JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1986, p. 1045–1049
0095-1137/86/121045-05$02.00/0

Vol. 24, No. 6

Demonstration of Immunoglobulin M Class Antibodies to *Toxoplasma gondii* Antigenic Component p35000 by Enzyme-Linked Antigen Immunosorbent Assay

E.-G. LINDENSCHMIDT

Institute of Medical Microbiology and Immunology, University of Hamburg, 2000 Hamburg 20, Federal Republic of Germany

Received 21 January 1986/Accepted 10 September 1986

On the basis that 89% of 48 acute-phase toxoplasmosis patients showed immunoglobulin M (IgM) class antibodies to the 35,000-molecular-weight antigenic component (p35000) of *Toxoplasma gondii*, as demonstrated by IgM immunoblotting, the antigen was purified by sucrose gradient centrifugation and enzyme labeled for use in an enzyme-linked antigen immunosorbent assay (ELA) for the demonstration of IgM class antibodies to the p35000 component. The ELA showed a specificity of 96% with 139 serum specimens at a serum dilution of only 1:5. The test serologically detected 73 symptomatic acute-phase toxoplasmosis patients; 64 were positive in the 19S IgM indirect immunofluorescent-antibody test, and 9 were negative, although they showed IgM antibodies to p35000, as demonstrated by IgM immunoblotting. Also, the ELA turned out to be independent of IgM rheumatoid factors in six acute-phase toxoplasmosis serum specimens.

The demonstration of immunoglobulin M (IgM) class antibodies to *Toxoplasma gondii* is of clinical value in the assessment of symptomatic or asymptomatic acute toxoplasmosis (9). Standard techniques established for this purpose are the 19S IgM indirect immunofluorescent-antibody test (IIF) and the double-sandwich IgM enzyme-linked immunosorbent assay (ELISA) (11). The specificities of both tests appear to be comparable (7), and the sensitivity of the ELISA is greater than that of the 19S IgM IIF (19). However, these tests are laborious to establish and prone to disturbances due to IgM class rheumatoid factors (IgM RFs) and antinuclear factor (3, 10). In previous studies on the demonstration of specific IgM class antibodies, it was proposed that IgM RFs do not interfere with an ELISA to detect specific IgM if an enzyme-linked antigen immunoassay (ELA) is used (16, 25). The studies presented here were designed to demonstrate the advantages of using an ELA to detect IgM class antibodies to the 35-kilodalton (kDa) major antigenic polypeptide component of *T. gondii* (IgM-p35000-ELA).

MATERIALS AND METHODS

Sera. Serum specimens were investigated from 130 patients showing lymphadenopathy and IgG antibody titers ≥1:4,000 to *T. gondii* by IIF, which raised clinical suspicion of symptomatic acute toxoplasmosis. Also, sera of 139 healthy blood donors were used that showed various titers (≤1:1,000) of these specific IgG antibodies and no IgM class antibodies to the protozoan.

IIF. The IIF was performed to demonstrate IgG or IgM antibodies to *T. gondii*. Specific IgG antibodies were evaluated quantitatively, starting with a serum dilution of 1:4. Commercial lyophilized *T. gondii* cells (bioMérieux lot 0311) were used as antigen. Fluorescein isothiocyanate-labeled anti-human IgG reagent (Dako, Copenhagen, Denmark) from rabbits was used at a dilution of 1:80 in phosphate-buffered saline (PBS) (pH 7.4). IgM class antibodies to the protozoan were detected in the 19S IgM serum fractions obtained after gel chromatography using Ultrogel AcA34 and PBS for the elution. Columns were loaded with 700 μl of serum, and four or five 19S IgM fractions of 1.3 ml each (total <7 ml) were collected, which indicated a less than 1:10 dilution of serum during gel chromatography. Specific IgM class antibodies complexed with membrane antigens of the commercial *T. gondii* cells were demonstrated with fluorescein isothiocyanate-coupled anti-human IgM reagent (Dako) diluted 1:40 in PBS.

