Immunoglobulin M-Immunosorbent Agglutination Assay for Diagnosis of Infectious Diseases: Diagnosis of Acute Congenital and Acquired *Toxoplasma* Infections

GEORGES DESMONTS,¹ YEHUDITH NAOT,^{2,3} AND JACK S. REMINGTON^{2,3*}

Laboratoire de Serologie Neonatale et de Recherche sur la Toxoplasmose, Institut de Puericulture de Paris, Paris, France¹; Division of Allergy, Immunology and Infectious Diseases, Palo Alto Medical Research Foundation, Palo Alto, California 94301²; and Department of Medicine, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, California 94305³

Received 25 February 1981/Accepted 2 June 1981

An immunoglobulin M (IgM)-immunosorbent agglutination assay (IgM-IS-AGA) was negative in all sera from individuals negative in the Sabin-Feldman dye test, in sera from individuals with chronic *Toxoplasma* infection, and in cord blood samples from uninfected infants. In contrast, all sera that were obtained from individuals with a recent history of acute *Toxoplasma* infection and from infants with congenital *Toxoplasma* infection and that were positive in both the dye test and the IgM-indirect fluorescent-antibody (IgM-IFA) test were positive in IgM-ISAGA. A total of 21 (67.7%) of 31 sera that were negative in the IgM-IFA test, despite being obtained from individuals with recently acquired *Toxoplasma* infection, and 8 (72.7%) of 11 sera that were negative in the IgM-IFA test and obtained from infants with congenital *Toxoplasma* infection were positive in IgM-ISAGA. The presence of rheumatoid factor, antinuclear antibodies, or both did not cause false-positive results in the IgM-ISAGA but did so in the IgM-IFA test. Thus, IgM-ISAGA is both more sensitive and more specific than the IgM-IFA test for detection of IgM antibodies to *Toxoplasma gondii* and, therefore, for the diagnosis of acute congenital and acquired *Toxoplasma* infections.

The fact that detection of immunoglobulin M (IgM) antibodies is valuable for the diagnosis of a variety of acute infectious diseases has been an impetus for the development of specific and sensitive assays for detection of this class of immunoglobulin antibodies to all classes of infectious agents. This has been most important for those infections in which isolation of the infecting organism is either not easily achieved or must be done in a specialized laboratory.

The IgM-indirect fluorescent-antibody (IgM-IFA) test has been the most satisfactory method available for the demonstration of IgM antibodies to *Toxoplasma gondii* and for the diagnosis of acute congenital and acquired infections with *T. gondii* (4, 7, 14, 20-22, 26). Recently, we developed a double-sandwich IgM-enzyme-linked immunosorbent assay (DS-IgM-ELISA) which is a marked improvement over the IgM-IFA test (15-17). DS-IgM-ELISA is easy to perform and read and is more sensitive and specific than both the conventional IgM-ELISA (3) and the IgM-IFA test. It avoids false-positive results due to the presence of rheumatoid factor (RF) and antinuclear antibodies (ANA) known to oc-

cur in the latter two assays (2, 3, 5, 12, 24, 25). The greater sensitivity and specificity of DS-IgM-ELISA are attributable to the initial step of the assay, in which the IgM fraction of the patient's serum is separated from other serum components by selective absorption of the IgM fraction onto microtiter plates in which the wells are precoated with specific antibodies to human IgM. Another serological test, the *Toxoplasma* agglutination test (9, 10) as modified by Desmonts and Remington (6), is accurate, inexpensive, and simple to perform, but is useful only for demonstration of IgG antibodies and thus cannot be used by itself for the early diagnosis of acute *Toxoplasma* infection. Without the modification, the agglutination test had disadvantages, since false-positive results were common due to naturally occurring IgM antibodies to *T. gondii* in the normal population. We have combined certain features of *Toxoplasma* DS-IgM-ELISA (15-17) and the direct *Toxoplasma* agglutination test (6, 9, 10) in an attempt to obtain a sensitive and specific method that does not require use of an enzyme conjugate and that allows for use of a particulate

antigen preparation for demonstration of IgM antibodies. The IgM-immunosorbent agglutination assay (IgM-ISAGA) that we describe here combines the advantages of both the direct agglutination test and DS-IgM-ELISA in its specificity and sensitivity for demonstration of IgM antibodies to *T. gondii* and should prove useful for the diagnosis of other infectious diseases as well.

