JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2004, p. 4627–4631 Vol. 42, No. 10
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.10.4627–4631.2004

Evaluation of the Immunoglobulin G Avidity Test for Diagnosis of Toxoplasmic Lymphadenopathy

Jose G. Montoya,[1,2]* Heather B. Huffman,[1] and J. S. Remington[1,2]

Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto,[1] *and Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford,*[2] *California*

Received 12 April 2004/Returned for modification 8 June 2004/Accepted 21 June 2004

Toxoplasmic lymphadenopathy (TL) is the most common clinical manifestation of acute acquired toxoplasma infection in normal individuals. The diagnosis is established by serologic methods and lymph node biopsy. Recently, tests for avidity of toxoplasma immunoglobulin G (IgG) antibodies have been introduced to help discriminate between recently acquired and distant infection with the parasite. We studied an avidity test to define the usefulness of this method and to determine the evolution of the IgG avidity in TL. Seventy-three consecutive patients diagnosed as having TL were studied. IgG avidity test titers were noted to be time dependent from the clinical onset of lymphadenopathy. Low IgG avidity test results were observed in patients who had developed lymphadenopathy from <1 month to 17 months prior to the sampling of sera, emphasizing that low IgG avidity test results are not reliable for diagnosis of recently acquired infection. In contrast, high IgG avidity test results were observed only in patients who had developed lymphadenopathy at least 4 months earlier. Thus, a high IgG avidity test result in an individual who has recent onset of lymphadenopathy (e.g., within 2 to 3 months of sera sampling) suggests a cause other than toxoplasmosis. In such cases, further workup is warranted in order to determine the cause of the lymphadenopathy.

In the primary care setting, lymphadenopathy (LN) is a not an uncommon complaint or finding on physical examination (6, 17). It may be discovered during routine physical examination or incidentally by the patient and constitute the sole reason for a medical visit. In either setting, because of the wide variety of diseases that cause lymph node enlargement, defining the etiology can present a diagnostic challenge. The cause is frequently obvious, and most patients can be diagnosed on the basis of a careful history and physical examination. In others, diagnosis may require laboratory tests and/or biopsy of the involved node(s).

Infection with *Toxoplasma gondii* is a cause of clinically significant, nontender and nonsuppurative LN. Infection with this organism usually causes regional enlargement of lymph nodes (most commonly suboccipital and cervical) rather than generalized LN (11). Although the diagnosis can be made when the classic histopathologic findings are present on lymph node biopsy (4), we have continued to seek serologic methods to make the diagnosis so as to avoid biopsy in most cases. In a previous study by our group, it was also demonstrated that the diagnosis of toxoplasmic lymphadenopathy (TL) can be established by the use of a panel of serological tests, i.e., the toxoplasma serological profile (TSP) (13). Little value has been added by performing isolation procedures (3) or the PCR (21).

The kinetics of the individual tests included in the TSP are unique, and results in these tests are time dependent and vary from the onset of lymphadenopathy to the time of drawing of the serum sample (13). A TSP consistent with a recently acquired infection (i.e., high titers in the dye test [DT], i.e., >1,024; positive immunoglobulin G [IgM], IgA, and IgE findings; and acute pattern in the AC/HS) is strongly suggestive of TL and is essentially found only in patients whose LN was detected within 2 to 3 months of the sampling of the serum (13). In these patients a lymph node biopsy is usually not indicated unless the LN persists, other symptoms develop, or some other diagnosis is being considered.

Equivocal results in the TSP (i.e., DT titer of ≤1,024, low-positive or equivocal results in the IgM, IgA, or IgE enzyme-linked immunosorbent assay [ELISA] results, and an equivocal pattern in the AC/HS) are more difficult to interpret. In such cases, *T. gondii* should not be ruled out or implicated as the etiological agent of the patient's LN (13). TSP results consistent with a chronic infection acquired in the distant past—i.e., a DT titer of ≤256, equivocal or negative IgM, IgA, and IgE levels, and a chronic pattern in the AC/HS—essentially excludes *T. gondii* as the causative agent of a lymph node detected to be enlarged within the previous 3 months of the drawing of the serum sample (13).