Demonstration of IgM RFs directed to human IgG antibodies. Demonstration of IgM RFs directed to human IgG antibodies was performed by a slightly modified ELISA, as described previously (7). For this test, IgG antibodies to *T. gondii* were purified from serum specimens of acute-phase toxoplasmosis patients (positive for 19S IgM and also for IgG ≥1:256,000 by IIF) by using ion-exchange chromatography over DEAE-Sephacel. Fractions of purified IgG to *T. gondii* showed titers of 1:1,000 to 1:2,000, and no IgM class antibodies were detectable by immunodiffusion or by 19S IgM IIF. Dynatech microtiter plates were coated with (per cup) 5 μg of purified IgG antibodies to *T. gondii* diluted in isotonic bicarbonate buffer (pH 9.6; coating buffer) during an 18-h incubation at 4°C, and the antibodies were then denatured for 20 min at 74°C (18) in a moist chamber. Test sera were diluted 1:5, 1:50, 1:500, and 1:5,000 in PBS and incubated for 2 h at 37°C in a moist chamber to have antibodies captured at the solid phase which were directed to human IgG. After three washes with 0.5% Tween 20 in PBS (washing solution), 50 μl of anti-human IgM reagent (Dako) coupled with horseradish peroxidase (type VI; Sigma Chemical Co., St. Louis, Mo.) and diluted 1:500 in 0.5% Tween 20 in PBS was added to each cup. This immunoreaction at the solid phase was allowed to occur for 18 h at room temperature and was conceived to detect IgM class antibodies complexed with IgG to *T. gondii* via the Inv or Gm allotypes (1). After three washing steps, the enzyme reaction was performed for 5 min at room temperature in the dark using 100 μl of *o*-phenylenediamine (10 mg/10 ml of 0.1 M Na_2PO_4–0.1 M K_2HPO_4) per cup and was stopped with 100 μl of 4 M H_2SO_4. Extinctions at 492 nm were read automatically in a Multiscan (Titertek). Negative control sera were negative for RFs in the hemagglutination test of

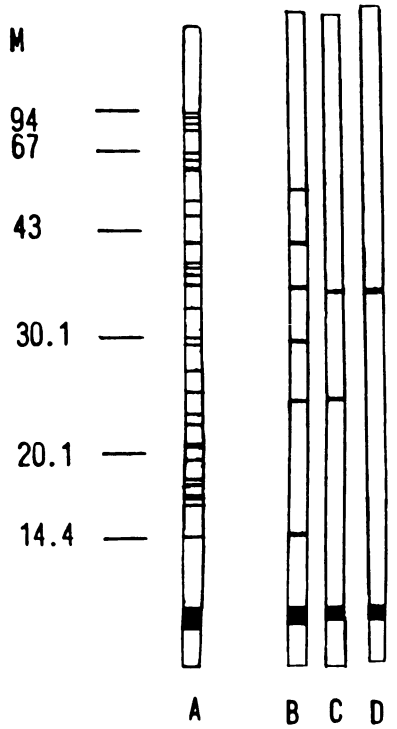

FIG. 1. Demonstration of antigenic components of *T. gondii* by IgG immunoblotting (lane a) and by IgM immunoblotting for three acute-phase toxoplasmosis patients (lanes B through D). The positions of molecular size markers (M) in kilodaltons are shown on the left.

Waaler (24) and devoid of IgG and IgM antibodies to *T. gondii*. Extinctions exceeding the double mean value of at least eight negative controls indicated a positive ELISA for IgM RF antibodies to IgG antibodies directed to *T. gondii*.