MATERIALS AND METHODS

Antigen. Purified tachyzoites of the RH strain of *T. gondii* were obtained either as described previously for the direct agglutination test (6) or from peritoneal exudates of mice infected 2 days earlier (19, 27).

Serological tests. The Sabin-Feldman dye test (DT) (11, 23), the *Toxoplasma* IgM-IFA test (26), and DS-IgM-ELISA (15-17) were performed by the methodology given in the respective references. RF titers were determined by the latex agglutination method (Rapi-Tex RF kit; Behring Diagnostics, American Hoechst Corp., Somerville, N.J.). Fluorescent ANA titers were determined by the method described by Johnson et al. (13). To remove RF activity, sera were absorbed with latex particles coated with human IgG (12).

Anti-human IgM antibodies. The IgG fraction of rabbit anti-human IgM (μ -chain specific) was obtained from Cappel Laboratories, (Downingtown, Pa.). The specific binding of human IgM by this antibody preparation was confirmed as previously described (17).

IgM-ISAGA. Wells of disposable, U-shaped, rigid styrene microtiter plates (Dynatech Laboratories, Alexandria, Va.) were coated with 100 μ l of an IgG fraction of rabbit anti-human IgM (μ -chain specific) diluted in 0.1 M carbonate buffer (pH 9.8), and the plates were incubated overnight at 4°C. The optimal dilution used for coating the wells was determined in preliminary tests as previously described (17). The wells coated with anti-human IgM antibodies were washed three times for 5 min each in phosphate-buffered saline (PBS) containing 0.05% Tween 20. Wells were postcoated with 1% bovine serum albumin in PBS containing 0.05% Tween 20, and plates were incubated for 1 h at 37°C and washed again. Samples of 150 μ l of serum dilutions in PBS (sera to be tested were diluted fourfold beginning with a dilution of 1:16) were added to washed wells, and the plates were incubated for 1 h at 37°C and washed twice in PBS containing 0.05% Tween 20 and twice in PBS. Suspensions of tachyzoites were diluted in alkaline buffer, pH 8.7 (7.02 g of NaCl, 3.09 g of H_3BO_3 , 24 ml of 1 N NaOH, 4 g of bovine serum albumin, 1 g of NaN_3 , and sufficient distilled water to effect a final volume of 1 liter) (6), to a concentration of 3.3×10^7 to 3.6×10^7 organisms per ml. Fifty microliters of this suspension was added to each well, and the plates were incubated overnight at 37°C.

The pattern of agglutination and the sensitivity of the test are greatly modified when working at different temperatures. Incubation in the cold (4°C) results in increased titers and in spontaneous agglutination in negative controls. Caution must be exercised when the

plates are incubated overnight at room temperature if there is no temperature control. It is for this reason that incubation in an incubator is recommended.

We do incubations at 37°C. Care must be taken to prevent drying of the plates. Plates may be read after 16, 24, 48, or even 72 h without a significant change in the titers as long as the wells have not dried.

The plates are read against a black background with a lateral light and are read by pattern. A smooth button at the bottom of the well is recorded as negative (0). A complete carpet is recorded as positive (+++). Intermediate readings from $\pm$ (doubtful) to ++ are also noted. In serial fourfold dilutions, two or three tubes are usually noted to be ++ to $\pm$, between definitely positive (+++) and completely negative (0). The titers are expressed as the highest serum dilution exhibiting a definite +++ pattern. In each test, a positive control and a negative control were included.