The avidity of *T. gondii*-specific IgG antibodies (in the patient's serum) for defined toxoplasma antigens (purified and used to capture and bind *T. gondii*-specifc IgG antibodies) has been found to gradually increase after primary infection with the parasite (7). IgG avidity can be measured by the resistance of the antibody-antigen complex to the disrupting force of 6 M urea (7). Avidity has been primarily studied in the setting of pregnancy (8, 9, 12). Low or equivocal avidity test results can persist for months to more than 1 year after primary infection (16). The time of conversion from low or equivocal to high avidity test results is highly variable among different individuals. However, high avidity test results are observed only in individuals infected for at least 3 to 5 months (this time window

* Corresponding author. Mailing address: Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto, CA 94301. Phone: (650) 853-6061. Fax: (650) 329-9853. E-mail: samja@stanford.edu.

varies according to the method used to measure avidity antibodies). In studies in pregnant women, high avidity test results by the IgG VIDAS avidity test (VIDAS Toxoplasma IgG Avidity test [bioMérieux, Marcy l'Etoile, France]), for example, have been reported to be present only in individuals who have been infected for at least 4 months (12, 16).

The goal of the present study was to attempt to define the kinetics and the clinical utility of the IgG VIDAS test in patients with TL.

(Presented in part at the 40th Annual Meeting of the Infectious Diseases Society of America, Chicago, Ill., in October 2002.)

MATERIALS AND METHODS

A total of 104 serum samples were studied from 73 consecutive, nonpregnant patients with TL. The sera were submitted to the Toxoplasma Serology Laboratory of the Palo Alto Medical Foundation between January 1994 and October 2002. LN was self-reported by the patients to their physicians or detected by their physicians. Physicians provided the date of onset of LN and demographics of their patients. The data were analyzed by the month the serum sample was drawn after clinical onset of LN. The interval between the clinical onset of LN and the time the serum was drawn was rounded off to the nearest month (i.e., patients whose sera were collected within 15 days of the onset of LN were assigned the "zero-month" time point and those whose sera were collected ≥15 days were assigned to the "one-month" time point).

Lymph node biopsy was performed in 45 (61.6%) of the 73 patients, and the histology slides were available to our group and to the Stanford Pathology Department for review. In each of these 45 cases, histological features of the lymph nodes were consistent with the diagnosis of TL (4). In the other 28 patients, clinical and serological data were available, but lymph node biopsy was not performed. Demographic and serological data for both groups of patients were analyzed together and separately. The results were similar compared for patients with or without biopsy. A two-tailed Student *t* test was used to assess whether the differences between these two groups were statistically significant.

Serum samples were routinely tested on receipt by the Toxoplasma Serology Laboratory of the Palo Alto Medical Foundation by using the TSP, which includes the Sabin-Feldman dye test (19), the double sandwich IgM ELISA (14), the IgA ELISA (20), the IgE ELISA (23), the IgE ISAGA (23), and the AC/HS test (2). Each test was performed as previously described (13). In the double-sandwich IgM ELISA (14), a result of ≥2.0 was interpreted as positive, a result of 1.7 to 1.9 was considered equivocal, and a result of ≤1.6 was considered negative. The following titers were considered positive, negative, and equivocal, respectively: IgA ELISA, ≥2.1 and ≤1.4 (equivocal, 1.5 to 2.0); IgE ELISA, ≥1.9 and ≤1.4 (equivocal, 1.5 to 1.8); and IgE ISAGA, ≥4 and ≤2 (equivocal, 3). The AC/HS test was interpreted as previously described (2) by comparing titers obtained with formalin-fixed tachyzoites (HS antigen) with those obtained with acetone-fixed tachyzoites (AC antigen) (2). IgG antibodies formed early in infection recognize stage-specific antigens in the AC preparation which are distinct from those formed later in infection. Each of the 73 patients had a TSP consistent with the diagnosis of TL in at least one of the serum samples (13).

