Rapid Immunochromatographic Test Using Recombinant SAG2 for Detection of Antibodies against *Toxoplasma gondii* in Cats

Xiaohong Huang,1,2 Xuenan Xuan,1 Haruyuki Hirata,1 Naoki Yokoyama,1 Longshan Xu,2 Naoyoshi Suzuki,1 and Ikuo Igarashi1*

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan,1 and Fujian Center for Disease Control and Prevention, Fuzhou 350001, China2

Received 24 April 2003/Returned for modification 19 August 2003/Accepted 13 September 2003

An immunochromatographic test using recombinant truncated surface antigen 2 for detection of antibodies against *Toxoplasma gondii* was developed. Evaluation of detection of the antibody in mice and cats suggests that this test is rapid, simple, accurate, relatively inexpensive, and suitable for use under field conditions.

*Toxoplasma gondii* is one of the most polyxenous parasites known to date. One of the transmission routes of the parasites among animals is oral ingestion of infectious oocysts from the environment (13). Recently, some outbreaks of acute toxoplasmosis in humans have been associated with oocyst contamination (1, 3, 11, 12). Therefore, the role of domestic cats in *T. gondii* transmission should be monitored, because these animals shed the infectious oocysts in their feces.

Serological tests have been considered important methods for detection of *T. gondii* infection. The latex agglutination test (LAT) is a commercially available test for the diagnosis of feline toxoplasmosis. In previous studies, the surface antigen 2 (SAG2) of *T. gondii* expressed either in *Escherichia coli* or in insect cells was validated as a useful antigen which promised a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) (6, 7). However, the performance of ELISA, which is laborious and time-consuming and requires expertise and equipment, still remains to be improved. Reported here is the development and evaluation of a simple, rapid, and reliable immunochromatographic test (TgICT) for detection of specific antibodies against *T. gondii* in domestic cats.

Generally, a higher concentration and purity of the antigen are required for the ICT. A truncated SAG2 without the highly hydrophobic signal peptide and C terminus was thus cloned and expressed to improve the yield of the soluble recombinant antigen. Briefly, a 438-bp DNA fragment encoding the truncated SAG2 was amplified by PCR with two oligonucleotide primers, 5′-ACGAATTCGTCCACCACGAGACG-3′ and 5′-ACGAATTTCCACCCACCCGAGACC-3′ (10), and template DNA extracted from tachyzoites of *T. gondii* strain RH (9). Then, the PCR product was inserted into an *Eco*RI site of *pGEX-4T-3*. The expression of the SAG2 fusion protein with glutathione S-transferase (GST) (G-rSAG2t) in *E. coli* by the recombinant plasmid and the purification of G-rSAG2t and rSAG2t without GST (Fig. 1) were performed as described previously (8), except that the temperature for expression was modified from 37 to 25°C to increase the yield of the soluble protein.

