Enzyme-Linked Immunosorbent Assay That Uses Labeled Antigen for Detection of Immunoglobulin M and A Antibodies in Toxoplasmosis: Comparison with Indirect Immunofluorescence and Double-Sandwich Enzyme-Linked Immunosorbent Assay

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A direct enzyme-linked immunosorbent assay (ELISA) is described that uses horseradish peroxidase-labeled antigen for detection of immunoglobulin M (IgM) and IgA antibodies to toxoplasma. In this assay, polystyrene microtiter plates were sensitized with anti-human IgM or IgA antibody to separate IgM or IgA from other classes of antibody. The presence of IgM or IgA antibodies to toxoplasma (Tox-IgM, Tox-IgA) was then detected by sequential addition of soluble horseradish peroxidase-labeled toxoplasma antigen and substrate. As judged by examining sucrose gradient-fractionated sera, the assay was specific for IgM or IgA classes of antibody. In contrast to the indirect immunofluorescence for IgM antibodies to toxoplasma, no inhibition of IgM reactivity by specific IgG antibodies could be detected. Furthermore, rheumatoid factor did not cause false-positive results. Of 80 single sera with high antibody titer to toxoplasma in indirect immunofluorescence and complement fixation, 40 were positive in the direct ELISA for Tox-IgM, 36 were positive in the double-sandwich ELISA, and only 21 were positive in the indirect immunofluorescence for Tox-IgM when whole serum was used. In the indirect immunofluorescence, another 13 sera became positive after sucrose gradient fractionation. The direct ELISA for IgG antibodies to toxoplasma was positive in 43 sera, of which 39 were positive in the direct ELISA for Tox-IgM. High levels of IgM antibodies were found within 3 months after the onset of symptoms, slowly decreasing thereafter. Tox-IgM may persist for more than 1 year after infection.

Detection of immunoglobulin M (IgM) antibodies to toxoplasma (Tox-IgM) by the indirect immunofluorescence (IIF) assay (21) has been shown to yield false-positive results due to rheumatoid factor (13) and false-negative results due to competitive inhibition by specific IgG antibodies (5, 9, 20). These problems may be solved by additional, more or less laborious treatment of sera, such as sucrose gradient centrifugation (33), gel filtration (9, 20), or absorption with insolubilized immunoglobulin (13), protein A (4), or anti-human γFc (12). A more simple procedure to overcome these difficulties is the use of solid-phase immunoassays in which the IgM class of antibodies is separated from IgG antibodies by adsorption to a solid phase (7, 10, 15) sensitized with anti-IgM antibody. Bound anti-IgM is then sequentially allowed to react with patient serum and antigen. Binding of the antigen indicates the presence of specific IgM antibodies in serum. This may be demonstrated by using enzyme-labeled antigen (24, 29), labeled F(ab')₂ fragments of an antiserum against antigen (8, 17), or erythrocytes in tests that use hemagglutinating antigens (15, 27). A number of assays in which one of these principles has been applied have been developed at our laboratory for detection of IgM antibodies to viral antigens including rubella virus (27), mumps virus (26), cytomegalovirus (29), and herpes simplex virus (30). These assays were shown to be specific for the IgM class of antibodies and no interference by rheumatoid factor (IgM-RF) or specific IgG antibody was found. This report describes the application of a direct enzyme-linked immunosorbent assay (ELISA) that uses enzyme-labeled antigen for detection of IgM and IgA antibodies to toxoplasma. The results are compared with those of an IIF assay with whole serum and density gradient-fractionated sera and with those of a double-sandwich ELISA (DS-ELISA) (8, 18).
MATERIALS AND METHODS

