1% BSA and 0.2% sodium azide] and stored at 4 °C.

TgSAG3-4D5 MAb was diluted to 1.5 mg/mL with 0.02 M sodium PBS (pH 8.5). The diluted MAb and 1 mg/mL goat anti-mouse IgG were transferred onto the NC membrane (Millipore, China) with a volume of 1 μL/cm to form the test (T) and the control (C) lines, respectively. The test strips were dried at 37 °C for 1 h 2% BSA was used as the blocking buffer.

2.5. Preparation of the immunochromatographic strip

The composition of the immunochromatographic test strip is described in Fig. 1.

The sample pad and glass fiber were saturated with 0.02 M PBS solution (pH 8.5) containing 0.2% Tween-20 and 1.5% (w/v) BSA and dried at 37 °C before use. The colloidal gold probe was diluted (1:5, v/v) with 0.02 M PBS (pH 8.5) containing 5% (w/v) sucrose and 1.5% (w/v) BSA. The colloidal gold probe was then added to the conjugate pad, dried at 37 °C for 1 h. The diluted MAb and goat anti-mouse IgG were transferred onto the NC membrane respectively described as above. The NC membrane was dealt with 0.02 M PBS containing 1.0% BSA, dried for 2 h at 37 °C. Pure cellulose fiber was used as the absorbent pad. The PVC plate was used as the bottom of the test strip. The sample pad, conjugate pad, immobilized NC membrane, and absorbent pad were assembled, as described in Fig. 1. These strips were cut into 6 mm width and stored in a desiccator at 4 °C for future use.

After the samples (100 μL) were added dropwise to the sample pad and allowed to pass through the NC membrane, the positive result was denoted by the appearance of two red lines in the test and control regions after 10 min. The negative result was denoted by the appearance of only one red line in the control region. The appearance of only a single red line in the test region or the absence of a line in test strip was considered an invalid test.

2.6. Sensitivity, specificity, and stability of the immunochromatographic test

Ten-folded dilutions of the standard positive porcine serum samples containing *T. gondii* in range of 1 mg/mL to 1 ng/mL were diluted by 0.9% NaCl (pH 7.2) to evaluate the limit of the immunochromatographic strip. For the sensitivity assay, 100 μL of the porcine serum samples without *T. gondii* was used as the negative control.

The specificity of the immunochromatographic strip was examined by positive porcine serum samples for *T. gondii*, *Cryptosporidium suis* (*C. suis*), *Mycoplasma suis* (*M. suis*), *Streptococcus suis* (*S. suis*), *Salmonella choleraesuis* (*S. choleraesuis*), *Cysticercus cellulosae* (*C. cellulosae*), *Isospora suis* (*I. suis*), and *Trichinella spiralis* (*T. spiralis*) kept in our laboratory.

All immunochromatographic strips were stored at 4 °C for 16 weeks to evaluate the stability of the immunochromatographic strips. 1 mg/mL of *T. gondii* serum samples were used to detect, and 0.9% NaCl (pH 7.2) was used as the blank control.

2.7. Detection of clinical samples

310 porcine serum samples collected in Zhejiang province, China were detected. All samples were also detected by the ELISA kit (R&D, USA) to evaluate the accuracy of the immunochromatographic test.

3. Results

3.1. Production of rTgSAG3-GST and rTgSAG3-His proteins

DNA sequencing confirmed that the recombinant plasmid pET30a-TgSAG3-His and pGEX-4-T-1-TgSAG3-GST were constructed successfully. SDS-PAGE showed that the recombinant protein rTgSAG3-GST and rTgSAG3-His were 64 kDa and 38 kDa, respectively after IPTG induction and purification as described (Fig. 2). Western blot analysis indicated that the rTgSAG3-His protein can be detected using mouse Toxoplasmosis positive serum (Fig. 2), and it has good immunogenicity. Western blot was also conducted in order to detect the GST tag of the recombinant protein using anti-GST antibody. The band with the molecular weight of 64 kDa demonstrated a positive signal which confirmed the successful production of rTgSAG3-GST (Fig. 2), the band of 26 kDa indicated single GST protein.