Human sera. A total of 168 serum samples were tested in Palo Alto. Of these, 50 sera were tested in both the Palo Alto and the Paris laboratories. Each sample was tested at least twice on different days and on different plates. Results were recorded by two individuals without prior knowledge of other serological test results in the samples. Criteria for the diagnosis of acute congenital or acquired toxoplasmosis or *Toxoplasma* infection were as described previously (1, 7, 20-22, 26).

Of 73 sera tested and obtained from individuals with a recent history of acute acquired toxoplasmosis, 28 sera were the first samples in which a seroconversion was noted in the DT (1, 20-22, 26). A total of 34 individuals had lymphadenopathy, and lymph node biopsies performed on 24 of these patients were diagnostic of toxoplasmic lymphadenitis (7). Nine sera were obtained from women who gave birth to infants with congenital toxoplasmosis. Two samples were obtained from patients who underwent heart transplantation and from whom *Toxoplasma* organisms were isolated (20). All 13 samples obtained from infants with a diagnosis of congenital toxoplasmosis were collected from infants born to mothers in whom seroconversion was observed during pregnancy. Diagnosis in these infants was based on isolation of *Toxoplasma* organisms from placentas or the blood of newborn infants (20).

A serum sample that was obtained from a patient 1 month after the clinical onset acute toxoplasmosis and that had a titer of 1:16,384 in the DT, 1:1,280 in the IgM-IFA test, and 1:16,384 in DS-IgM-ELISA served as the positive control.

A pool of seven sera that were obtained from healthy individuals and that were negative in the DT, IgM-IFA test, and DS-IgM-ELISA and also negative for RF and ANA served as the negative control.

RESULTS

Optimal conditions for IgM-ISAGA. Preliminary experiments performed in our laboratories in Palo Alto and Paris indicated that the *Toxoplasma* antigen concentration used is critical for both sensitivity and specificity. The effects of various antigen concentrations on the

results obtained with positive and negative control sera are shown in Table 1. It is evident that the range of optimal antigen concentrations yielding a sensitive and specific response is narrow. False-positive results occur at lower antigen concentrations, although false-negative results might occur at higher antigen concentrations in sera containing low concentrations of IgM antibodies to *T. gondii*. To attain a high degree of specificity and sensitivity, it was found that a *Toxoplasma* antigen concentration of 3.3 to 10^7 to 3.6×10^7 organisms per ml was optimal.

Results of IgM-ISAGA in sera. After the optimal antigen concentrations were defined, the following five groups of sera were tested in IgM-ISAGA: (A) 25 sera that were obtained from uninfected individuals and that were negative in the DT; (B) 25 sera that were obtained from individuals with chronic (latent) infection and that were positive in the DT but negative in both the IgM-IFA test and DS-IgM-ELISA; (C) 25 sera that were obtained from individuals with a history of recent acute *Toxoplasma* infection and that were positive in the DT, negative in the IgM-IFA test, and positive in DS-IgM-ELISA; (D) 25 sera that were obtained from individuals with a history of recent acute *Toxoplasma* infection and that were positive in the DT, positive in the IgM-IFA test, and positive in DS-IgM-ELISA; and (E) 50 sera that were obtained from uninfected individuals and from patients with congenital or acute acquired *Toxoplasma* infections and that were tested in IgM-ISAGA in both the Palo Alto and the Paris laboratories.

The IgM-ISAGA results in sera from groups

A and B were all negative. In contrast, IgM-ISAGA was positive in 41 (82%) of 50 sera from groups C and D (Table 2). Of these 50 sera, 25 were negative in the IgM-IFA test despite being obtained from individuals with recently acquired *Toxoplasma* infection. These 25 sera were all positive in DS-IgM-ELISA; 16 of them were positive in IgM-ISAGA. The remaining 25 sera obtained from individuals with a recent history of acute acquired toxoplasmosis were all positive in the DT, the IgM-IFA test, DS-IgM-ELISA, and IgM-ISAGA.