Preparation and demonstration of *T. gondii* antigenic components. Preparation and demonstration of *T. gondii* antigenic components was done initially using tachyzoites of the RH strain cultivated in a human epithelial (HEp) cell line (23) in Eagle minimal essential medium supplemented with fetal calf serum (2.5%), gentamicin (80 mg/liter), and nystatin (4 mg diluted in 1 ml of dimethylformamide per liter). Cytopathic effect was observed 2 to 3 days after inoculation, and *T. gondii* cells were harvested mainly from days 4 to 12. They were separated from HEp cells by passage through a filter (Selecta 47108; pore size, 3 μm) and purified from the medium by five washes in PBS, each followed by a centrifugation at 3,000 × *g* for 10 min at 4°C in a Christ centrifuge. The cells were lysed first by freezing (−80°C) and thawing (4°C) three times, then by an overnight incubation in 2 mM Tris (pH 7.4) diluted in bidistilled water, and finally by six ultrasonication steps for 45 s each in an ice bath. Residual particles were sedimented (10 min at 5,000 × *g*). The supernatant contained soluble antigens of *T. gondii* at a protein concentration of 8 to 12 mg/ml. Protein estimations were done by the method of Lowry et al. (8). Soluble antigens were dialyzed against Laemmli buffer (5), and 100-μl aliquots were stored at −80°C.

Dissolved antigenic components were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by the method of Laemmli (5) with an 11% slab gel. Electrophoresis was performed for 3 h at 25 mA, staining was done with 0.001% Coomassie blue in 50% methanol–10% acetic acid for 18 h, and destaining was done in 5% methanol–7% acetic acid for 24 h.

IgG and IgM class antibodies to different antigenic components of *T. gondii* were demonstrated by immunoblotting, performed as described previously (13, 17). The antigens separated by SDS-polyacrylamide gel electrophoresis were transferred electrophoretically (22) onto nitrocellulose paper at 200 mA for 14 h at 4°C in 192 mM glycine–20% (vol/vol) methanol–25 mM Tris hydrochloride (pH 8.3). Blocking was done first in 0.5% Tween 20 in PBS for 15 min and later in 10% skim milk powder (Oxoid Ltd., London, England) in 0.5% Tween 20 in PBS for 30 min. Nitrocellulose strips (2 to 3 mm) were exposed to human test sera diluted 1:50 in 0.5% Tween 20 in PBS for 18 h and, after an additional blocking procedure for 4 h, to anti-human IgG (Fig. 1, lane A) or anti-human IgM (Fig. 1, lanes B through D) reagent labeled with alkaline phosphatase (Dako; 1:1,000 in 0.5% Tween 20 in PBS). The enzyme reaction was performed with Nitro Blue Tetrazolium (0.1 mg/ml) and 0.01% indolylphosphate dissolved in 0.1 M veronate in acetic acid (pH 9.6) for 3 to 5 min in the dark.

Preparation of the p35000 antigenic component of *T. gondii*. Protein (10 mg) from a *T. gondii* whole-cell lysate was adjusted to 192 mM glycine–2% mercaptoethanol–1% SDS–25 mM Tris hydrochloride (pH 8.3), boiled for 3 min, loaded on a sucrose gradient (15 to 60% in the same reducing medium), and centrifuged in an SW40 rotor in a Beckman L-50 centrifuge for 72 h at 38,000 rpm at 10°C. Later, 15 or 16 gradient fractions of about 850 μl each were collected (Fig. 2).

The fractions were investigated for antigenic components of *T. gondii* by an ELISA as follows. Samples of the fractions (50 μl) were diluted in coating buffer and allowed to adsorb to the solid phase of Dynatech microtiter plates for 18 h at 4°C. After three washes, the cups were incubated with 100 μl of a 19S IgM fraction positive by IIF at a 1:40 dilution. Specific IgM complexed with antigenic components of the protozoan at the solid phase was detected with horseradish peroxidase-labeled anti-human IgM reagent, as described for the ELISA established to demonstrate IgM RFs. The fractions showing a positive ELISA for antigenic components of *T. gondii* were investigated for the p35000 polypeptide by SDS-polyacrylamide gel electrophoresis and IgM immuno-