Antigens. Antigen was obtained from cultures of human larynx carcinoma cells (HEp-2) infected with a strain of *Toxoplasma gondii* isolated in The Netherlands (strain Deelen) as described previously (31). Parasites were harvested at days 7 and 10 after infection. At each harvest, parasites were collected from the supernatant by centrifugation at 1,000 × g for 10 min, forced through a 27-gauge needle to disrupt complete cells, and washed three times with phosphate-buffered saline (PBS) at pH 7.2. After sonication, the disrupted material was clarified by centrifugation at 5,000 × g for 15 min. Control antigen was prepared by similar sonication of uninfected HEp-2 cells and subsequent removal of debris by centrifugation at 5,000 × g for 15 min. Toxoplasma and HEp-2 antigens were labeled with horseradish peroxidase (HRPO) (RZ 3.0, Sigma Chemical Co., St. Louis, Mo.) by the peroxidase method of Wilson and Nakane (34). The method was modified as reported previously (29).

Briefly, the antigen suspension was dialyzed against 0.05 M sodium carbonate buffer (pH 9.5). Next, 7 mg of HRPO was dissolved in 1 ml of distilled water, followed by the addition of 0.2 ml of a freshly prepared 0.1 M NaIO₄ solution. This solution was stirred for 0.5 h at room temperature. Then, the reaction mixture was passed over a Sephadex G-25 column (PD 10; Pharmacia, Sweden) equilibrated with 1 mM sodium acetate buffer (pH 4.4) to remove the periodate. To this solution of activated HRPO was added 5 mg of antigen in 2.5 to 5.0 ml of sodium carbonate buffer (pH 9.5). After the reaction mixture was stirred for 2 h at room temperature, 0.1 ml of a freshly prepared NaBH₄ solution (4 mg/ml) was added, and the mixture was left for 3 h at 4°C. After extensive dialysis against PBS (pH 7.2), fetal bovine serum was added to a concentration of 2%. The labeled antigens were stored at 4°C.

Recently, further purification of parasites was achieved by treating them with 0.5% trypsin (2 × 10⁶ parasites per ml) for 45 min at 37°C and subsequent centrifugation through an isonicterine PColl (Pharmacia, Sweden) layer with a density of 1.060 g/ml for 20 min at 1,500 × g. The pellet containing the parasites was resuspended in PBS (pH 7.2), sonicated, and clarified by centrifugation for 15 min at 5,000 × g. The yield of purifying parasites by this procedure was approximately 50%, as determined both by counting the parasites and by a sandwich ELISA for toxoplasma antigen. Furthermore, antigenic determinants of HEp-2 cells that were abundantly present in the starting material as determined by a sandwich ELISA were completely removed by this procedure (data not shown).

Sera. Four groups of sera were studied. The sera were submitted to our laboratory for diagnostic purposes. After being examined by IIF and complement fixation (CF), the sera were stored at −20°C. The sera included (i) 80 single sera from patients suspected for toxoplasmosis and with high antibody titers in IIF and CF, (ii) serial serum specimens from 24 patients with recent toxoplasma infection as demonstrated by the presence of specific IgM in IIF, (iii) 78 sera from healthy laboratory personnel, and (iv) 43 single sera from patients with infectious mononucleosis (n = 13) or with rheumatoid factor and high titers of toxoplasma antibody in IIF and CF (n = 15), antinuclear antibody (n = 10), or antibody to double-stranded DNA (n = 5) in their serum.

