Interlaboratory Evaluation of Indirect Enzyme-Linked Immunosorbent Assay, Antibody Capture Enzyme-Linked Immunosorbent Assay, and Immunoblotting for Detection of Immunoglobulin M Antibodies to Toxoplasma gondii

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One hundred and fifteen serum samples from healthy laboratory personnel and 50 consecutive samples from 19 patients with anamnestic clinical signs of toxoplasmosis were assayed by four laboratories for the presence of immunoglobulin M antibodies to Toxoplasma gondii by an indirect enzyme-linked immunosorbent assay (ELISA), an antibody capture assay with peroxidase-labeled toxoplasma antigen, and an immunoblotting assay. In addition, a commercially available antibody capture ELISA was used. Highly significant correlation coefficients were obtained between the four laboratories and the commercial test. The indirect ELISA and antibody capture ELISA showed equal sensitivity in detection of immunoglobulin M antibodies to toxoplasma in early-stage serum samples. However, in this study, the antibody capture assay discriminated better between serum samples obtained at early or late stages of toxoplasma infection.

Detection of specific antibodies, preferably of the immunoglobulin M (IgM) class, to Toxoplasma gondii in patient serum is generally used to diagnose toxoplasmosis. Since the introduction by Sabin and Feldman (11) of the dye test for detection of toxoplasma antibodies, many other techniques, like the complement fixation, indirect hemagglutination, and indirect immunofluorescence antibody tests, have been used (e.g., see references 3, 4, and 10). More recently, the enzyme-linked immunosorbent assay (ELISA) has been introduced for the detection of IgG and IgM antibodies to toxoplasma. Three ELISA techniques for detection of IgM antibodies in cases of toxoplasmosis are in use: the indirect ELISA (1, 9), the IgM antibody capture assay with a peroxidase-labeled antitoxoplasma serum (6, 17), and the IgM antibody capture assay with peroxidase-labeled toxoplasma antigen (15).

We describe an interlaboratory evaluation of the indirect ELISA in comparison with the antibody capture assay with peroxidase-labeled antigen for the detection of IgM antibodies to T. gondii in longitudinal serum samples from patients suspected of toxoplasmosis and in samples from healthy persons. In addition, the usefulness of the immunoblotting assay for the detection of IgM antibodies to toxoplasma was evaluated.

MATERIALS AND METHODS

Toxoplasma antigens. Toxoplasma antigens were prepared either from mice infected with the RH strain of T. gondii (2) or from cultures of human larynx carcinoma cells (HEp-2) infected with a strain of T. gondii isolated in The Netherlands, i.e., strain Deelen (16). Control antigen was purified in a similar way from uninfected HEp-2 cells. For the antibody capture ELISA, toxoplasma antigen was labeled with horse-radish peroxidase as described previously (15).

Serum samples. A total of 165 serum samples was studied. One hundred and fifteen serum samples were obtained from healthy laboratory personnel. Fifty samples were from 19 patients with clinical symptoms suggestive of toxoplasmosis. These samples were selected in laboratory B and tested under code by the other three laboratories. Of these 19 patients, 8 had clinical signs of recent toxoplasmosis, which had existed no longer than 3 months. These patients showed a fourfold or greater increase in IgG antitoxoplasma antibody titer during this period. In 10 patients, the onset of clinical signs that could be due to toxoplasmosis was less clear. No increase in IgG antitoxoplasma antibody titer was found in consecutive serum samples. In one patient, no IgG antibodies to toxoplasma were found in consecutive samples. This patient had suffered from general malaise for more than a year. No clinical diagnosis could be made.

Indirect ELISA for IgG antibody. The indirect ELISA for detection of IgG antitoxoplasma antibodies with the RH strain was performed as described by Ruitenberg and van Knapen (9) and with the Deelen strain as described by Van Loon and van der Veen (16). Results were expressed either as ELISA titers, using the mean absorption value of 20 healthy Sabin-Feltman-negative donors plus twice the standard deviation as the threshold value, or as international units per milliliter.