Previously, rSAG2t was used to develop the ICT; however, no good result was obtained. Therefore, G-rSAG2t was used to replace rSAG2t, and GST was used as a control antigen. The purified G-rSAG2t was conjugated with a gold colloid (British BioCell International, Cardiff, United Kingdom) (1:10, vol/vol) at pH 6.5 by incubation at room temperature for 10 min. Then 0.05% polyethylene glycol 20000 (PEG) and 1% bovine serum albumin (BSA) were added to stabilize and block the conjugate particles. The supernatant was discarded by 90% after centrifugation at 18,000 × g for 20 min. The pellet was resuspended by sonication and washed with phosphate-buffered saline (PBS) containing 0.5% BSA and 0.05% PEG. After centrifugation, the pellet was resuspended in PBS with 0.5% BSA and 0.05% PEG. The concentration of the conjugate was adjusted until the absorbance at 520 nm reached 5. The conjugate was diluted in 10 mM Tris-HCl (pH 8.2) with 5% sucrose, sprayed on the glass fiber (Schleicher & Schuell, Keene, N.H.), and dried in a vacuum oven. Mouse anti-rSAG2t IgG was purified with an Econo-Pac protein A kit (Bio-Rad Laboratories, Hercules, Calif.) from sera of BALB/c mice immunized with rSAG2t. Mouse anti-rSAG2t immunoglobulin G (IgG; 1.5 mg/ml), G-rSAG2t (0.5 mg/ml), and GST were jetted linearly on nitrocellulose (NC) (Schleicher & Schuell) as shown in Fig. 2, lane 1, by using a Biojet 3050 quanti-dispenser (BioDot Inc., Irvine, Calif.). Then the membrane was dried at 50°C for 30 min and blocked by using 0.5% casein in a 50 mM bovine serum albumin (BSA) containing 0.05% PEG. The concentration of the conjugate was adjusted until the absorbance at 520 nm reached 5. The conjugate was diluted in 10 mM Tris-HCl (pH 7.4) containing 0.5% sucrose and 0.05% sodium cholate, the membrane was dried in air overnight. Sequentially, the NC, absorbent pad, conjugate pad, and sample pad were assembled on an adhesive card (Schleicher & Schuell) and cut into 3-mm-wide strips by using a BioDot cutter as shown in Fig. 2, lane 1. Detection was performed by pipetting 50 μl of the diluted serum (1:2 in PBS) on the sample pad. The result was judged within 15 min and recorded as shown in Fig. 2. LAT and ELISA were performed as described previously (6). Relative sensitivity, specificity, and agreement with LAT and ELISA were calculated as described by Griner et al. (5).

Sera from 9, 6, 2, and 1 mice infected with *T. gondii* strains Beverley, Gail, PLK, and S-273, respectively, from 3 mice in-
fected with *Neospora caninum*, and from 12 specific-pathogen-free (SPF) mice were examined by the TgICT. These sera were collected 30 to 270 days postinfection. All sera from mice infected with one of the four strains of *T. gondii*, but none of those from 3 *N. caninum*-infected and 12 SPF mice, were positive. This result suggests that the TgICT not only could detect antibodies against various strains of *T. gondii*, although its antigen was encoded by the gene from strain RH, but also could distinguish between toxoplasmosis and neosporosis, which is important because some companion animals and livestock (for example, dogs, cattle, sheep, and horses) can be infected naturally with both *T. gondii* and *N. caninum* (2).

Sera from 30 SPF cats (CSK, Suwa, Japan) were all negative by TgICT detection. Sera collected from four experimental cats before infection were negative, whereas all of those collected 35 to 110 days after infection with *T. gondii* strain Beverley (9) were positive, indicating that the TgICT had high sensitivity and specificity.

One hundred seventy-nine field serum samples collected from domestic cats in Japan (4) were examined by the TgICT, and the result was compared with those of LAT and ELISA (Table 1). The relative sensitivity and specificity of TgICT were 100 and 94.5% when LAT was used as a reference and 97.2 and 95.8% when ELISA was used as a reference. The relative agreements were 95.5% between the TgICT and LAT and 96.1% between the TgICT and ELISA. These high agreements suggest that the TgICT would be reliable. Theoretically, the TgICT could detect various classes of immunoglobulin. Therefore, it might be more sensitive than the ELISA. Three field cat sera (1.7%) were found positive to both G-rSAG2t and GST in the TgICT (Fig. 2, lane 4), and these positive reactions were due to antibodies to GST but not to specific antibodies to *T. gondii*. Therefore, these sera were considered negative for *T. gondii* infection.

In conclusion, the TgICT was rapid, simple, sensitive, and specific. It would be a suitable diagnostic tool for detection of the specific antibodies in *T. gondii* infection in cats under field conditions. To our knowledge, this study is the first report of the serodiagnosis of *T. gondii* infection by use of an ICT. Further improvements would include the following: (i) use of whole-blood samples in the TgICT to simplify the test even more, (ii) evaluation of potential utility for detection of the specific antibodies in humans, and (iii) development of a TgICT device for detection of both IgM and IgG against *T. gondii* in order to distinguish between acute and chronic infections.

This study was supported by a grant from the 21st Century COE Program (A-1), by the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by Grants-in-Aid for Scientific Research (A) from the Japan Society for the Promotion of Science (13356007).

**REFERENCES**