ELISA procedures. (i) Direct ELISA-IgM. The direct ELISA for toxoplasma IgM antibodies (ELISA-IgM) was performed essentially as described before (29, 30). Briefly, wells of polystyrene microtitration plates (Cooke M 29 AR) were sensitized with 0.12 ml of immunoaffinity-purified goat anti-human IgM (Cappel Laboratories, Cochranville, Pa.) at a 1:4,000 dilution in 0.01 M Tris-hydrochloride buffer (pH 9.0). After overnight incubation at 4°C, the plates were washed four times with 0.01 M PBS at pH 7.2 containing 0.1 M NaCl and 0.05% Tween 20 and shaken dry. Next, 0.1 ml of patient serum at a dilution of 1:100 in PBS-Tween with 1% bovine serum albumin was added to each of two wells. After incubation for 2 h at 37°C, the plates were washed again four times with PBS-Tween. After a 0.1-ml amount of anti-IgM sensitive antigen at a 1:500 dilution in 0.01 M PBS-Tween buffer (pH 7.2) with 2% fetal bovine serum was added to each of two wells and incubated overnight at 4°C. After washing the plates four times with PBS-Tween, 0.1 ml of substrate solution was added to each well. Substrate solution was prepared immediately before use by dissolving 1 mg of orthophenylenediamine per ml in 0.1 M PBS (pH 6.0), followed by the addition of 0.1% of 30% H₂O₂. After 15 min of incubation at room temperature, the reaction was stopped by adding 0.2 ml of 3 N H₂SO₄. The absorbance of the reaction was determined by spectrophotometry (Titertek Multiskan; Flow Laboratories Ltd., Irvine, Scotland) with the buffer control as a blank. A weakly positive serum was used as a reference serum to determine the positive/negative cutoff value in each plate. The serum was also used to determine the positive/negative cutoff value in the direct ELISA-IgA and the double-sandwich ELISA-IgM (DS-ELISA-IgM) described below.

(ii) Direct ELISA-IgA. The direct ELISA for toxoplasma IgA antibodies (Tox-IgA) was carried out as described above for detection of Tox-IgM. Instead of using anti-human IgM, immunoaffinity-purified goat anti-human IgA (a gift from Cappel Laboratories) was used to sensitize microtitration plates. Furthermore, HRPO-labeled antigen was used at a dilution of 1:200.

(iii) DS-ELISA-IgM. The procedure for the DS-ELISA-IgM for detection of IgM antibodies to toxoplasma (8, 18) was similar to the procedure described above. Unlabeled toxoplasma antigen was used instead of labeled antigen. The unlabeled antigen was detected by reaction with HRPO-labeled F(ab’)₂ fragments of rabbit IgG antibodies to toxoplasma. Briefly, after incubation of anti-IgM sensitive antigen on plates with patient serum at a dilution of 1:100, plates were washed four times with PBS-Tween. Next, 0.1 ml of a solution of 0.01 M PBS with 2% fetal bovine serum and containing simultaneously toxoplasma antigen (10 μg/ml) and HRPO-labeled F(ab’)₂ fragments of rabbit IgG antibody to toxoplasma at a dilution of 1:50 were added to each well (F. Wielaard, personal communication). The plates were incubated overnight at 4°C. Thereafter, the test was performed exactly as described above.

(iv) Indirect ELISA for IgG antibody. The indirect ELISA was essentially that previously described for detection of IgG antibodies to toxoplasma (31). Sera were examined in four fourfold dilutions in PBS-Tween with 1% bovine serum albumin at a starting
dilution of 1 in 200. HRPO-labeled anti-human IgG (Institut Pasteur, Paris, France) was used at a dilution of 1:1,000 in 0.01 M PBS-Tween with 2% FBS. A positive serum pool containing 90 IU/ml served as a reference serum to calculate antibody concentrations from the titration curve of each serum in a manner analogous to the method described by Leinikki et al. (16). A reference absorbance value was defined as the absorbance that was obtained with the 1:800 dilution of the reference serum. Then, the dilution ratio between patient serum and reference serum was determined at the reference absorbance, and antibody concentrations were determined by multiplying this ratio by the antibody concentration of the reference serum. A 1:800 dilution of the reference serum was chosen because this dilution gave absorbance values that were within the linear part of the titration curve of most sera. This permitted an accurate estimation of antibody levels of both positive and negative sera.

**IF tests for toxoplasma antibodies.** The procedure for detection of total antibody to toxoplasma by IIF was that described previously (32). Cryostat sections of brain tissue from mice infected intracerebrally 3 days previously with the Deelen strain of toxoplasma were allowed to react with a patient’s serum, washed, and incubated with a polyvalent fluorescein-conjugated anti-human antiserum (Progressive Laboratories, Inc., Baltimore, Md.) at a dilution of 1:40.