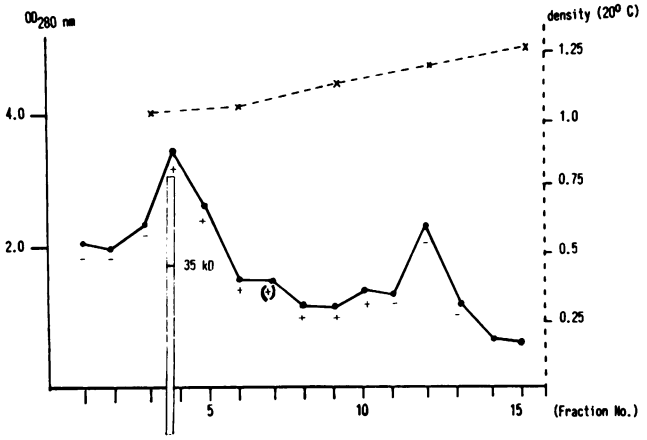

FIG. 2. Detection of p35000 antigenic component of *T. gondii* after sucrose gradient centrifugation. Results of ELISA for the demonstration of *T. gondii* antigen are shown as positive (+) or negative (−). For the molecular size marker, see Fig. 1.

TABLE 1. Occurrence of IgM class antibodies to different antigenic components of *T. gondii* in 48 acute-phase toxoplasmosis patients

Mol wt of antigenic component	No. (%) of patients with IgM class antibodies to antigenic component
50,000	33 (69)
40,000	39 (81)
35,000	43 (89)
30,000	15 (39)
25,000	12 (25)
14,000	3 (6)
6,000	47 (97)

blotting (Fig. 2). Two nonprotein bands due to 2-mercaptoethanol at the 54- and 68-kDa positions were visible on the gel, as described by others (21).

Antigen labeling of the p35000 antigenic component. The fractions containing the antigenic component p35000 obtained after sucrose gradient centrifugation were dialyzed against 192 mM glycine–2% 2-mercaptoethanol–25 mM Tris hydrochloride (pH 8.4) to remove SDS. The protein was labeled with horseradish peroxidase (type VI; Sigma) by the periodate method of Wilson and Nakane (26). Labeled antigen was dissolved in Laemmli buffer without SDS but with 5% fetal calf serum and 0.001% Merthiolate and stored as 50-μl aliquots at −80°C.

ELISA for demonstration of IgM class antibodies to p35000. Dynatech microtiter plates were coated with anti-human IgM reagent (Dako) diluted 1:1,000 for 18 h at 4°C and washed three times afterward. Test serum specimens were diluted 1:5, 1:25, 1:125, 1:625, etc., in PBS and incubated for 2 h at 37°C in a moist chamber. Enzyme-labeled p35000 antigen diluted 1:1,000 in 0.5% Tween 20 in PBS (50 μl per cup) was allowed to react for 2 h at 37°C in a moist chamber. After three washing steps, the enzyme reaction was performed and interpreted as described for the IgM RF ELISA. Negative controls did not show any specific IgG or IgM class antibodies, as demonstrated by IIF.

RESULTS

IgM class antibodies to different antigenic components of *T. gondii* during acute toxoplasmosis. The IgM immunoblots for 3 of 48 investigated acute-phase toxoplasmosis patients are shown in Fig. 1, lanes B through D. The first shows IgM class antibodies complexed with the 6-, 14-, 25-, 30-, 35-, 40-, and 50-kDa polypeptides of *T. gondii*; the second shows IgM class antibodies to only the 6-, 25-, and 35-kDa components; the third shows IgM class antibodies to the 6-kDa component and 35-kDa polypeptide.

The frequency of detection of the different IgM class antibodies directed to various *T. gondii* antigens in 48 acute-phase toxoplasmosis patients is presented in Table 1. IgM class antibodies to the 6-kDa polypeptide were seen in 97% of the patients, and IgM class antibodies to the 35-kDa component were seen in 89% of the patients.

