JOURNAL OF CLINICAL MICROBIOLOGY, July 1996, p. 1606–1609 Vol. 34, No. 7
0095-1137/96/$04.00+0

Bicentric Evaluation of Access Toxo Immunoglobulin M (IgM) and IgG Assays and IMx Toxo IgM and IgG Assays and Comparison with Platelia Toxo IgM and IgG Assays

ANNE DECOSTER[1]* AND BERTRAND LECOLIER[2]

Laboratoire de Microbiologie, Hôpital St. Vincent, 59044 Lille Cedex,[1] and Hôpital Notre Dame du Bon Secours, 75674 Paris Cedex 14,[2] France

Received 21 September 1995/Returned for modification 10 November 1995/Accepted 2 April 1996

The recent Access immunoanalysis system (Sanofi Diagnostics Pasteur) for the serological diagnosis of toxoplasmosis was compared with the Abbott Toxo IMx EIA system, taking the Platelia Toxo immunoglobulin G (IgG) and Platelia Toxo IgM systems as references and using as confirmation methods an indirect fluorescence assay or a dye test for IgG and an immunosorbent agglutination assay (ISAGA) for IgM. A total of 1,461 serum samples were studied, of which 128 were collected from 42 recently seroconverted patients. Sensitivity and specificity rates of the Access system were 97.7 and 99.5%, respectively, for IgM and 98.6 and 100%, respectively, for IgG. Sensitivity and specificity rates of the Abbott IMx EIA system were 91 and 100%, respectively, for IgM and 92.5 and 100%, respectively, for IgG. The Access Toxo IgG and IgM EIA systems were found to be more sensitive than the Abbott Toxo IgG and IgM IMx EIA systems.

Toxoplasmosis, a ubiquitous protozoan infection, is caused by an intracellular parasite, *Toxoplasma gondii*. It is generally benign in healthy people, but it can be serious in the context of immunodeficiency, especially in the case of AIDS and bone marrow or heart transplant patients or in children infected in utero. Early diagnosis of acute toxoplasma infection in pregnant women is of the utmost importance if clinicians are to carry out effective antitoxoplasmic therapy. The prevention of congenital infection requires the identification of nonimmune women at the beginning of pregnancy and serological monitoring of the women to the time of delivery. The diagnosis of acquired infection is based on serological tests which detect immunoglobulin M (IgM) and IgG antibodies. A number of techniques are used to distinguish between recently acquired acute toxoplasmosis and chronic toxoplasmosis (3, 5, 6, 10, 17). In recent years, clinical laboratories have seen a drastic increase in demand for automated detection of antibodies to *T. gondii* by assays based on immunoassay technology (1, 18). In the present study, the performance characteristics of the new Access Toxo IgG and IgM antibody assays on the Access automated analyzer (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were compared with those of IMx Toxo IgG and IgM antibody assays on the IMx automated analyzer (Abbott, Rungis, France) and with those of the Platelia Toxo IgG and IgM (Sanofi Diagnostics Pasteur) systems with respect to reproducibility, relative sensitivity, specificity, and accuracy.

MATERIALS AND METHODS

Access Toxo IgM and IgG antibody immunoassays. Microparticles were coated with *T. gondii* antigen enriched with membrane antigen. These paramagnetic microparticles were left to react with a patient specimen that had undergone instrument-controlled dilution in buffer. Anti-*T. gondii* antibodies bound with the coated microparticles to form an antigen-antibody complex. This reactive mixture was submitted to a magnetic field and was washed to remove unbound materials. Next, anti-human IgM or IgG alkaline phosphatase conjugate was dispensed and bound to the antigen-antibody complex. A luminescent substrate was added, and the luminescence was measured. The reaction depends on the chemiluminescent substrate reagent 4-methoxy-4-(3-phenylphosphate) spiro(1,2-dioxetane-3,2′-adamantane) in LumiPhos 530 (LumiGen, Detroit, Mich.). The results are qualitative and are expressed in international units (IgG) or arbitrary units (AU) (IgM) per milliliter.