ELISA for IgM detection. The characteristics and basic principles of the indirect ELISA, antibody capture ELISA with peroxidase-labeled toxoplasma antigens, and antibody capture ELISA with peroxidase-labeled antitoxoplasma serum for detection of IgM antibodies to T. gondii are shown in Fig. 1A, B, and C, respectively.

(i) Indirect ELISA for IgM antibodies. Briefly, in the indirect ELISA (Fig. 1A), toxoplasma antigen is coated on

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the wells of microtiter plates. IgM antibodies present in the patient serum can bind during incubation and can be detected by the use of a peroxidase-labeled anti-human IgM antiserum followed by the addition of substrate (5-amino salicylic acid). In fact, this ELISA was carried out as described for IgG antitoxoplasma antibodies, with a peroxidase-labeled anti-human IgM antiserum (heavy chain specific: Pasteur Institute, Paris) instead of anti-human IgG. The RH strain was used to prepare the antigen. The absorbance value of a weakly positive reference serum sample, selected on the basis of the frequency distribution between positive and negative serum samples, was used as the threshold value. This test was performed in laboratories A, B, and D.

(ii) Antibody capture ELISA for IgM antibodies with peroxidase-labeled toxoplasma antigen. In the antibody capture ELISA with peroxidase-labeled toxoplasma antigen (Fig. 1B), microtiter plates are coated with anti-human IgM antiserum. IgM antibodies present in the patient serum can bind during incubation. If IgM antibodies against toxoplasma are present, these will bind the added peroxidase-labeled toxoplasma antigen, which can be detected by adding substrate (ortho-phenylenediamine). The antibody capture assay for IgM antitoxoplasma antibodies with the Deelen strain was performed in laboratory C as described before (15). In laboratory D, the RH strain was used to prepare the antigen. The absorbance of a weakly positive reference serum sample was used as the threshold value.

(iii) Commercial antibody capture ELISA for IgM antibodies with peroxidase-labeled antitoxoplasma serum. The antibody capture ELISA with peroxidase-labeled antitoxoplasma antiserum (Fig. 1C) is essentially like the antibody capture ELISA with peroxidase-labeled toxoplasma antigen. However, instead of peroxidase-labeled toxoplasma antigen, unlabeled toxoplasma antigen is added, followed by the addition of a peroxidase-labeled antitoxoplasma serum sample. This assay was performed in laboratory B according to the description of the manufacturer (Organon Teknika, Boxtel, The Netherlands).

In fact, the antibody capture ELISA measures the percentage in serum of IgM reactive with toxoplasma antigen, whereas the indirect ELISA measures an absolute concentration of IgM antibodies to toxoplasma antigen.

Immunoblotting assay. (i) Polyacrylamide gel electrophoresis. Antigen preparations from the RH strain of *T. gondii* were electrophoresed in 10 to 20% acrylamide gradient slab gels by means of the discontinuous sodium dodecyl sulfate buffer system described by Laemmli (5). Phosphorylase B, bovine serum albumin, ovalbumin, soybean trypsin inhibitor, and aprotinin (all obtained from Boehringer GmbH, Mannheim, Federal Republic of Germany) were used as molecular weight standards. Briefly, samples were diluted in a buffer containing 0.05 M Tris (pH 6.8), 4% sodium dodecyl sulfate, 5% mercaptoethanol, and 0.01% bromophenol blue and were then boiled for 3 min before application. The running buffer contained 25 mM Tris, 200 mM glycine, and 0.1% sodium dodecyl sulfate. Gels were run at 6 V/cm at room temperature until the tracking dye reached the bottom of the gel. Separated polypeptides were then transferred to a nitrocellulose membrane.

(ii) Electrophoretic transfer. For electrophoretic transfer, modification of the technique first described by Towbin et al. (14) was used. After polyacrylamide gel electrophoresis, gels were applied to a sheet of nitrocellulose membrane (0.45-μM pore diameter; Schleicher and Schuell, Dassel, Federal Republic of Germany) and electrophoresed for 3 h at 7 V/cm. Thereafter the blots were soaked in 0.3% Tween 20 in phosphate-buffered saline (PBS). Strips were cut from the nitrocellulose membrane and incubated with human sera diluted 1:100 in 0.05% Tween 20 in PBS for 90 min at room temperature. The strips were washed and incubated with peroxidase-labeled goat anti-human IgM (diluted 1:1,000 in PBS-0.05% Tween 20) for 1 h at room temperature. The strips were washed and developed in a solution of 0.06% diaminobenzidine, 0.03% 9-chloronaphthol, and 0.01% hydrogen peroxide in PBS. The reaction was stopped by washing with distilled water. The stained strips were dried in the dark and then photographed.