Table 3 summarizes results of IgM-ISAGA performed in our laboratories with the 50 sera in group E. The data demonstrate that whereas negative IgM-ISAGA results were obtained in both laboratories in sera from uninfected individuals or uninfected infants, positive results were obtained in sera from patients with the congenital or acute required infection. Among young infants who have IgG maternal antibody, IgM-ISAGA and DS-IgM-ELISA differentiated between those who were infected and those who were not infected.

Results of IgM-ISAGA in sera containing RF or ANA or both RF and ANA. When 18 serum samples that were obtained from individuals with rheumatoid arthritis or systemic lupus erythematosus and that were positive for RF or for ANA or for both RF and ANA were tested in IgM-ISAGA, all 18 sera were negative (Table 4).

Reproducibility. The negative and positive control sera were tested in IgM-ISAGA 40 times on different plates over a period of 2 weeks. The

TABLE 1. Effects of concentrations of *Toxoplasma* antigen preparations on sensitivity and specificity of IgM-ISAGA^a

Antigen concn (organisms per ml)	Control serum used	Agglutination pattern at serum dilution (reciprocal)							
		16	64	256	1,024	4,096	16,384	65,536	262,144
6.6×10^7	Positive	+++	++	++	+	0	0	0	0
	Negative	0	0	0	0	0	0	0	0
4.4×10^7	Positive	+++	+++	+++	++	++	+	0	0
	Negative	0	0	0	0	0	0	0	0
3.3×10^7	Positive	+++	+++	+++	+++	+++	+++	++	+
	Negative	0	0	0	0	0	0	0	0
2.2×10^7	Positive	+++	+++	+++	+++	+++	+++	+++	++
	Negative	+	+	+	0	0	0	0	0
1.6×10^7	Positive	+++	+++	+++	+++	+++	+++	+++	+++
	Negative	++	++	+	+	0	0	0	0
0.8×10^7	Positive	+++	+++	+++	+++	+++	+++	+++	+++
	Negative	+++	++	+	+	0	0	0	0

^a See the text for a description of positive and negative controls and for patterns of agglutination.

TABLE 2. Results of IgM-ISAGA in sera from uninfected individuals, individuals with chronic *Toxoplasma* infection, and individuals with a recent history of acute toxoplasmosis

Source of sera	No. tested	No. positive (range of titers ^a)			
		DT	IgM-IFA	DS-IgM-ELISA	IgM-ISAGA
Uninfected individuals	25	0 (negative < 4)	0 (negative < 16)	0 (negative < 4)	0 (negative < 16)
Individuals with chronic <i>Toxoplasma</i> infection	25	25 (8–8,000)	0 (negative < 16)	0 (negative < 4)	0 (negative < 16)
Individuals with acute <i>Toxoplasma</i> infection					
Negative IgM-IFA test	25	25 (32–16,384)	0 (negative < 16)	25 (4–16,384)	16 (4–1,024)
Positive IgM-IFA test	25	25 (8–32,768)	25 (8–1,024)	25 (4–16,384)	25 (4–16,384)

^a Titers expressed as reciprocal of serum dilution.

TABLE 3. Results of IgM-ISAGA in 50 sera tested in two different laboratories

Source of sera	No. tested	No. positive (range of titers) in tests performed in:				
		Paris			Palo Alto	
		DT (IU/ml) ^a	IgM-IFA ^b	IgM-ISAGA ^b	IgM-ISAGA ^b	DS-IgM-ELISA ^b
Uninfected individuals	4	0 (negative < 2)	0 (negative < 10)	0 (negative < 10)	0 (negative < 16)	0 (negative < 16)
Individuals with recently acquired <i>Toxoplasma</i> infection	23	23 (2–1,600)	17 (50–1,000)	23 (10–10,000)	22 (16–16,384)	22 (16–16,384)
Uninfected infants (cord blood)	10	10 (100–1,600)	0 (negative < 10)	0 (negative < 10)	0 (negative < 16)	0 (negative < 16)
Infants with congenital <i>Toxoplasma</i> infection	13	13 (100–1,600)	2 (50–100)	10 (10–10,000)	10 (16–256)	10 (64–4,096)