3.2. Production and screening of MAbs

In this study, we harvested three mAbs against rTgSAG3-His, but only two mAbs could react with the natural antigen of *T. gondii* (Fig. 3), so we selected the two different mAbs to continue this assay. As a result, the purification was successful and the bands of light and heavy chains of the mAb were seen clearly. The heavy chain was about 45 kDa and the light chain was about 25 kDa. The distinct bands showed by Western blot analysis also indicated that TgSAG3-4D5 MAb and TgSAG3-3A7 MAb can both react specifically with the natural antigen of *T.*

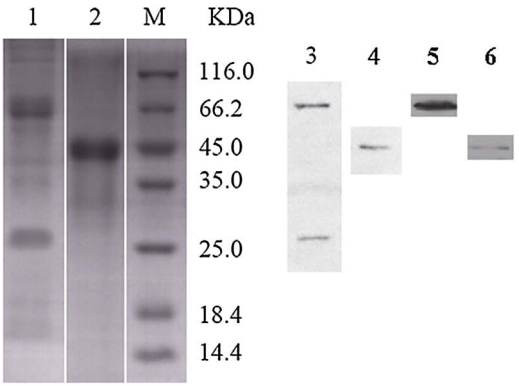

Fig. 2. SDS-PAGE Analysis and Western blot Assay of Two Recombinant Proteins. Lane 1 and 2: rTgSAG3-GST and rTgSAG3-His were analyzed by SDS-PAGE respectively; lane 3 and 4: rTgSAG3-GST and rTgSAG3-His were confirmed by Western blot, the first antibodies were anti-GST and anti-His antibodies respectively. Lane 5 and 6: rTgSAG3-GST and rTgSAG3-His were confirmed by Western blot, the first antibody was mouse anti-toxoplasma antibody.

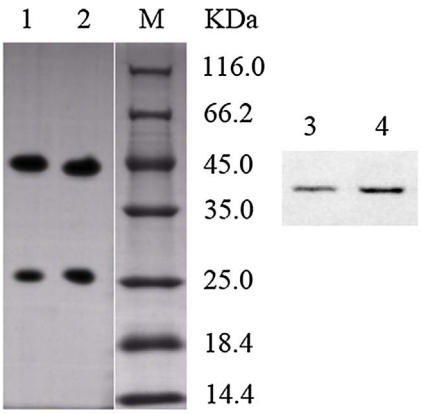

Fig. 3. SDS-PAGE Analysis and Western blot Assay of Two MAbs. Lane 1 and 2: SDS-PAGE analysis of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb successively; Lane 3 and 4: Western blot assay of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb successively.

gondii.

3.3. Identification and hypo types and epitopes' specificities of the MAbs

The hypo types, titers and epitopes' specificities of purified ascites were showed in Table 1. Because only two different mAbs (TgSAG3-4D5 and TgSAG3-3A7) were identified in the research. So we only analyzed the isotypes of these two mAbs and they were IgG_3 and IgG_1 respectively. The Ka (affinity constant) of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb are 7.02 mL/ng,5.32 mL/ng. So we selected the TgSAG3-4D5 MAb as the capture MAb in the following established immunochromatographic test. The value of A.I was 63%, demonstrating the different targeting epitopes of the two MAbs according to the ELISA overlap tests described previously.

3.4. Sensitivity, specificity, and stability of the immunochromatographic test

The result of sensitivity of the ICT was showed in Fig. 4 (A). As we can see, the red line on the testing region can be oberserved clearly when the concentration of the standard positive porcine serum samples containing *T. gondii* ranged from 1 mg/mL to 100 ng/mL, illustrating that the limit of the detection was 100 ng/mL.

Other porcine serum samples infected different pathogens were used to evaluate the specificity of the ICT. The specificity test (Fig. 4B) showed that only the positive porcine serum for *T. gondii* presented two red lines in both test and control regions compared with others presenting only one red line in control region.

The red line could been seen clearly after the same batch of ICT stored at 4 °C for 16 weeks to assess their stability by evaluating the sensitivity and specificity (Fig. 5). The strips were proved to be stable for at least 16 weeks at 4 °C.