**Statistical analysis results.** Results were compared by the chi-square test and the nonparametric Mann-Whitney U test as indicated below.

**RESULTS**

IgM antibodies to *T. gondii* in healthy persons. To investigate the prevalence of IgM antibodies to *T. gondii* in a healthy population, 115 serum samples from laboratory personnel were tested in all four laboratories by the indirect ELISA (laboratories A, B, and D) or the antibody capture assay (laboratories C and D). Each laboratory used its own weakly positive reference serum sample to set the threshold value.

One hundred and eleven serum samples were found negative by all four laboratories. One sample was found positive.

<table>
<thead>
<tr>
<th>IgM Antibodies to T. gondii</th>
<th>Laboratory A</th>
<th>Laboratory B</th>
<th>Laboratory C</th>
<th>Laboratory D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human light</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-human light</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM Antitoxoplasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 1.** Characteristics of indirect ELISA (A) and antibody capture ELISA with peroxidase-labeled toxoplasma antigen (B) or peroxidase-labeled antitoxoplasma serum (C). For details, see Materials and Methods.
by three laboratories (A, C, and D). In the fourth laboratory (B), an absorbance value just below the absorbance value of the reference serum sample was found. Three other serum samples were positive only in the antibody capture assay, by one or both laboratories (C and D). These three serum samples contained IgG antibodies to toxoplasma but were negative for IgM antibodies to the 6-kilodalton antigen in the immunoblotting assay. The correlation coefficients between the IgM ELISA results from the four laboratories are shown in Table 1. Highly significant positive correlations were obtained (0.97 ≤ r ≤ 0.99; P < 0.00001; χ² test).

IgM antibodies to *T. gondii* in patient serum samples. To compare the indirect ELISA for IgM antibodies to *T. gondii* with the IgM antibody capture assay, 50 follow-up serum samples taken from 19 patients were assayed under code by the four laboratories. In addition, laboratory D also tested the serum samples for IgM antibodies to toxoplasma by the immunoblotting assay. Some typical examples of positive results are shown in Fig. 2.

The correlation coefficients between the results obtained in the assays in the four laboratories are shown in Table 2.

Highly significant positive correlations were obtained between the results in the indirect ELISA and the antibody capture assay (0.78 ≤ r ≤ 0.98; P < 0.002; χ² test), both with the ELISAs developed in the laboratories and with the commercial ELISA.

In addition, results obtained in the indirect ELISAs and antibody capture ELISAs from the laboratories were compared with the results of the immunoblotting assay. Again, highly significant correlation coefficients were obtained, both between the indirect ELISA and the immunoblotting assay (0.85 ≤ r ≤ 0.95; P < 0.002; χ² test) and between the antibody capture ELISA and the immunoblotting assay (0.88 ≤ r ≤ 0.98; P < 0.002; χ² test). In Fig. 3, the absorbance values obtained in the indirect ELISA from laboratory B are compared with the ratios obtained in the IgM antibody

![Image](http://jcm.asm.org/)

**FIG. 2.** Reaction patterns obtained for IgG (A) and IgM (B) antibodies to *T. gondii* by the immunoblotting assay. Serum samples that reacted with the 6-kilodalton band were considered positive. Lanes: a, consecutive serum samples from patient a; b, consecutive serum samples from patient b; c, serum samples from healthy controls.