The VIDAS Toxoplasma IgG Avidity test was added to our TSP in 1999. It was performed retrospectively in those patients in whom it had not been performed at the time of original testing. The IgG avidity test was performed and interpreted according to directions of the manufacturer (16). The test uses a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) system (VIDAS Toxoplasma IgG Avidity). It is performed by the VIDAS instrument that automatically produces results and their interpretation. Interpretation was as follows: <0.200, low avidity ("may be seen in acute primary infection with *T. gondii*"); 0.200 to <0.300, borderline avidity ("primary infection during the last 6 months is possible"); and >0.300, high avidity ("excludes primary infection within the last 16 weeks"). The manufacturer states that, with this method, a high avidity result in a pregnant woman excludes a recently acquired infection in the prior 16 weeks from serum sampling (16).

RESULTS

The mean age of the patients was 34.5 years (range, 14 to 74 years). The female/male ratio was 6:5. Each of the 73 patients had serological test results consistent with a diagnosis of TL (data not shown). Avidity data are shown in Tables 1 and 2 and in Fig. 1. Avidity titers were time dependent from the clinical onset of LN and steadily rose over time after the onset of LN (Fig. 1). There was a progressive increase in the IgG avidity test values over time.

TABLE 1. Toxoplasma IgG avidity titers in 73 patients following onset of TL

Time from onset to sampling of sera (mo)[a]	No. tested	Mean avidity test result (range)
0	5	0.050 (0.014–0.132)
1	22	0.066 (0.020–0.268)
2	27	0.076 (0.009–0.262)
3	9	0.094 (0.035–0.291)
4	7	0.177 (0.085–0.310)
5	4	0.235 (0.082–0.487)
6	5	0.204 (0.102–0.339)
7–12	8	0.307 (0.150–0.517)
13–24	10	0.467 (0.162–0.646)
25–36	2	0.469 (0.340–0.598)
37–48	5	0.526 (0.391–0.652)

[a] Interval from the onset of LN to date that serum samples were collected (rounded off to the nearest month).

The mean age and the female/male ratio for the 45 patients in whom lymph node biopsies were performed were 36 years and 11:10, respectively, and for the 28 patients in whom lymph node biopsies were not performed, the corresponding values were 31 years and 11:10. The differences in age were not statistically significant. There were no significant differences when avidity test results were compared between those who had lymph node biopsy performed and those who did not (data not shown).

Ninety-seven percent of the low avidity test results were observed in the 61 patients who had developed LN from 0 to 6 months prior to the first sampling of their sera.

Of the 67 sera with low-avidity test results within the first 6 months after the onset of LN, 62 (92.5%) were in sera drawn within the first 4 months (Table 2). Low avidity results were also observed as late as 17 months after onset of TL.

Eighty-two percent of the high avidity test results were observed in the 17 patients who had developed LN at least 7 months prior to the first sampling of their sera (Table 2).

Of the 24 sera with high avidity test results, 21 (84%) were observed in patients whose LN had developed at least 4 months prior to the sampling of sera.

TSP results consistent with an infection acquired in the recent or distant past have been shown by our group to successfully diagnose or exclude TL (13). Comparison of avidity test results and those in the TSP is shown in Table 3.

A chronic pattern in the TSP essentially establishes that the patient has an infection acquired in the distant past. In this setting, LN that developed within the prior 3 to 4 months would probably not be due to toxoplasmosis. Equivocal results in the TSP are more difficult to interpret. In many of these patients, follow-up sera are required to determine whether a rise in IgG antibody titers has occurred. In the present study, 9 (56.3%) of 16 serum samples with equivocal results in the TSP had high avidity test results, showing that these nine

