Fast Dipstick Dye Immunoassay for Detection of Immunoglobulin G (IgG) and IgM Antibodies of Human Toxoplasmosis

Si Jin,1,2 Zhu Yin Chang,2* Xu Ming,2 Cao Li Min,3 He Wei,2 Liang You Sheng,2 and Guan Xiao Hong4

Institute of Medical Molecular Biology, Nanjing Medical University, Nanjing,1 and The Key Lab on Molecular Biology of Parasites, Jiangsu Institute of Parasitic Diseases, Wuxi,2 Jiangsu, and Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei,3 People’s Republic of China

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Toxoplasma gondii infection is widespread in humans, although its prevalence varies widely from place to place. In the United States and the United Kingdom, it is estimated that 16 to 40% of the population are infected, whereas in Central and South America and continental Europe, estimates of infection range from 50 to 80% (4). Most infections in humans are asymptomatic but the parasite can produce devastating disease. In pregnancy, infection can result in congenital infection with severe sequelae or late-onset eye disease, and it is a frequent cause of encephalitis in severely immunosuppressed patients with AIDS (1, 12). Toxoplasmosis is also a serious complication following organ transplantation (2). In addition to being a major source of infection for humans, it is also of considerable importance in domestic animals and is responsible for abortions in sheep and swine (16). Therefore, there is an urgent need to develop an effective diagnostic kit and vaccine.

For clinical purposes, toxoplasmosis can be divided for convenience into five infection categories: (i) those acquired by immunocompetent patients, (ii) those acquired during pregnancy, (iii) those acquired congenitally, (iv) those acquired by or reactivated in immunodeficient patients, and (v) ocular infections. In any category, clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered. Furthermore, methods of diagnosis and their interpretations may differ for each clinical category.

Diagnosis of T. gondii infection or toxoplasmosis in humans is made by biological, serological, histological, or molecular methods or by some combination of these. Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic for a definite diagnosis. In fact, toxoplasmosis mimics several other infectious diseases. Detection of T. gondii antibodies (mainly immunoglobulin G [IgG] and IgM) in patients may aid diagnosis. IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 months, decline at various rates, and usually persist for life (6, 8). IgM antibodies may appear earlier and decline more rapidly than IgG antibodies, so the detection of IgG antibodies may be helpful for diagnosis of chronically infected patients, if IgM antibodies are negative. An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past; because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed (3, 9, 17).

There are numerous serological procedures available for the detection of humoral antibodies; these include the Sabin-Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay, the direct agglutination test, the latex agglutination test, the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (13). Most of these immunodiagnostic tests are not easy to apply in the field, e.g., the ELISA or the indirect fluorescence antibody assay, since these techniques require special equipment and reagents. Performing any of these tests even in the laboratory generally takes time, sometimes with overnight incubation steps; otherwise, enzyme reagents would need a cold chain for delivery. In such situations, a rapid, simple, and inexpensive colorimetric assay with robust reagents and no instrumentation could have many diagnostic applications.

In this study, the dipstick dye immunoassay (DDIA) for detection of IgG or IgM antibodies of human toxoplasmosis was developed. Sheep anti-human IgG or rabbit anti-human IgM conjugated with a colloidal dye produced in China served as the color-detecting reagents, and a soluble antigen of tachyzoites of T. gondii strain RH (TSA) on a nitrocellulose paper (NCP) membrane dipstick was used as the capture antigen. The DDIA assay for the detection of IgG or IgM anti-
bodies of human toxoplasmosis was found to be rapid, simple, cheap, and effective.

**MATERIALS AND METHODS**

**Serum samples.** Twenty-five serum samples were provided by Jack S. Remington, Toxoplasma Serology Laboratory, Palo Alto Medical Foundation Research Institute. Each of these sera was positive for both IgG (detected by the Sabin-Feldman dye test) and IgM (detected by IgM-ELISA), with different titers ranging from 1:128 to >1:16,000 (IgG) and different absorbance values ranging from 2.9 to 9.1 (IgM). Fifty serum samples negative for *T. gondii* were obtained from healthy subjects in the central blood station, Wuxi, China. Each serum sample was confirmed by ELISA to be negative for both anti-*T. gondii* IgG and IgM antibodies. A total of 172 serum samples were obtained from a group carrying out an epidemiological investigation of toxoplasmosis by serological methods. All sera were stored in aliquots at −20°C.