In tests for IgM antibodies to toxoplasma (IF-IgM), a goat anti-human IgM antiserum conjugated with fluorescein isothiocyanate (Hyland Laboratories, Inc., Costa Mesa, Calif.) was used at a 1:80 dilution in 0.01 M PBS (pH 7.2). Sera with antibody titers exceeding 1:16 were considered to be positive.

For the IIF assay of fractionated sera, sucrose gradient centrifugation was performed as described before (29). IgM fractions were examined according to the procedure described above. Fractions with IgM antibody titers equal to or exceeding 1:4 were considered to be positive.

**Other serological tests.** The CF procedure was carried out according to the microtechnique of Casey (3), with toxoplasma antigen harvested from the peritoneal cavity of mice infected 3 days before with the Deelen strain of toxoplasma (31). IgM-RF was determined by a direct ELISA with HRPO-labeled human IgG, as described before (29). IgM antibody to the capsid antigen of Epstein-Barr virus (EBV-IgM) was detected by an indirect immunofluorescence technique, as described before (29). Antinuclear antibody (ANA) and antibody to double-stranded DNA (anti-dsDNA) were determined by an IIF assay (M.A. Bioproducts, Walkersville, Md.), using human epithelial cells as substrate for ANA and *Crithidia luciliae* cells as substrate for anti-dsDNA.

**Rabbit hyperimmune serum.** Conventionally raised rabbits (New Zealand White) were infected with 10^5 trophozoites of the Deelen strain of *Toxoplasma gondii* by intraperitoneal inoculation. After 2 months, they were given a booster injection of 10^5 trophozoites. Two weeks later they were bled. The antibody titer in the IIF test was 1:64,000. The IgG fraction was prepared by passing the serum through a Sepharose 4B-protein A column (Pharmacia, Upssala, Sweden) and by eluting with 0.1 M glycine hydrochloride (pH 2.8), F(ab')_2 fragments of rabbit IgG antibodies to toxoplasma were prepared by pepsin digestion and conjugated with HRPO by the two-step glutaraldehyde method (2).

**RESULTS**

**Specificity for IgM class of antibodies.** To determine the specificity of the direct ELISA-IgM for antibodies of the IgM class, sera were fractionated on sucrose gradients. The fractions were examined by the direct ELISA-IgM and by the indirect ELISA for IgG antibodies. The results of examining fractions of a positive serum are shown in Fig. 1. The direct ELISA for Tox-IgM was positive only in the IgM fractions, whereas the indirect ELISA for toxoplasma IgG detected positive reactions only in the IgG fractions.

**Dose-response relationship of the direct ELISA-IgM.** A dose-response test was performed by examining serial fourfold dilutions of the weakly positive reference serum, four positive and three negative sera (Fig. 2). Absorbances decreased with increasing serum dilutions. The leveling of the curves at high serum concentrations was probably due to saturation of the IgM binding sites on the solid phase. The test appeared to be quite sensitive, since it still detected IgM antibodies at serum dilutions of 1:10,000 or more.

**Effect of RF and specificity for toxoplasma antigen.** To study the effect of RF and the specificity of the direct ELISA-IgM for toxoplasma antigen, 78 sera from healthy laboratory personnel, 15 sera with IgM-RF and high titers of IgG antibody to toxoplasma, 13 sera from patients with infectious mononucleosis as determined by detection of IgM antibodies to Epstein-Barr virus (EBV), and 15 sera with ANA...
or anti-dsDNA were examined. The results were compared with those of 80 sera from patients suspected for toxoplasmosis and with high antibody titers in IIF and CF. The log₂ geometric mean titer of the latter sera in IIF was 12.0 ± 2.3 (Fig. 3). All sera with IgM-RF, IgM antibody to EBV, ANA, or anti-dsDNA were negative in the direct ELISA for Tox-IgM. Of the 78 sera from the group of healthy laboratory personnel, 3 were found to be positive. These three sera had high antibody titers in IIF-immunoglobulin and CF. Two of the latter three sera were also positive in IIF-IgM. All three sera were negative in the direct ELISA for IgM antibodies with control antigen.