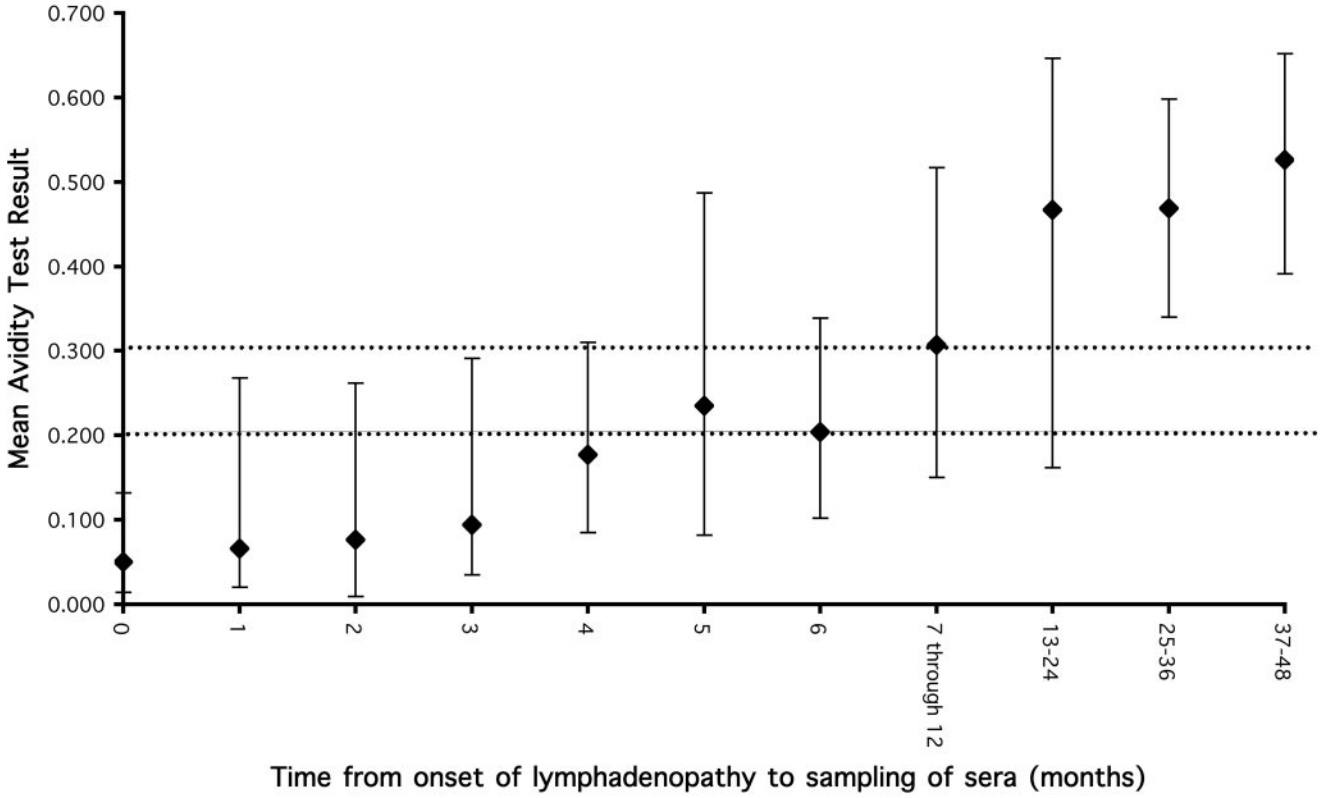

FIG. 1. Mean and range of VIDAS Toxoplasma IgG Avidity test titers after onset of lymphadenopathy in 73 nonpregnant consecutive patients. The time from the onset of lymphadenopathy to sampling of sera is rounded off to the nearest month. Interpretation of avidity test results: <0.200, low; 0.200 to <0.300, borderline; >0.300, high.

patients had an infection that had been acquired in the distant past.

Of note, only 8 (10%) of 80 serum samples with an acute pattern in the TSP had a high avidity test result, suggesting that these eight patients had an infection acquired in the distant past. Of 24 patients with high avidity test results, 16 (67%) had an acute or equivocal pattern in the TSP.