3.5. Clinical application of the developed immunochromatographic test

310 porcine serum samples were collected from farms in Zhejiang, China. They were detected by the developed strip and by the commericially ELISA kit respectively. 23 positive samples were found by the developed strip with the rate of 7.42% comparing with 22 positive samples detected by the commercially ELISA kit which the positive rate

Table 1
Immunological Properties of TgSAG3-4D5 MAb and TgSAG3-3A7 MAb.

mAb	Isotype	titer	A.I
4D5	IgG_3	1:409600	63%
3A7	IgG_1	1:204800	

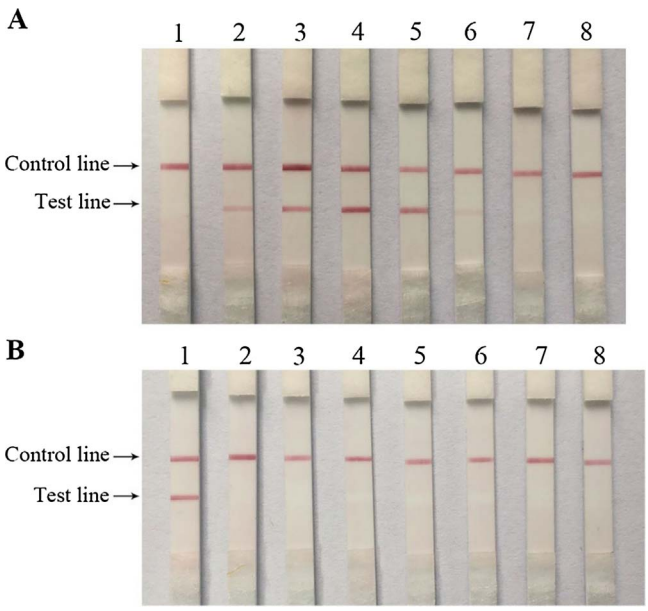

Fig. 4. Sensitivity and Secificity Testing of Immunochromatographic Test. (A) Sensitivity of the novel ICT. Lane 1: Negative control; 2: *T. gondii*, 1 mg/mL; 3: *T. gondii*, 100 μg/mL; 4: *T. gondii*, 10 μg/mL; 5: *T. gondii*, 1 μg/mL; 6: *T. gondii*, 100 ng/mL; 7: *T. gondii*, 10 ng/mL; 8: *T. gondii*, 1 ng/mL. (B) Specificity of the novel ICT. Lane 1: *Toxoplasma gondii*; 2: *Cryptosporidium suis*; 3: *Mycoplasma suis*; 4: *Streptococcus suis*; 5: *Salmonella choleraesuis*; 6: *Cysticercus cellulosae*; 7: *Isospora suis*; 8: *Trichinella spiralis*.

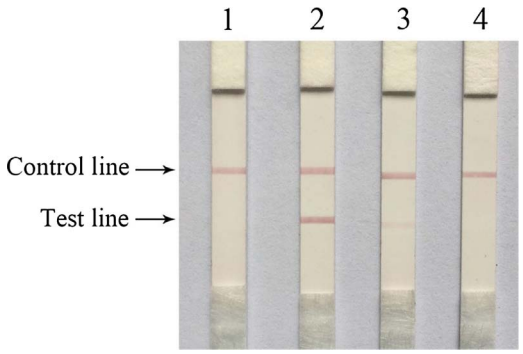

Fig. 5. Stability Testing of Immunochromatographic Test. Lane 1: Negative control; 2: *T. gondii*, 10 μg/mL; 3: *T. gondii*, 100 ng/mL; 4: The porcine serum sample infected *Streptococcus suis*.

Table 2
Results of Positive Porcine Serum Sample Analyzed with Different Methods.

	The No. (%) of *T. gondii* positive samples by		Predictive value(%)
	Test Strip	ELISA	
Positive	23 (7.42)	22 (7.1)	95.65
Negative	287 (92.58)	288 (92.9)	99.65

was 7.1% (Table 2). The relative sensitivity and specificity of this developed ICT were 100% and 99.65%, and the relative agreement was 99.68% when ELISA was used as a gold standard.

4. Discussion

In this study, we developed a novel ICT to detect toxoplasmosis, which exhibited high specificity, sensitivity, and agreement with the results of ELISA. We used TgSAG3 to target for the detection of *T. gondii* because of its well antigenic properties. Previous reports (Jacquet et al.,

2001) showed that TgSAG3 could be detected in acute and chronic infection. What's more, TgSAG3 is expressed by all infectious stages of *T. gondii* and is abundant on the surface of *T. gondii* (Jiang et al., 2008). Numerous of TgSAG3 would be released increasingly when *T. gondii* invaded the host cells and play an important role in the invasion (Jacquet et al., 2001). Thus, TgSAG3 is an excellent serological marker for the detection of toxoplasmosis. To our knowledge, this is the first report to use TgSAG3 as serological antigen for ICT.