^a Titers expressed as international units per milliliter for results of DT performed in Paris. DT titers in Palo Alto were the same. Sufficient sera for testing in the IgM-IFA test in Palo Alto were not available. Qualitative agreement of results in the IgM-IFA test between the two laboratories is greater than 99%.^b Titers expressed as reciprocal of serum dilution.

negative control serum exhibited negative results 38 times. In the other two tests, the negative control serum exhibited a doubtful ($\pm$) result at 1:16 and 1:64 dilutions. The positive control serum had a titer of 1:16,384 in 31 tests, a titer of 1:4,096 in 3 tests, and a titer of 1:65,536 in 6 tests. The variation in titers observed for positive test sera was not greater than fourfold in tests performed on different plates on different days with both low- and high-positive sera.

DISCUSSION

This study describes the development of IgM-ISAGA and its application for the diagnosis of acute acquired and congenital *Toxoplasma* infections. This method is more specific and sensitive than the IgM-IFA test. Serum samples obtained from uninfected individuals, cord blood samples obtained from uninfected infants, and

sera obtained from individuals with chronic (latent) infection with *T. gondii* were all negative in IgM-ISAGA. Furthermore, sera that contained RF or ANA or both RF and ANA were also negative in IgM-ISAGA. These results revealed that IgM-ISAGA is more specific than the IgM-IFA test in which false-positive results occur in certain sera containing RF or ANA or both (1, 5, 12). In addition to avoiding false-positive results due to RF and ANA, IgM-ISAGA avoids those false-positive results known to occur in the direct *Toxoplasma* agglutination test due to "natural" IgM antibodies (9, 10). We have previously noted that such false-positive results can be eliminated in the direct agglutination test by the use of 2-mercaptoethanol in the diluent for the serum samples (6). However, ablation of IgM activity by the use of 2-mercaptoethanol restricts the direct agglutination test

TABLE 4. Results of IgM-ISAGA in sera containing RF or ANA or both

Patient no.	Serological test results					
	RF ^a	Fluorescent ANA ^b	DT ^c	IgM-IFA ^c	DS-IgM-ELISA ^c	IgM-ISAGA ^c
1	pos	neg	128	256 ^d	neg	neg
2	pos	neg	512	64 ^d	neg	neg
3	pos	neg	1,024	32	neg	neg
4	pos	neg	256	32	neg	neg
5	pos	neg	64	32	neg	neg
6	pos	neg	64	32	neg	neg
7	pos	neg	128	32	neg	neg
8	neg	pos	neg	neg	neg	neg
9	neg	pos	32	neg	neg	neg
10	neg	pos	256	neg	neg	neg
11	neg	pos	neg	neg	neg	neg
12	neg	pos	neg	neg	neg	neg
13	pos	pos	neg	neg	neg	neg
14	pos	pos	neg	neg	neg	neg
15	pos	pos	neg	neg	neg	neg
16	pos	pos	neg	neg	neg	neg
17	pos	pos	neg	neg	neg	neg
18	pos	pos	512	256 ^d	neg	neg

^a Positive (pos) titer = >20.^b Positive (pos) titer = >40.^c Titers expressed as reciprocal of serum dilution; negative (neg) = DT (<4), IgM-IFA (<16), DS-IgM-ELISA (<4), IgM-ISAGA (<16).^d Sera were negative in the IgM-IFA test after absorption of sera with latex particles coated with human IgG (12). DT titers did not change after absorption, indicating that these sera were obtained from patients with chronic toxoplasmosis.

to the detection of IgG antibodies to *T. gondii*. IgM-ISAGA detects these IgM antibodies which are so significant for diagnostic purposes.