TABLE 2. Toxoplasma IgG avidity test results following onset of TL

Time from onset to sampling of sera (mo)[a]	No. serum samples tested	No. of IgG avidity results that were:		
		Low	Equivocal	High
0	5	5	0	0
1	22	21	1	0
2	27	26	1	0
3	9	8	1	0
4	7	3	3	1
5	4	2	1	1
6	5	3	1	1
7–12	8	1	2	5
13–24	10	1	0	9
25–36	2	0	0	2
37–48	5	0	0	5
Total	104	70	10	24

[a] Interval from the onset of LN to date that serum samples were collected (rounded off to the nearest month).

DISCUSSION

In the present study, avidity test results gradually and consistently rose over time after the onset of LN; a decrease to lower values thereafter was not observed. Our findings emphasize the importance of knowing the interval between clinical onset of LN and the date the first serum sample was drawn for testing in order to properly interpret the avidity test results. Whereas low avidity test results were most frequently observed in patients with recent infection, high avidity test results were demonstrable only in patients whose LN was detected ≥4 months prior to sampling their sera.

It should be noted that at certain time points a relatively small number of sera were available for study. In addition, information on the onset of LN was subject to the limitations imposed by how accurately and promptly patients reported the

TABLE 3. Comparison of the VIDAS IgG Avidity test and TSP results in 104 serum samples from 73 consecutive patients with TL

Avidity	TSP (no. of results)			
	Acute	Equivocal	Chronic	Total
Low	66	3	1	70
Equivocal	6	4	0	10
High	8	9	7	24
Total	80	16	8	104

LN to their physicians. Despite these limitations, there was a high degree of consistency between the mean avidity test titers and their trend to increase over time. This consistency was apparent when data observed in different patients or data from the same patient at different time points were compared.

A study of the IgG avidity test in the setting of TL (and chorioretinitis) has been reported by Paul (15). In her study, IgG avidity was determined with the *T. gondii* IgG avidity EIA kit (Labsystems, Helsinki, Finland) and by using a noncommercial ELISA developed in the Clinic of Parasitic and Tropical Diseases in Poznan, Poland (15). Of 19 patients with LN in that study, 6 exhibited low avidity test results for as long as 5 months after their first serological examination. Low avidity test results were also documented in two of our patients whose LN had developed for as long as 8 and 17 months, respectively, before sampling of their sera. Our findings and those of Paul reveal that low avidity test results alone cannot be used to diagnose recently acquired infection in patients with LN.

In the study by Paul, 53% of individuals with high avidity test results had LN for more than 5 months (15). In the present study, high avidity tests results were only observed in patients whose LN had developed at least 4 months prior to the date their sera were first drawn. However, most (82%) of the high avidity test results were observed in patients whose LN had developed later than 7 months from the date their sera were first drawn. These findings in patients with LN are consistent with those of Pelloux et al., who observed that a high avidity result in the VIDAS Toxoplasma IgG Avidity test in pregnant women excludes the acute infection having been acquired in the previous 16 weeks (16). The time window for exclusion of recently acquired infection by a high avidity test result varies with the method used. Therefore, it is crucial for clinicians using the avidity test to know the time window of exclusion for the method used. For instance, whereas a high avidity test result in the Labsystems test (Labsystems, Helsinki, Finland) kit excludes acute infection in the 3 months prior the sampling of sera, a high avidity result in the VIDAS test excludes acute infection in the 4 months prior to the sampling of sera.

We compared results in the avidity test with those of the TSP rather than with the IgM test alone because a combination of tests (such as in the TSP) has been shown to be superior for the diagnosis of toxoplasmosis (13, 18). There was good agreement between low avidity test results and an acute TSP pattern. The data suggest that the transition from a low to a high avidity test result occurs earlier than does the transition from an acute to a chronic TSP pattern. Among patients whose initial sera had already exhibited a high avidity test result, 66.7% had an acute or equivocal pattern in the TSP. In contrast, of patients whose initial sera had a chronic pattern in the TSP, 87.5% had a high avidity test result (see Table 3).