Comparison with IIF and DS-ELISA. Further information on specificity and sensitivity of the direct ELISA for Tox-IgM was obtained when the results of the above-mentioned 80 sera in direct ELISA for Tox-IgM were compared with those obtained with whole sera, with density gradient-fractionated sera in IF-IgM, and with those in DS-ELISA-IgM (Table 1). In addition, all 80 sera were tested for IgA antibodies to toxoplasma, IgM-RF, and IgM and IgA antibodies to control antigen (uninfected HEp-2 cells). Fourteen sera were positive in IIF-IgM only after density gradient fractionation. This was probably due to competitive inhibition by specific IgG antibodies. Thirteen sera became negative in IIF-IgM after fractionation. Eleven of these contained IgM-RF and probably represented false-positive reactions due to IgM-RF.

A total of 40 sera were positive in the direct ELISA for Tox-IgM, and 36 of these also reacted positively in DS-ELISA-IgM. Seven sera were negative in IIF-IgM, using either whole or fractionated serum, and positive in the direct ELISA for Tox-IgM. These sera were positive for Tox-IgA and negative for IgM or IgA antibodies to control antigen. Four of these sera were positive by DS-ELISA-IgM. Of the remaining three sera, one was obtained from a patient whose serum was found to be positive for Tox-IgM by IIF-IgM and by direct ELISA 2 months previously. The other two sera had high antibody titers to toxoplasma by CF (>1:128 and 1:64) and IIF (1:32,000 and 1:4,096).

A positive reaction in the direct ELISA for Tox-IgA was found in 43 of the 80 sera. Thirty-nine of these 43 sera were positive for Tox-IgM by the direct ELISA.

A positive reaction for HEp-2-IgM was found in 4 of these 80 sera. The two strongest reacting sera, which also contained HEp-2-IgA, were negative in the ELISAs for Tox-IgM; the other

FIG. 2. Titration of the weakly positive control serum (O---O), four positive (O—O), and three negative sera (●) in the direct ELISA for Tox-IgM antibodies.

FIG. 3. Reactivity in direct ELISA for Tox-IgM of sera from patients with suspected toxoplasmosis (n = 80), infectious mononucleosis (n = 13), IgM-RF (n = 15), ANA (n = 10) or anti-dsDNA (n = 5), and in sera (n = 78) from healthy laboratory personnel.
TABLE 1. Comparative results of IIF-IgM, using whole and fractionated sera and two ELISAs for detection of IgM antibodies to toxoplasma in 80 sera from patients with high antibody titers in CF and IIF.

<table>
<thead>
<tr>
<th>IIF-IgM sera</th>
<th>Result of direct ELISA-IgM (no. of sera)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole serum +; fraction +</td>
<td>18 (18) 1c</td>
</tr>
<tr>
<td>Whole serum -; fraction +</td>
<td>13 (12) 1d</td>
</tr>
<tr>
<td>Whole serum +; fraction -</td>
<td>2 (2)b 11*</td>
</tr>
<tr>
<td>Whole serum -; fraction -</td>
<td>7 (4) 27</td>
</tr>
</tbody>
</table>

a Numbers in parentheses indicate number of sera positive by DS-ELISA-IgM.
b Both sera had low titers in IIF-IgM with whole serum (1:64 and 1:32).
c Serum with strong positive reaction in direct ELISA for IgM and IgA antibodies to control antigen.
d Serum with borderline absorbance; positive on repeat testing.
e All sera positive for IgM-RF.