**Antibodies for labeling and quality control.** Sheep anti-human IgG (IgG fragment of antiserum) was obtained from the Sino-American Biotech Company; the concentration of protein was 5 mg/ml. Rabbit anti-human IgM (IgG fragment of antiserum) (Sigma) was purchased from the Beijing Superior Chemical & Instruments Co., Ltd.; the protein concentration was 4 mg/ml. Sheep anti-rabbit IgG and rabbit anti-sheep IgG were purchased from the Beijing Biodee Biotech Co., Ltd.; the protein concentration of each was 2 mg/ml.

**Antigen preparation.** The original inocula of *T. gondii* RH strain were peritoneal exudates from infected mice maintained in our laboratory. The exudates were obtained from infected mice maintained in our laboratory. The exudates of human toxoplasmosis was found to be rapid, simple, cheap, and effective.

**Colloidal dye preparation.** The colloidal dye (D-1) produced in China was used as described previously (18). The stock colloidal dye D-1 suspension was prepared as described by Snowden and Hommel (15). Briefly, 0.5 g of D-1 dye was suspended in 30 ml of deionized water and stirred overnight at room temperature. The suspension was washed six times by centrifugation at 20,000 × g for 30 min at room temperature, and the pellet was resuspended in an equal volume of double-distilled water. Aggregated colloidal particles were removed by low-speed centrifugation (500 × g; 30 min). This stock colloidal dye solution, with 0.01% thimerosal added as a preservative, was stored at 4°C.

**Labeling sheep anti-human IgG or rabbit anti-human IgM with colloidal dye D-1.** A total of 5 ml of prepared dye suspension was mixed with 100 µl of sheep anti-human IgG (5 mg/ml) or 125 µl of rabbit anti-human IgM (4 mg/ml). The mixture was incubated overnight at room temperature. Then, bovine serum albumin was added at a final concentration of 5% to the mixture for another 2 h, after which it was centrifuged at 18,000 rpm for 30 min. The pellet was washed twice with PBS and dispersed in 5 ml of PBS with 0.01% thimerosal.

**Dipstick preparation.** NCP membranes were purchased from Millipore (Bedford, Mass.). The dipsticks were prepared from NCP membranes (3.0 by 0.3 cm). At the top and bottom of the dipstick, paper pads of 2.0 and 0.5 cm, respectively, were attached. At 1.0 cm from the bottom of the NCP, a band of TSA (about 3 µl per each band, at a concentration of 1 mg/ml) was applied as the detection band. At sites that were 1.0 cm from the top, another band of rabbit anti-sheep IgG (for IgG detection) or sheep anti-rabbit IgG (for IgM detection) was added as a control band.

**IgG-DDIA and IgM-DDIA.** The IgG-DDIA kit included the sheep anti-human IgG conjugated with dye, prepared dipsticks (the control band was rabbit anti-sheep IgG), and cups, while the IgM-DDIA kit included the rabbit anti-human IgM conjugated with dye, prepared dipsticks (the control band was sheep anti-rabbit IgG), and cups. The detection procedure for the IgG-DDIA and IgM-DDIA was as follows: 10 µl of serum sample was placed in a small plastic cup (0.6 by 1.0 cm), and 50 µl of antibody-conjugated dye was added and mixed. The prepared dipstick was inserted in the cup for 5 to 15 min at room temperature. If both the detection band and the control band were colored purple-blue, the sample was recorded as positive. If the control band was colored purple-blue but the detection band was not colored, it was recorded as negative. If neither band was colored, the test reagents were assumed to be invalid (Fig. 1).