ELISA for demonstration of IgM-RFs directed to human IgG. Of 73 acute-phase toxoplasmosis patients positive for 19S IgM to *T. gondii* by IIF, 20 (27%) showed IgM class antibodies complexed with IgG of *T. gondii* (IgM RFs), as detected by the ELISA. These antibodies were demonstrated at a titer of 1:5 in six patients (30%), 1:50 in nine patients (45%), 1:500 in three patients (15%), and ≥1:5,000 in 2 patients (10%).

Specificity and sensitivity of IgM-p35000-ELA. The specificity of the IgM-p35000-ELA, investigated with sera of 139 healthy blood donors negative for 19S IgM to *T. gondii* by IIF, is shown in Table 2. Using a dilution of 1:5, a specificity of 133/139 (96%) was calculated. However, all 139 serum specimens had a negative ELA at a dilution of 1:25, indicating a specificity of >99% at this test dilution. The data in Table 2 also show the IgM-p35000-ELA to be independent of the IgG titers to *T. gondii* in sera.

TABLE 2. Specificity of IgM-p35000-ELA determined with sera of 139 healthy blood donors negative for 19S IgM to *T. gondii*

IgG IIF titer	No. of sera tested	No. of sera with indicated IgM-p35000-ELA result at serum dilution:	
		1:5 (positive)	≥1:25 (negative)
1:16	14	1	14
1:64	11		11
1:256	20	2	20
1:1,000	35	1	35
1:4,000	20	1	20
1:16,000	19	1	19
1:64,000	16		16
1:256,000	4		4

The accordance of the 19S IgM IIF and the IgM-p35000-ELA for sera of 130 patients with lymphadenopathy and specific IgG titers of ≥1:4,000, indicating clinical suspicion of acute toxoplasmosis, is presented in Table 3. Of 72 serum specimens positive for 19S IgM to *T. gondii* by IIF, 64 (89%) were also reactive in the IgM-p35000-ELA. Of the eight differing specimens, six were demonstrated by ELISA to be positive for IgM RFs. After adsorption of RFs to latex particles coated with human IgG (Behring, Marburg, Federal Republic of Germany), as described previously (6), the six serum specimens were actually negative for 19S IgM by IIF. The other two serum specimens were negative for IgM class antibodies to the p35000 antigenic component by IgM immunoblot. Of 58 serum specimens with a negative 19S IgM IIF, however, 9 showed IgM class antibodies to p35000 both in the IgM-p35000-ELA and by IgM immunoblot.

DISCUSSION

The demonstration of IgM class antibodies to *T. gondii* is urgently needed in any microbiologic laboratory. 19S IgM IIF requires separation of IgG and IgM antibodies and also prevention of IgM RF interference. The double-sandwich ELISA for the detection of IgM to *T. gondii* needs soluble antigens of the protozoan, purified and enzyme-labeled IgG to *T. gondii*, and control for IgM RF as well as antinuclear factor (11). IgM-p35000-ELA was easier to establish and provided us with several advantages over the double-sandwich IgM ELISA. Advantages of the ELA were also reported recently by Neurath et al. (12) in detecting antibodies to the p24 component of human T cell lymphotropic virus type III by using enzyme-labeled core protein (p24) which they precipitated in polyethylene glycol 6000 after an extraction of the p24 antigen from the virus.

Earlier investigations revealed that the p35000 antigenic component of the *T. gondii* membrane (2, 4, 15, 17) is the

TABLE 3. Accordance of 19S IgM IIF and IgM-p35000-ELA

Result of 19S IgM IIF	No. of sera at indicated dilution with IgM-p35000-ELA result:	
	Positive (≥1:25)	Negative (1:5)
Positive	64	8
Negative	9	49

most immunogenic constituent of tachyzoites, representing 3 to 5% of the total protein of the protozoan (13). Our investigations on the demonstration of different IgM class antibodies to different antigens of *T. gondii* in 48 acute-phase toxoplasmosis patients by using IgM immunoblotting showed that those directed to the p35000 component were detectable in almost 90% of the patients. This was lower only than the frequency of detection of antibodies directed to the 6-kDa antigenic component (97%). The 6-kDa antigenic component did not appear suitable for ELA use since it is assumed to be a minor protein component of the protozoan, consisting mainly of carbohydrates (17), and since its purification from the 14-kDa component seems to be difficult. We succeeded in isolating the p35000 antigenic component by isopycnic sucrose gradient centrifugation, keeping the epitope stable in the reducing buffer of Laemmli et al. (5).