**FIG. 3.** Comparison of absorbance values and IgM ratios as obtained by indirect ELISA in laboratory B and antibody capture ELISA in laboratory C, respectively, for 50 patient serum samples.
TABLE 3. Time course of IgG and IgM antibodies to T. gondii in follow-up serum samples from three patients with clinical signs of toxoplasma infection

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (mo) after onset of clinical signs</th>
<th>IgG titer</th>
<th>IgM absorbance</th>
<th>IgM ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>640</td>
<td>1.80</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20,480</td>
<td>0.98</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>40,960</td>
<td>0.80</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>40,960</td>
<td>0.56</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>160</td>
<td>1.49</td>
<td>2.8</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1,280</td>
<td>1.46</td>
<td>3.2</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>5,120</td>
<td>1.24</td>
<td>2.8</td>
</tr>
<tr>
<td>3.5</td>
<td>3.5</td>
<td>10,240</td>
<td>0.81</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10,240</td>
<td>0.50</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2,560</td>
<td>1.92</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10,240</td>
<td>1.80</td>
<td>1.9</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>2,560</td>
<td>1.44</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Determined by indirect ELISA in laboratory B.
* Determined by indirect ELISA in laboratory B. The threshold value for positivity was 0.60.
* Determined by antibody capture ELISA in laboratory C. The threshold ratio for positivity was 1.0 (absorbance value for patient serum divided by absorbance value for weakly positive reference serum).
* NT, Not tested.

capture assay in laboratory C. Similar results were obtained with the results from the other laboratories.

By using Spearman's rank correlation coefficient, a moderate correlation between absorbance value and ratio was obtained ($r_s = 0.68; P < 0.0001$). Two serum samples were positive in the indirect ELISA and negative in the antibody capture assay. One of these samples was also weakly positive in the immunoblotting assay. The other sample was from a patient with a history of toxoplasmosis. The four samples that were positive in the antibody capture assay and negative in the indirect ELISA were all from patients with a less recent toxoplasma infection. Previous samples from these patients, taken sooner after the onset of clinical signs, were positive in both assays. Three of these four serum samples were positive in the immunoblotting assay. The other sample showed a nonspecific reaction pattern.

Time course of IgM antibodies in follow-up serum samples from patients with toxoplasmosis. The persistence of IgM antibodies to T. gondii in follow-up sera from eight patients with a well-known onset of clinical symptoms varied from 3 to 13 months or more. Table 3 shows the results of the longitudinal study for the presence of IgG and IgM antibodies to T. gondii in three patients. These results illustrate the different time courses of IgM antibodies to T. gondii in the patients. In some patients, antitoxoplasma IgM levels decreased rapidly to the threshold value within 3 to 4 months; in others, toxoplasma IgM levels remained high for more than a year after infection.

Comparison of indirect and antibody capture ELISAs for discrimination between early and late stages of toxoplasma infection. Of the 50 serum samples tested, 29 were obtained from the eight patients for whom the onset of clinical symptoms was well-known. Consecutive samples from these patients showed a fourfold or greater rise in IgG antitoxoplasma titer. To compare the ability of the indirect ELISA and the antibody capture ELISA to discriminate between serum samples obtained in an early or a late stage after infection, these 29 samples were divided into two groups: one group contained serum samples obtained within 3 months after onset of clinical symptoms, and the other group contained samples obtained in a late stage.

FIG. 4. Comparison of indirect (a) and antibody capture (b) ELISAs for discriminating between serum samples obtained at early (within 3 months of onset of clinical symptoms) and late stages of toxoplasma infection. IgM absorbance values as determined by indirect ELISA are from laboratory B. IgM ratios as determined by antibody capture ELISA are from laboratory C.
Figure 4a and b show the results obtained by both methods. The antibody capture ELISA discriminated better between serum samples taken in early and late stages of infection than did the indirect ELISA. (P < 0.001 and P = 0.05, respectively; Mann-Whitney U test).