**Sample detection with SERION ELISA classic *T. gondii* IgG and IgM kits.**

**RESULTS**

**Sensitivity and specificity.** Twenty-five serum samples that were positive for both IgG and IgM were used to test the sensitivity of the IgG-DDIA and IgM-DDIA; 50 serum samples from healthy subjects were also used to detect specificity (Table 1). The sensitivity and specificity of IgG-DDIA were 100 and 96%, respectively; the sensitivity and specificity of IgM-DDIA were 100 and 94%, respectively.

**Comparison between DDIA and ELISA.** A total of 172 serum samples obtained from an epidemiological study of toxoplasmosis were used to compare DDIA and ELISA (Table 2). The total rate of correspondence (i.e., the rate of positive correspondence and the rate of negative correspondence) for IgG detection between IgG-DDIA and IgG-ELISA was 97.7% (86 of 88 samples), 100% (52 of 52 samples), and 94.4% (34 of 36 samples), respectively. For IgM detection, the total rate of correspondence (the rate of positive correspondence and the rate of negative correspondence) for IgG detection between IgG-DDIA and IgG-ELISA was 97.7% (86 of 88 samples), 100% (52 of 52 samples), and 94.4% (34 of 36 samples), respectively.

**TABLE 1. Sensitivity and specificity test of IgG- and IgM-DDIA**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>No. of samples</th>
<th>IgG-DDIA</th>
<th>IgM-DDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>50</td>
<td>2</td>
<td>48</td>
</tr>
</tbody>
</table>

* Pos, positive; Neg, negative.
TABLE 2. Comparison of detection results obtained by DDIA and ELISA

<table>
<thead>
<tr>
<th>Results by IgG- or IgM-DDIA (n)</th>
<th>Pos</th>
<th>Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-DDIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos (54)</td>
<td>52</td>
<td>2</td>
</tr>
<tr>
<td>Neg (34)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>IgM-DDIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pos (37)</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>Neg (47)</td>
<td>0</td>
<td>47</td>
</tr>
</tbody>
</table>

* The serum samples were tested by SERION ELISA classic T. gondii IgG or IgM kit, respectively. Pos, positive; Neg, negative.

The diagnosis of T. gondii infection or toxoplasmosis can be established by serologic tests, PCR, histological examination, or isolation of the parasite. T. gondii infection can be asymptomatic, and the clinical manifestations of patients with symptomatic toxoplasmosis are protean and nonspecific. The choice of the appropriate diagnostic method(s) and its (their) interpretation may differ for each clinical category. This study describes the rapid, simple, and inexpensive serodiagnostic tests for detection of IgG and IgM antibodies to human toxoplasmosis. The sensitivity and specificity were similar to those produced by ELISA (SERION ELISA classic T. gondii IgG and IgM kits), but the DDIA technique was more rapid and simpler to carry out, taking just 5 to 15 min and not requiring special equipment.

In the past, a number of modifications to the ELISA have been described in efforts to produce a more field-applicable assay format. The dot immunobinding assay, using an NCP membrane as a test matrix, is becoming widely used in simple assay formats. The dot immunobinding assay, using an NCP membrane dipstick based on immunochromatography to produce an inexpensive, robust, NCP-assay, colloidal dye-labeled antigen or antibody, and immunochemical detection of antibodies specific for T. gondii was rapid, easier to operate, and did not require special equipment.

Recently, some colloidal dye particles were used in dot blot assays to avoid use of antibody conjugates is similar to or even simpler than antibody-coupled gold particles. The dye-labeled antibodies can maintain reactivity for at least 6 months when stored at 4°C in a liquid suspension, and the dipstick can also keep its detective capacity after storage at 4°C for at least 6 months.

It is important to mention that the presented data are only preliminary, but they give us a first impression about the suitability of such a test system for routine diagnostics. In other words, the DDIA which utilize sheep anti-human IgG or rabbit anti-human IgM-colloidal dye conjugates as the agents visualized could also be used for detection of specific antibodies in other infectious diseases, if there were specific diagnostic antigens.

Acknowledgments

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References