The ELA established for the demonstration of IgM class antibodies to the p35000 component provided us with several advantages over the standard techniques to detect IgM class antibodies to *T. gondii*. First, this test appears to allow specific detection of the IgM class antibodies at an initial serum dilution of only 1:5 (Table 2). This is different from the double-sandwich IgM ELISA, which should not be used at a serum dilution lower than 1:40 (6). Second, the IgM-p35000-ELA appears to be independent of specific IgG antibodies. A specificity of >95% for this test at a dilution of 1:5 at both low and high titers of IgG to *T. gondii* is shown in Table 2. Also, competition of high-titered IgG antibodies with specific IgM class antibodies, resulting in reduced IgM titers or false-negative IgM tests (20, 25), is not to be expected if the monospecificity of the anti-human IgM reagent at the dilution applied is guaranteed. Monospecificity was confirmed for our studies by a negative IIF for the 7S IgG fractions obtained after gel chromatography of an acute-phase toxoplasmosis serum (IgG titer, $1:4 \times 10^6$ by IIF), using our fluorescein isothiocyanate-labeled anti-human IgM reagent diluted 1:1,000. Third, this test was shown to be independent of IgM RFs in six cases. Fourth, it was suggested previously (14) that the demonstration of IgM class antibodies to the p35000 antigenic component of *T. gondii* is highly indicative of a rather early stage of infection. Therefore, a negative IgM-p35000-ELA does not completely rule out acute toxoplasmosis (Table 1) if serum samples are taken late during IgM-positive disease. Such samples need to be tested by 19S IgM IIF or IgM ELISA if clinical signs are highly indicative of acute disease. For these reasons and because of its ease of performance and the stability of the purified p35000 antigenic component in Laemmli buffer at −80°C for at least 3 months, we prefer to use the IgM-p35000-ELA to screen for early acute toxoplasmosis.

ACKNOWLEDGMENTS

The RH strain of *T. gondii* was kindly provided by K. Mannweiler, Director, Department of Bacteriology/Serology, Bernhard Nocht Institute, Hamburg, Federal Republic of Germany. G. Winterseel performed the standard tests skillfully. H. von Wulffen kindly read this paper critically.