Does IgG avidity testing represent an important addition to the serological diagnosis of TL? There is no single serological test that can be used to confirm conclusively whether *T. gondii* is the etiologic agent of a patient's LN. The absence of *T. gondii*-specific IgG antibodies usually rules out toxoplasmosis as the cause of LN. However, it is important to recognize that immunocompetent patients may be initially negative for IgG *T. gondii* antibodies when the serum sample is obtained close to the onset of the LN; although this is unlikely, a follow-up serum sample is required to rule out seroconversion in this situation.

The presence of toxoplasma-specific IgG antibodies establishes that the patient was exposed to the parasite but does not clarify whether the exposure was acquired in the recent or more distant past. A positive IgG test for *T. gondii* alone should not necessarily lead to the conclusion that the patient's LN (of recent onset, i.e., within 3 months) is a manifestation of toxoplasmosis since IgG antibodies can remain positive for the life of the individual. Similarly, positive IgM test results cannot be used to establish that a recent onset LN is due to toxoplasmosis. Positive IgM test results have been observed in patients who have been infected with the parasite for many months and even years (1, 5, 13, 22). In addition, several commercial kits to detect *T. gondii*-specific IgM have a high rate of false-positive results (10).

With a panel of tests such as the TSP, however, results consistent with an acute infection in a patient whose LN developed within the previous 3 to 4 months is highly suggestive of *T. gondii* as the etiologic agent (13). In this same patient but with TSP results consistent with an infection acquired in the distant past (chronic pattern), etiologies other than toxoplasmosis should be excluded. Equivocal test results in the TSP in such a patient represent a diagnostic challenge, and it is for this patient that IgG avidity testing was found to represent an important addition to the serological diagnosis of TL. A significant number of these patients had high avidity test results, thereby placing their infection to ≥4 months prior to the sampling of sera. In patients with recent onset LN (i.e., onset within the prior 3 months), high avidity test results should caution physicians from attributing the recently detected LN to toxoplasmosis. In theses cases lymph node biopsy may be necessary to clarify the cause of the LN.

ACKNOWLEDGMENT

This study was supported by the U.S. Public Health Service grant AI04717 from the National Institutes of Health.

REFERENCES

1. **Bobic, B., D. Sibalic, and O. Djurkovic-Djakovic.** 1991. High levels of IgM antibodies specific for. *Toxoplasma gondii* in pregnancy 12 years after primary toxoplasma infection. Gynecol. Obstet. Investig. **31:**182–184.
2. **Dannemann, B. R., W. C. Vaughan, P. Thulliez, and J. S. Remington.** 1990. Differential agglutination test for diagnosis of recently acquired infection with *Toxoplasma gondii*. J. Clin. Microbiol. **28:**1928–1933.
3. **Diego, J. A., J. J. Vazquez, P. Penin, J. Fernandez, S. Sanchez, and C. Gamallo.** 1993. Use of murine subinoculation for the diagnosis and isolation of toxoplasmosis in HIV-infected patients with persistent lymphadenopathy. Ann. Trop. Med. Parasitol. **87:**179–184.
4. **Dorfman, R. F., and J. S. Remington.** 1973. Value of lymph-node biopsy in the diagnosis of acute acquired toxoplasmosis. N. Engl. J. Med. **289:**878–881.
5. **Food and Drug Administration.** 1997. Public Health Advisory: limitations of toxoplasma IgM commercial test kits 1–3. Food and Drug Administration, Washington, D.C.
6. **Habermann, T. M., and D. P. Steensma.** 2000. Lymphadenopathy. Mayo Clin. Proc. **75:**723–732.
7. **Hedman, K., M. Lappalainen, I. Seppala, and O. Makela.** 1989. Recent primary *Toxoplasma* infection indicated by a low avidity of specific IgG. J. Infect. Dis. **159:**736–739.
8. **Lappalainen, M., P. Koskela, M. Koskiniemi, P. Ämmälä, V. Hiilesmaa, K. Teramo, K. O. Raivio, J. S. Remington, and K. Hedman.** 1993. Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. J. Infect. Dis. **167:**691–697.
9. **Liesenfeld, O., J. G. Montoya, S. Kinney, C. Press, and J. S. Remington.** 2001. Effect of testing for IgG avidity in the diagnosis of *Toxoplasma gondii* infection in pregnant women: experience in a U.S. reference laboratory. J. Infect. Dis. **183:**1248–1253.
10. **Liesenfeld, O., C. Press, J. G. Montoya, R. Gill, J. L. Isaac-Renton, K.**