Methods and clinical specimens. (i) Clinical specimens. For the anti-toxoplasma IgG investigation, five groups of sera were studied. A total of 1,461 serum samples were used, including 604 serum samples collected from noninfected patients (group 1), 560 serum samples from chronically infected patients (group 2), 128 serum samples from 42 recently seroconverted patients (group 3), 54 serum samples from random blood donors (group 4), and 115 serum samples containing agents that may interfere or cross-react with the assay (group 5). The last group included sera from patients with infectious mononucleosis ($n = 4$), cytomegalovirus infection ($n = 3$), monoclonal gammopathy ($n = 10$), amebiasis ($n = 6$), malaria ($n = 10$), or AIDS ($n = 21$) or patients with rheumatoid factor ($n = 47$), antinuclear antibody ($n = 10$), antimitochondrial antibody ($n = 3$), or antichromosomal antibody ($n = 1$) in their sera. For the anti-toxoplasma IgM investigation, 1,026 serum samples were studied. A total of 609 serum samples were collected from either noninfected patients or chronically infected patients (group 6), 129 serum samples were collected from patients infected for more than 3 months (group 7), 128 serum samples were collected from 42 recently seroconverted patients (group 3), 54 serum samples were obtained from random blood donors (group 4), and 106 serum samples contained agents that may interfere or cross-react with the assay (group 5). This last group included sera from patients with infectious mononucleosis ($n = 3$), cytomegalovirus infection ($n = 3$), monoclonal gammopathy ($n = 10$), amebiasis ($n = 6$), malaria ($n = 10$), or AIDS ($n = 15$) or patients with rheumatoid factor ($n = 46$), antinuclear antibody ($n = 9$), antimitochondrial antibody ($n = 3$), or antichromosomal antibody ($n = 1$) in their sera.

(ii) Procedure. To evaluate reproducibility and precision, the 10 groups of serum samples were tested. They included negative samples as well as samples with low, medium, and high levels of IgG or IgM antibodies to *T. gondii*. They were tested by the Platelia tests to determine their titers prior to inclusion in the panel. For the IgG investigation, the titers in the serum samples were <6, 10 to 12, 70 to 120, 120 to 180, and 220 to 320 IU/ml in groups 1 to 5, respectively. For the IgM investigation, the titers in the serum samples were <100, 220 to 320, 300 to 400, 400 to 500, and 550 to 650 AU/ml in groups 1 to 5, respectively. They were all tested 30 times in 1 run and 3 times in 10 runs (2 runs per day for 5 days). After successfully completing reproducibility testing, evaluation of the clinical specimens began.

The clinical specimens were submitted to our laboratories for diagnostic purposes and were first characterized by an agglutination test with and without β-mercaptoethanol (Biomérieux, Marcy l'Etoile, France), an indirect IgG enzyme-linked immunosorbent assay (ELISA), and a double-sandwich ELISA for the detection of IgM antibodies directed against the *T. gondii* major surface protein P30 (Platelia Toxo IgG or IgM; Sanofi Diagnostics Pasteur). They were subsequently classified into groups according to the clinical and serological results and were stored at −20°C. Sera were first submitted in duplicate to the

* Corresponding author. Mailing address: Hôpital St Vincent, Laboratoire de Microbiologie, Bvd. de Belfort, 59044 Lille Cedex, France. Phone: 20.87.45.03.

TABLE 1. Reproducibility of Access Toxo IgM and IgG assays

Serum sample	Access Toxo IgM assay[a]			Access Toxo IgG assay[b]		
	Mean value (AU/ml)	Within-run CV (%)	Between-run CV (%)	Mean value (IU/ml)	Within-run CV (%)	Between-run CV (%)
Negative	4.12	NA[c]	NA	0.02	NA	NA
Low	296	5.38	7.13	13	7.96	14.57
Medium 1	362	4.15	7.74	101	8.76	17.14
Medium 2	499	4.54	5.87	135	10.21	16.01
High	716	3.23	4.29	248	8.17	15.91

[a] Cutoff, 100 AU/ml.
[b] Cutoff, 6 IU/ml.
[c] NA, not applicable.