Two recombinant proteins were obtained successfully in our study. The results showed that the rTgSAG3-His could be recognized by the positive porcine serum for *T. gondii* and exhibited good immunogenicity. His tag is also characterized by its smaller molecular weight than GST tag. So we decided to use rTgSAG3-His to immune BALB/c mice and rTgSAG3-GST to detect and screen MAbs by ELISA. This way made the screening process simple and timesaving.

Several serologic methods have been standardized for the detection of *T. gondii* infection. Among these methods, the indirect hemagglutination test (IHAT), the latex agglutination test (LAT), the indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA) are the most common. In these studies, the detection for antibody is the general method because of its high sensitivity. However, the antibody for *T. gondii* like IgG will maintain for a long time even for the whole life of the host (Hill and Dubey, 2002). Furthermore, the antibody for *T. gondii* in the immunocompromised host cannot be detected (Weiss and Dubey, 2009). Thus, the detection for antibody cannot distinguish the recent infection and previous infection, and will increase inevitable false positive or false negative results. In this study, we established an ICT assay by the double antibody sandwich method via detecting the TgSAG3 of *T. gondii*, which can further guarantee the decrease of the false positive or negative results effectively and can be applied for toxoplasmosis detection from different species of animals.

In this assay, we expressed and screened two MAbs successfully, and their titers and additivity indexes (A.I) were measured by ELISA. We used TgSAG3-4D5 MAb as the capture MAb because of its higher affinity. While TgSAG3-3A7 MAb was used as an antigen detector to conjugate with the colloidal gold as the low titer of antibody could not capture sufficient conjugated antigen to cause color reaction on the membrane.

The strips can be used for detection at least 16 weeks after storage at 4 °C indicating excellent stability with the limit of 1 μg/mL. Positive porcine serum samples for *T. gondii*, *C. suis*, *M. suis*, *S. suis*, *S. choleraesuis*, *C. cellulosae*, *I. suis*, and *T. spiralis* were used for specificity detection. Only positive porcine serum samples infected with *T. gondii* can be detected, showing no-cross reaction of the established ICT.

Terkawi developed an ICT based on dense granule protein 7 (GRA 7) for *T. gondii* infection (Terkawi et al., 2013). The relative sensitivity and specificity were 80% and 100% compared with iELISA for IgG. Wang established an ICT for the detection of *T. gondii* circulating antigens, in his study, the relative sensitivity and specificity were 98% and 100% compared with iELISA (Wang et al., 2011). Dubey analyzed relative sensitivity and specificity of various detections of *T. gondii* by the modified agglutination test (MAT), latex agglutination test (LAT), indirect hemagglutination test (IHAT) and ELISA. The relative sensitivity and specificity of these tests were calculated respectively to be: 82.9 and 90.29% for MAT, 29.4 and 98.3% for IHAT, 45.9 and 96.9% for LAT, and 72.9 and 85.9% for ELISA (Dubey et al., 1995). In our study, the relative sensitivity and specificity demonstrated to be higer than Terkawi's assay. Although our strip test presented relative higher sensitivity but relative lower specificity than Wang's strip test, the specificity of our strip test still achieve up to 99.65%. Our strip test remains in a relative high sensitivity and specificity among those tests from Dubey's analysis.

5. Conclusion

In conclusion, two MAbs: TgSAG3-4D5 and TgSAG3-3A7 were developed for the ICT for the detection of *T. gondii*. Although the specificity of the strip test described in this study was lower than that of ELISA, the sensitivity and specificity was better than other ICTs and the method proved to be a rapid, practical assay for detection of the infection and requires few equipment compared with ELISA, and other techniques. This developed assay is also distinguished by its early detection than other ICTs. The novel ICT based on two MAbs: TgSAG3-4D5 and TgSAG3-3A7 is a valid rapid method for the clinical investigation of *T. gondii* infection.

Acknowledgements

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