Hedman, and J. S. Remington. 1997. False-positive results in immunoglobulin M (IgM) toxoplasma antibody tests and importance of confirmatory testing: the Platelia toxo IgM test. J. Clin. Microbiol. **35:**174–178.
11. **McCabe, R. E., R. G. Brooks, R. F. Dorfman, and J. S. Remington.** 1987. Clinical spectrum in 107 cases of toxoplasmic lymphadenopathy. Rev. Infect. Dis. **9:**754–774.
12. **Montoya, J. G., O. Liesenfeld, S. Kinney, C. Press, and J. S. Remington.** 2002. VIDAS test for avidity of *Toxoplasma*-specific immunoglobulin G for confirmatory testing of pregnant women. J. Clin. Microbiol. **40:**2504–2508.
13. **Montoya, J. G., and J. S. Remington.** 1995. Studies on the serodiagnosis of toxoplasmic lymphadenitis. Clin. Infect. Dis. **20:**781–790.
14. **Naot, Y., and J. S. Remington.** 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. **142:**757–766.
15. **Paul, M.** 1999. Immunoglobin G avidity in diagnosis of toxoplasmic lymphadenopathy and ocular toxoplasmosis. Clin. Diagn. Lab. Immunol. **6:**514–518.
16. **Pelloux, H., E. Brun, G. Vernet, S. Marcillat, M. Jolivet, D. Guergour, H. Fricker-Hidalgo, A. Goullier-Fleuret, and P. Ambroise-Thomas.** 1998. Determination of anti-*Toxoplasma gondii* immunoglobulin G avidity: adaptation to the Vidas system (bioMerieux). Diagn. Microbiol. Infect. Dis. **32:**69–73.
17. **Peters, T. R., and K. M. Edwards.** 2000. Cervical lymphadenopathy and adenitis. Pediatr. Rev. **21:**399–405.
18. **Roberts, A., K. Hedman, V. Luyasu, J. Zufferey, M. H. Bessieres, R. M. Blatz, E. Candolfi, A. Decoster, G. Enders, U. Gross, E. Guy, M. Hayde, D. Ho-Yen, J. Johnson, B. Lecolier, A. Naessens, H. Pelloux, P. Thulliez, and E. Petersen.** 2001. Multicenter evaluation of strategies for serodiagnosis of primary infection with *Toxoplasma gondii*. Eur. J. Clin. Microbiol. Infect. Dis. **20:**467–474.
19. **Sabin, A. B., and H. A. Feldman.** 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (toxoplasma). Science **108:**660–663.
20. **Stepick-Biek, P., P. Thulliez, F. G. Araujo, and J. S. Remington.** 1990. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J. Infect. Dis. **162:**270–273.
21. **Weiss, L., Y. Chen, G. Berry, J. Strickler, R. Dorfman, and R. Warnke.** 1992. Infrequent detection of *Toxoplasma gondii* genome in toxoplasmic lymphadenitis: a polymerase chain reaction study. Hum. Pathol. **23:**154–158.
22. **Wilson, M., J. S. Remington, C. Clavet, G. Varney, C. Press, D. Ware, et al.** 1997. Evaluation of six commercial kits for detection of human immunoglobulin M antibiodies to *Toxoplasma gondii*. J. Clin. Microbiol. **35:**3112–3115.
23. **Wong, S. Y., M.-P. Hadju, R. Ramirez, P. Thulliez, R. McLeod, and J. S. Remington.** 1993. The role of specific immunoglobulin E in diagnosis of acute toxoplasma infection and toxoplasmosis. J. Clin. Microbiol. **31:**2952–2959.