Platelia Toxo IgG and IgM assays and were then tested by the Access Toxo IgG and IgM assays (Sanofi Diagnostics Pasteur). Among the serum samples, 481 were tested by the IMx Toxo IgM assay (Abbott, Rungis, France) and 737 were tested by the IMx Toxo IgG assay (Abbott). Results for sera with discrepant results for IgG antibodies were then verified by a dye test or an immunofluorescent assay (IFA IgG; Biomérieux), while an immunosorbent agglutination assay (ISAGA IgM; Biomérieux) was used to verify the results for serum samples with discrepant results for IgM antibody. All assays were conducted as recommended by the manufacturers, and the results were considered positive when they met the following criteria (expressed as an index, in international units, or in AU): index, ≥100 AU/ml for the Access Toxo IgM assay, AU/ml >0 for the Platelia Toxo IgM assay, ≥0.5 AU/ml for the IMx Toxo IgM, assay, and >8 AU/ml for ISAGA IgM; IgG antibody titer, ≥6 AU/ml for the Access Toxo IgG assay, ≥6 IU/ml for the Platelia Toxo IgG and the IMx Toxo IgG assays, >2 IU/ml for the dye test, and >5 IU/ml for the IFA IgG.

For the anti-toxoplasma IgG investigation, the specificity rate was calculated by testing 604 serum samples collected from noninfected patients (group 1), and the sensitivity rate was calculated by testing 560 serum samples collected from chronically infected patients (group 2). For the anti-toxoplasma IgM investigation, the specificity rate was calculated by testing 609 serum samples collected from both noninfected and chronically infected patients (group 6), and the sensitivity rate was calculated by testing 129 serum samples from patients infected for more than 3 months (group 7).

RESULTS

The within-run and between-run reproducibility rates, calculated as the coefficient of variation (CV), were between 3.23 and 7.74% for serum samples tested by the Access IgM and between 7.96 and 17.14% for those tested with the Access Toxo IgG assay. The results are presented in Table 1.

The relative sensitivity, specificity, and accuracy rates for sera from uninfected or chronically infected patients, patients infected for more than 3 months, recently seroconverted patients, random blood donors, and sera containing agents that may interfere or cross-react with the assay are summarized in Tables 2 to 4 and Tables 5 to 7, which illustrate the comparative performances of the Access, Platelia, and IMx Toxo IgG assays and the Access, Platelia, and IMx Toxo IgM assays, respectively. In the comparison study of the Platelia and Access Toxo IgG assays, 10 serum samples had discrepant results; 8 serum samples from group 2 and 2 serum samples from group 3 were Platelia Toxo IgG assay positive (titers, 12 to 21 IU/ml) but Access Toxo IgG assay negative (titers, 2.7 to 5.1 IU/ml). All of these serum samples were positive by the dye test (titers, 4 to 32 IU/ml) as well as IFA (titers, 5 IU/ml). In the study comparing the Platelia and IMx Toxo IgG assays, 31 serum samples had discrepant results: 23 serum samples from group 2 and 3, 4, and 1 serum samples from groups 3, 4, and 5, respectively. They were Platelia Toxo IgG assay positive (titers, 7 to 21 IU/ml) but IMx Toxo IgG assay negative (titers, 0 to 5.7 IU/ml). All of these serum samples were positive by IFA (titers, 5 to 25 IU/ml). In the comparison study of the IMx and Access Toxo IgG assays, 25 serum samples had discrepant results: 17 serum samples from group 2 and 3, 4, and 1 serum samples from groups 3, 4, and 5, respectively. They were IMx Toxo IgG assay negative (titers, 0 to 5.7 IU/ml) but Access Toxo IgG assay positive (titers, 6 to 28 IU/ml). All of these serum samples were positive by IFA (titers, 5 to 25 IU/ml). Data obtained for three serum samples collected early from seroconverted patients should be highlighted. Their titers, as measured by the Platelia, IMx, Access, and IFA IgG assays, were 14, 5, 28, and 10 IU/ml, respectively (serum collected 1 month after a negative result); 20, 0, 26, and 25 IU/ml, respectively (serum collected 1.5 months after a negative result); and 11, 0, 8, and 10 IU/ml, respectively (serum collected 1 month after a negative result).