LITERATURE CITED

1. **Franklin, E.** 1968. Structure and function of immunoglobulins. N.Y. State J. Med. **411**:411–424.
2. **Handman, E., J. W. Goding, and J. S. Remington.** 1980. Detection and characterization of membrane antigens of *Toxoplasma gondii*. J. Immunol. **124**:2578–2583.
3. **Hyde, B., E. V. Barnett, and J. S. Remington.** 1975. Method for differentiation of nonspecific from specific *Toxoplasma* IgM fluorescent antibodies in patients with rheumatoid factor. Proc. Soc. Exp. Biol. Med. **148**:1184–1188.
4. **Kasper, L. H., J. H. Crabb, and E. R. Pfefferkorn.** 1983. Purification of a major protein of *Toxoplasma gondii* by immunadsorption with a monoclonal antibody. J. Immunol. **130**:2407–2412.
5. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
6. **Lindenschmidt, E.-G.** 1984. Rheumatoid factor activity as disturbance factor in serological diagnosis of specific IgM-antibodies. Immun. Infekt. **12**:94–100.
7. **Lindenschmidt, E.-G.** 1985. Enzyme-linked immunosorbent assay for detection of soluble *Toxoplasma gondii* antigen in acute-phase toxoplasmosis. Eur. J. Clin. Microbiol. **4**:488–492.
8. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
9. **McCabe, R. E., and J. S. Remington.** 1983. The diagnosis and treatment of toxoplasmosis. Eur. J. Clin. Microbiol. **2**:95–104.
10. **Naot, Y., E. V. Barnett, and J. S. Remington.** 1981. Method for avoiding false-positive results occurring in immunoglobulin M enzyme-linked immunosorbent assays due to presence of both rheumatoid factor and antinuclear antibodies. J. Clin. Microbiol. **14**:73–78.
11. **Naot, Y., and J. S. Remington.** 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to toxoplasmosis. J. Infect. Dis. **142**:757–766.
12. **Neurath, A. R., N. Strick, P. Sproul, L. Baker, P. Rubinstein, C. E. Stevens, P. Taylor, R. C. Gallo, J. W. M. Gold, Y. S. Lee, and T. Nilsen.** 1985. Radioimmunoassay and enzyme-linked immunoassay of antibodies to the core protein (P24) of human T-lymphotropic virus (HTLV III). J. Virol. Methods **11**:75–86.
13. **Partanen, P., H. J. Turunen, R. Paasivuo, E. Forsblom, J. Suni, and P. O. Leinikki.** 1983. Identification of antigenic components of *Toxoplasma gondii* by an immunoblotting technique. FEBS Lett. **158**:252–254.
14. **Partanen, P., H. J. Turunen, R. T. A. Paasivuo, and P. O. Leinikki.** 1984. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulins G, M, and A antibodies at different stages of infection. J. Clin. Microbiol. **20**:133–135.
15. **Rodriguez, C., D. Afchain, A. Capron, C. Dissons, and F. Santoro.** 1985. Major surface protein of *Toxoplasma gondii* (p 30) contains an immunodominant region with repetitive epitopes. Eur. J. Immunol. **15**:747–749.
16. **Schmitz, H. V., U. Deimling, and D. Flehmig.** 1980. Detection of IgM antibodies to cytomegalovirus (CMV) using an enzyme-labeled antigen (ELA). J. Gen. Virol. **50**:59–68.
17. **Sharma, S. D., J. Mullenax, F. G. Araujo, H. A. Erlich, and J. S. Remington.** 1983. Western blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol. **131**:977–983.
18. **Shirodaria, P. V., K. B. Fraser, and C. F. Stanford.** 1973. Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein-conjugated anti-IgM. Ann. Rheum. Dis. **32**:53.
19. **Siegel, J. P., and J. S. Remington.** 1983. Comparison of methods for quantitating antigen-specific immunoglobulin M antibody with a reverse enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18**:63–70.
20. **Sulzer, A. J., E. L. Franco, E. Tahafuji, M. Benenson, K. W. Walls, and R. L. Greenup.** 1986. An oocyst-transmitted outbreak of toxoplasmosis: patterns of immunoglobulin G and M over one year. Am. J. Trop. Med. Hyg. **35**:290–296.
21. **Tasheva, B., and G. Dessev.** 1983. Artifacts in sodium dodecyl sulfate-polyacrylamide gel electrophoresis due to 2-mercaptoethanol. Anal. Biochem. **129**:98–102.
22. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
23. **van Loon, A. M., and J. van der Veen.** 1980. Enzyme-linked immunosorbent assay for quantitation of toxoplasma antibodies in human sera. J. Clin. Pathol. **33**:635-639.

24. **Waaler, E.** 1940. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. Acta Pathol. Microbiol. Scand. **17**:172–188.
25. **Walls, K. W., A. J. Sulzer, J. G. Soto, and E. L. Franco.** 1983. Diagnosis of acute acquired toxoplasmosis with the enzyme-labeled antigen reversed immunoassay for immunoglobulin M antibodies. J. Immunoassay **4**:373–393.
26. **Wilson, M. B., and P. K. Nakane.** 1978. Recent developments in the periodate method of conjugating horseradish peroxidase to antibodies, p. 115–124. *In* W. Knapp, K. Holubar, and G. Wick (ed.), Immunofluorescence and related staining techniques. Elsevier Science Publishing Co., Inc., New York.