Temporal course of IgM antibody in successive sera from patients with toxoplasmosis. To study the temporal course of Tox-IgM in direct ELISA, we examined follow-up sera from 24 patients with acute or recent toxoplasmosis as determined by the presence of IgM antibodies in IIF-IgM. In 21 patients, the first serum sample was still available. Tox-IgM was detected by direct ELISA-IgM in all patients (Fig. 4). High levels of Tox-IgM were found in sera collected in months 1 and 2 after collection of the first serum. Thereafter, Tox-IgM antibody levels slowly decreased and occasionally remained detectable for more than 1 year after infection. Comparison with IIF-IgM (data not shown) indicated that generally, Tox-IgM remained detectable longer by IIF-IgM than by direct ELISA. These sera were also examined by CF, IIF, and the indirect ELISA for Tox-IgG. In most cases, antibody titers in CF and IIF were already very high in the initial sera from these patients. Both CF and IIF detected only 3 patients with a significant rise of antibody titer, whereas significant antibody titer rises were found in 18 of 24 patients by the indirect ELISA for Tox-IgG. Representative results for six patients are shown in Table 2.

DISCUSSION

This study clearly underlines the two main problems of the IIF assay for Tox-IgM. The first is the occurrence of false-positive results due to the simultaneous presence of IgM-RF and specific IgG antibody (13). In our series of 80 sera with high antibody titers to toxoplasma, we found that 11 sera contained IgM-RF and were positive in IIF-IgM of whole serum, but became negative after separation of IgG and IgM antibodies. These 80 sera, however, were not taken completely at random with respect to the pres-
TABLE 2. Temporal course of antibodies to toxoplasma as measured by CF, IIF, and ELISA in six patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Mo after first serum</th>
<th>Reciprocal antibody titer</th>
<th>ELISA</th>
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<tr>
<td></td>
<td></td>
<td>CF</td>
<td>IIF- IgM</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>64</td>
<td>4,096</td>
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<tr>
<td></td>
<td>1</td>
<td>128</td>
<td>8,000</td>
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<tr>
<td></td>
<td>5</td>
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<td>16,000</td>
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<tr>
<td></td>
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<td></td>
<td>9</td>
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<td>8,000</td>
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<td>2</td>
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<td>128</td>
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<td>4.5</td>
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<td>4,096</td>
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<sup>a</sup> Antibody concentration in international units per milliliter.

<sup>b</sup> Expressed as absorbance at 492 nm. The positive/negative cutoff value was 0.33.

ence of IgM-RF; therefore, 11 of 80 sera is not a representative figure.

The second problem involves the competition between IgM and IgG antibodies, causing false-negative reactions (5). Particularly in toxoplasmosis, different authors have described the importance of this effect (9, 20). We found that of 48 sera negative for Tox-IgM by the IIF test on whole serum, 14 became positive after separation of IgG and IgM antibodies by sucrose gradient centrifugation.

Different solutions to these problems have been suggested (4, 9, 12, 13, 33). Only recently, elegant, efficient procedures that use an anti-IgM coated solid phase to separate IgG and IgM antibodies have been developed to solve these problems in the serological diagnosis of toxoplasmosis (8, 11, 18). We have applied this principle for detection of Tox-IgM by a direct ELISA that uses peroxidase-labeled antigen. Specificity for the IgM class of antibody was demonstrated by examining sucrose gradient fractionated sera. Only IgM fractions were positive. In addition, the direct ELISA for Tox-IgM appeared to be specific for toxoplasma antigen. Sera with IgM-RF, EBV-IgM, ANA, or anti-dsDNA were negative in all instances. It is true that 3 of 78 sera from our group of healthy laboratory personnel were positive in the direct ELISA for Tox-IgM, but subsequent examination suggested that these results represented specific reactions. This is not very surprising in view of the annual infection rate of 3% (28) in this population of mainly young adults and the relatively long persistence of Tox-IgM. Similarly, in a series of 100 healthy blood donors, Pyndiah et al. (20) found that 15 were seropositive for toxoplasma antibodies, of which 1 was also positive for Tox-IgM. In our series of 78 healthy persons who served as a control group, 32 were seropositive for toxoplasma antibodies in the indirect ELISA for IgG antibodies.
Apart from being more specific than IIF-IgM due to the absence of the above-mentioned false-positive and false-negative reactions, the direct ELISA appeared to be more sensitive. In addition to the 13 sera that became positive in IIF-IgM only after sucrose gradient centrifugation, we found that 7 sera were positive in the direct ELISA and negative in IIF-IgM. These results may be regarded as specific, since none of these sera contained IgM antibodies to control antigen. In addition, four of these sera were positive by DS-ELISA-IgM which used the F(ab')₂ fragment of specific IgG antibodies to toxoplasma, and another serum was obtained from a patient who was found to be positive for Tox-IgM by all IgM tests 2 months previously. It is of interest to note that 39 of 40 sera positive in the direct ELISA for Tox-IgM were also positive in the direct ELISA for Tox-IgA, including the 7 sera which were negative in IIF-IgM. In view of recent investigations indicating the significance of IgA antibodies in chronic, viral diseases (6, 23), further studies on production and persistence of IgA antibodies in toxoplasmosis are being undertaken at our laboratory.