The higher degree of sensitivity of IgM-ISAGA as compared with the IgM-IFA test was demonstrated by results obtained in sera from individuals with a recent history of acute toxoplasmosis. The sera that were positive in the IgM-IFA test were all positive in IgM-ISAGA. Furthermore, of 31 sera that were obtained from individuals with acute toxoplasmosis and that were all negative in the IgM-IFA test, 21 (67.7%) were positive IgM-ISAGA. When sera from infants with congenital toxoplasmosis were tested, 10 (76.9%) of 13 were positive in IgM-ISAGA, whereas only 2 (15.3%) of 13 were positive in the IgM-IFA test. In addition to the higher frequency of detection of IgM antibodies in both acutely acquired and congenitally infected patients, IgM-ISAGA exhibited higher titers of IgM antibodies as compared with titers in the IgM-IFA test. Thus, IgM-ISAGA is significantly more specific and sensitive than the IgM-IFA test in detection of IgM antibodies to *T. gondii*.

The greater specificity and sensitivity of IgM-ISAGA as compared with the IgM-IFA test can be attributed to the fact that serum IgM and IgG fractions are separated during the initial step of the test. (Separation of serum IgM from

IgG antibodies to *T. gondii* also results in increased sensitivity of the IgM-IFA test and DS-IgM-ELISA [8, 15-18].)

A comparison of the results of IgM-ISAGA with those of DS-IgM-ELISA recently developed by us (15-17) revealed that these two assays share the same degree of specificity. However, IgM-ISAGA appears to be slightly less sensitive than DS-IgM-ELISA. Of the group of 25 sera that were obtained from acutely infected individuals and that were all negative in the IgM-IFA test, all were positive in DS-IgM-ELISA and 16 were positive in IgM-ISAGA. It should be pointed out, however, that IgM-ISAGA is simpler to perform since it uses only *Toxoplasma* organisms instead of both the *Toxoplasma* sonicated antigens and the enzyme-conjugated antibodies to *T. gondii* which are used in DS-IgM-ELISA. The latter reagents also require careful titration to determine their optimal dilutions. Furthermore, DS-IgM-ELISA requires special and expensive equipment for determination of absorbance of the enzymatic product, whereas IgM-ISAGA results are easily and accurately recorded visually and thus enable the performance of the test in any laboratory.

Of the 50 sera tested in IgM-ISAGA in two different laboratories, there was excellent qualitative agreement in 49 samples. One sample was

found to be positive (1:100) in Paris and negative in Palo Alto. Since no serum remained for additional testings, we are unable to explain these differences. Our purpose in performing the tests in the same sera in both laboratories was solely to determine whether there was qualitative agreement. The quantitative differences may be more apparent than real since different reagents (e.g., anti-human IgM antibodies and different batches of antigen) were used in the two laboratories.

To achieve qualitative agreement of results, it will be necessary to standardize the reagents and the control sera used in the test. Certainly, this has been the case for all serological tests for toxoplasmosis (20), including the Sabin-Feldman DT. We conclude that IgM-ISAGA is an assay that is sensitive, specific, reproducible, and easy to perform and that can be used for the diagnosis of acute acquired and congenital infections with *T. gondii*. The advantages of this assay also suggest that IgM-ISAGA should be applied for the diagnosis of other infectious diseases.

ACKNOWLEDGMENTS

We thank Douglas Guptill for his excellent technical assistance.