DISCUSSION

In this study, two ELISA methods for detection of IgM antibodies to T. gondii, i.e., the indirect ELISA and the antibody capture ELISA, with two antigen preparations were compared in four laboratories. Results obtained by the four laboratories for 115 serum samples from healthy laboratory personnel showed a highly significant positive correlation. Four samples were considered positive for IgM antibodies by one to three of the laboratories. In the four laboratory, none of the samples from healthy laboratory personnel were considered positive. However, in this laboratory, three of the four positive serum samples had absorbance values just below the absorbance values of the reference serum. Since all four serum samples were positive for IgG antitoxoplasma antibodies and IgM ratios as obtained in the antibody capture assay were low (<2.0), the samples probably represent late-stage samples from individuals who had had a toxoplasma infection in the past. This may also be the reason that three of these sera were negative in the immunoblotting assay. Therefore, the discrepancies between the four laboratories were probably due to a different setting of the threshold value for positivity. Results obtained by the four laboratories for the 50 patient serum samples also showed a highly significant positive correlation. Of the 19 patients, 8 showed a fourfold or greater increase in IgG antitoxoplasma titer and high IgM absorbance values in consecutive serum samples. Clinically, these patients had signs of a recent toxoplasmosis. In all serum samples taken from these patients early after the onset of clinical signs, IgM antibodies were detected by all four laboratories. Discrepancies between the four laboratories occurred only in the samples obtained more than 3 months after the onset of clinical signs.

In 10 of the other 11 patients, the onset of symptoms was less clear. Since no increase in IgG antitoxoplasma titer was found in consecutive serum samples, they probably represent late-stage samples. Thus, the discrepancies between the four laboratories for the serum samples from these 18 patients have all been found in samples from a late stage of toxoplasma infection, probably due to a different setting of the threshold value for positivity.

Another possible explanation for the minor discrepancies between the four laboratories might be the difference in sensitivity between the indirect ELISA and the antibody capture ELISA, due to competition between toxoplasma-specific IgG and IgM antibodies in the indirect ELISA. Although many serum samples contained high concentrations of IgG antibodies to toxoplasma, this fact did not seem to influence the detection of toxoplasma-specific IgM antibodies in the early-stage serum samples. Some of the discrepancies found between the four laboratories with the late-stage serum samples, however, might, in addition to different threshold values for positivity, be due to competition of IgG antitoxoplasma antibodies in the indirect ELISA. Therefore, the sensitivities of the indirect and antibody capture ELISAs for detection of IgM antibodies in the early stage of infection appear to be similar.

Serum from one patient was found weakly positive for IgM antitoxoplasma antibodies exclusively in the indirect ELISA, independently of which laboratory performed the assay. In addition, this sample was weakly positive in the immunoblotting assay. In this serum sample, no IgG antibodies against toxoplasma could be detected. Follow-up serum samples remained positive for IgM and negative for IgG antitoxoplasma antibodies. These results might be due to IgM antibodies to mitochondria, which were shown to be present in this sample. This patient suffered from general malaise for more than a year. No clinical diagnosis could be made.

In addition to being tested by the indirect ELISA and antibody capture ELISA, samples were also tested by the immunoblotting assay for the presence of IgM antibodies to T. gondii. Our results show that recognition of the 6-kilodalton band as described by Sharma et al. (12) was highly correlated with detection of IgM antitoxoplasma antibodies by indirect ELISA or antibody capture ELISA.

The time course of IgM antibodies to T. gondii varied considerably, independently of the test used. In some patients, IgM antibodies to T. gondii persisted for only 3 to 4 months; in other patients, the antibodies persisted for more than a year. This finding has also been reported by other investigators (7, 8, 15). Either system was able to demonstrate the presence of IgM antibodies to toxoplasma for more than a year.

As stated by Siegel and Remington (13), the indirect ELISA measures an absolute concentration of IgM antitoxoplasma antibodies, whereas the antibody capture ELISA measures the immune load. They also reported that the immune load of toxoplasma IgM antibody as measured by the antibody capture assay discriminates better between serum samples obtained at early and late stages after toxoplasma infection than does the absolute concentration of IgM antitoxoplasma antibodies. The results obtained in our study for 29 serum samples from eight clinically well-defined patients are in agreement with those of Siegel and Remington (13).

In conclusion, the results presented in this study show that the indirect ELISA and the antibody capture ELISA had equal sensitivity in detecting IgM antibodies to toxoplasma early after infection, whereas the IgM immunoblotting assay seems useful as a confirmation assay. The antibody capture assay discriminated better between serum samples obtained in the early and late stages of toxoplasma infection.

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