Our direct ELISA for Tox-IgM was found to be slightly more sensitive than a DS-ELISA-IgM. The latter detected 36 Tox-IgM-positive sera, which were all positive by direct ELISA. A further four sera, positive by direct ELISA, were negative by DS-ELISA-IgM, whereas no sera which were negative by direct ELISA were positive by DS-ELISA-IgM. Three of these four sera were weakly positive in direct ELISA (absorbance <0.440), and the absorbance in DS-ELISA-IgM was near the cutoff value. Therefore, further optimization of our DS-ELISA-IgM might reduce this apparent difference in sensitivity. Another reason for this difference might be that the human IgM bound to the solid phase, and the rabbit IgG antibodies which are used to detect bound antigen react with different antigen epitopes. Furthermore, the direct ELISA for Tox-IgM enabled us to use less antigen and did not give the relatively high background levels which we observed in the DS-ELISA-IgM. Moreover, DS-ELISA-IgM requires additional treatment of a hyperimmune sera to obtain F(ab')₂ fragments to avoid interference by IgM-RF and ANA (17). On the other hand, the direct ELISA requires purified labeled antigens, otherwise false-positive reactions due to ANA (1) may occur. A purified, soluble toxoplasma antigen preparation was easily obtained in a reproducible fashion, particularly by applying the recent extension of the procedure.

In our series from 80 patients with high antibody titers to toxoplasma, four sera were strongly positive for IgM antibodies to control antigen (uninfected HEp-2 cells). Two of these sera were negative in the direct ELISA, whereas both other sera were positive in all tests for Tox-IgM, including DS-ELISA-IgM. Since HEp-2 cells may be considered to be a suitable substrate for detecting ANA (14), this further indicates the absence in our direct ELISA of false-positive reactions due to ANA.

In accordance with other investigators (20, 22), we found that the persistence of Tox-IgM varied considerably. Twenty-one of the 24 patients with Tox-IgM in their initial sera became negative within 1 year after the first serum. On the other hand, Tox-IgM persisted for longer periods in three patients, though at low levels. The findings in this study and a previous study (25)—that prolonged IgM responses may occur occasionally—emphasizes the importance of comparing the results of ELISA-IgM tests with those of IIF, CF, or other laboratory tests to determine the approximate onset of the disease and of interpreting the laboratory results in light of clinical details.

In contrast to CF and IIF, the indirect ELISA for Tox-IgG detected a significant rise in antibody level in 75% of our longitudinally studied patients. However, the production of Tox-IgG as measured by indirect ELISA was relatively slow and only reached peak values several months after infection (19). This somewhat reduces its value as a diagnostic test that could be used for detection of significant antibody titer rises in acute toxoplasmosis. Nevertheless, in conjunction with a sensitive test for toxoplasma IgM, it could be a useful test for a more accurate determination of the approximate onset of toxoplasmosis.

ACKNOWLEDGMENTS

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LITERATURE CITED

6. Cradock-Watson, J. E., M. K. S. Ridehalgh, and M. S.