LITERATURE CITED

1. Anderson, S. E., and J. S. Remington. 1975. The diagnosis of toxoplasmosis. *South. Med. J.* **68**:1433-1443.
2. Araujo, F. G., E. V. Barnett, L. O. Gentry, and J. S. Remington. 1971. False-positive anti-Toxoplasma fluorescent-antibody tests in patients with antinuclear antibodies. *Appl. Microbiol.* **22**:270-275.
3. Camargo, M. E., A. W. Ferreira, J. R. Mineo, C. K. Takiguti, and O. S. Nakahara. 1978. Immunoglobulin G and immunoglobulin M enzyme-linked immunosorbent assays and defined toxoplasmosis serological patterns. *Infect. Immun.* **21**:55-58.
4. Camargo, M. E., P. G. Leser, and W. S. Leser. 1976. Diagnostic information from serological tests in human toxoplasmosis. I. A comparative study of hemagglutination, complement fixation, IgG and IgM-immunofluorescence tests in 3,752 serum samples. *Rev. Inst. Med. Trop. Sao Paulo* **18**:215-226.
5. Camargo, M. E., P. G. Leser, and A. Rocca. 1972. Rheumatoid factors as a cause for false positive IgM anti-Toxoplasma fluorescent tests. A technique for specific results. *Rev. Inst. Med. Trop. Sao Paulo* **14**:310-313.
6. Desmonts, G., and J. S. Remington. 1980. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *J. Clin. Microbiol.* **11**:562-568.
7. 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.
8. Filice, G. A., A. S. Yeager, and J. S. Remington. 1980. Diagnostic significance of immunoglobulin M antibodies to *Toxoplasma gondii* detected after separation of immunoglobulin M from immunoglobulin G antibodies. *J. Clin. Microbiol.* **12**:336-342.
9. Fulton, J. D. 1965. Micro-agglutination test for Toxoplasma antibodies. *Immunology* **9**:491-495.
10. Fulton, J. D. 1965. Studies on agglutination of *Toxoplasma gondii*. *Trans. R. Soc. Trop. Med. Hyg.* **59**:694-704.
11. Handman, E., and J. S. Remington. 1980. Serological and immunochemical characterization of monoclonal antibodies to *Toxoplasma gondii*. *Immunology* **40**:579-588.
12. 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.
13. Johnson, G. D., E. J. Holborow, and J. Dorling. 1978. Immunofluorescence and immunoenzyme techniques, p. 15.1-15.30. In D. M. Weir (ed.), *Handbook of experimental immunology*, 3rd ed. Blackwell Scientific Publications, Ltd., Oxford.
14. Nahmias, A. J. 1974. The Torch complex. *Hosp. Pract.* **9**:65-72.
15. 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.
16. Naot, Y., G. Desmonts, and J. S. Remington. 1981. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital Toxoplasma infection. *J. Pediatr.* **98**:32-36.
17. 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.
18. Pyndiah, N., U. Krech, P. Price, and J. Wilhelm. 1979. Simplified chromatographic separation of immunoglobulin M from G and its application to Toxoplasma indirect immunofluorescence. *J. Clin. Microbiol.* **9**:170-174.
19. Remington, J. S., M. M. Bloomfield, E. Russell, Jr., and W. S. Robinson. 1970. The RNA of *Toxoplasma gondii*. *Proc. Soc. Exp. Biol. Med.* **133**:623-626.
20. Remington, J. S., and G. Desmonts. 1976. Toxoplasmosis, p. 191-332. In J. S. Remington and J. O. Klein (ed.), *Infectious diseases of the fetus and newborn infant*. The W. B. Saunders Co., Philadelphia.
21. Remington, J. S., M. J. Miller, and I. Brownlee. 1968. IgM antibodies in acute toxoplasmosis. I. Diagnostic significance in congenital cases and a method for their rapid demonstration. *Pediatrics* **41**:1082-1091.
22. Remington, J. S., M. J. Miller, and I. Brownlee. 1968. IgM antibodies in acute toxoplasmosis. II. Prevalence and significance in acquired cases. *J. Lab. Clin. Med.* **71**:855-866.
23. 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.
24. Voller, A., D. E. Bidwell, A. Bartlett, D. G. Fleck, M. Perkins, and B. Oladehin. 1976. A microplate enzyme-immunoassay for Toxoplasma antibody. *J. Clin. Pathol.* **29**:150-153.
25. Walls, K. W., S. L. Bullock, and D. K. English. 1977. Use of the enzyme-linked immunosorbent assay (ELISA) and its microadaptation for the serodiagnosis of toxoplasmosis. *J. Clin. Microbiol.* **5**:273-277.
26. Welch, P. C., H. Masur, T. C. Jones, and J. S. Remington. 1980. Serologic diagnosis of acute lymphadenopathic toxoplasmosis. *J. Infect. Dis.* **142**:256-264.
27. Wilson, C. B., V. Tsai, and J. S. Remington. 1980. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. *J. Exp. Med.* **151**:328-346.