In the study comparing the Platelia and Access Toxo IgM assays, 16 serum samples had discrepant results: 8 were Platelia Toxo IgM assay negative but Access Toxo IgM assay doubtful or positive (titers, 101 to 224 AU/ml), and all of them were ISAGA IgM negative (index, 4 to 6); 8 were Platelia Toxo IgM assay positive (index, 6 to 9.4) but Access Toxo IgM assay negative (titers, 27 to 99 AU/ml), while 4 were ISAGA IgM positive (index, 8 to 12). In the study comparing the Platelia and IMx Toxo IgM assays, 5 serum samples had discrepant results: all were Platelia Toxo IgM assay positive (index, 2 to 5) but were IMx Toxo IgM assay negative (index, 0.21 to 0.47), while all of them were ISAGA IgM positive (index, 8 to 9). In the study comparing the IMx and Access Toxo IgM assays, 10 serum samples had discrepant results: 7 were IMx Toxo IgM assay negative (index, 0.21 to 0.57) but Access Toxo IgM assay positive (index, 166 to 224 AU/ml), while 6 of them were ISAGA IgM positive (index, 8 to 9); 3 serum samples were IMx Toxo IgM assay positive (index, 1.27 to 1.51) but Access Toxo IgM assay negative (index, 36 to 78), while all serum samples

TABLE 2. Comparison of performances of Access and Platelia Toxo IgG assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated results			% Agreement
		Platelia Toxo IgG negative, Access negative	Platelia Toxo IgG positive		
			Access negative	Access positive	
1	604	604			100
2	560		8	552	98.6
3	128	49	2	77	98.4
4	54	30		24	100
5	115	19		96	100
Total	1,461	702	10	749	99.3

[a] $r = 0.74$ between the two assays.

TABLE 3. Comparison of performances of IMx and Platelia Toxo IgG assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated results			% Agreement
		Platelia Toxo IgG negative, IMx negative	Platelia Toxo IgG positive		
			IMx negative	IMx positive	
1	302	302			100
2	309		23	286	92.5
3	61	20	3	38	95
4	25	5	4	16	84
5	40	11	1	28	97.5
Total	737	338	31	368	95.8

[a] $r = 0.47$ between the two assays.

TABLE 5. Comparison of performances of Access and Platelia Toxo IgM assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated results				% Agreement
		Platelia Toxo IgM		Platelia Toxo IgM positive		
		Access negative	Access positive	Access negative	Access positive	
6	609	605	4			99.5
7	129			4	125	97.7
3	128	43		1	84	99.2
4	54	53	1			98.1
5	106	100	3	3		94.3
Total	1,026	801	8	8	209	98.4

[a] $r = 0.87$ between the two assays.

were ISAGA IgM positive (index, 12). In addition, one of the serum samples with a discrepant result, collected 1 month after seroconversion, showed the following results: an index of 9 by the Platelia Toxo assay, an index of 1.51 by the IMx Toxo assay, titer of 78.5 AU/ml by the Access Toxo assay, and an index of 12 by ISAGA IgM.

The difference between the mean for the entire population that was clinically negative for *T. gondii* and the Access Toxo IgM assay cutoff of 100 AU/ml was calculated to be 4.86 standard deviations, and 97.7% of the serum samples had an index lower than 50 (i.e., mean value, 2.2 standard deviations). The difference between the mean for the entire population that was clinically negative for *T. gondii* and the Access Toxo IgG assay cutoff of 6 IU/ml was calculated to be 4 standard deviations, and 98% of the serum samples had a titer of less than 2 IU/ml.

Table 8 illustrates the performances (specificity, sensitivity, percent agreement, and correlation coefficient) of the three assays for the total population tested.

DISCUSSION

A number of techniques have been applied to detect IgM and IgG antibodies in patients with toxoplasma infections, including the dye test (4), direct agglutination assay (5, 7), IFA (8, 16), ELISA (2, 17, 20, 21), IgG avidity analysis (10, 11), ISAGA (6, 15), immunoblotting (9, 12, 14, 19), and enzyme-linked immunofiltration assay (13). Because of the practicability, rapidity, and reliability of immunoassays, automated detection of antibodies to *T. gondii* on the basis of immunoassay technology has gained widespread use over the past few years in clinical laboratories (1, 18).

The analysis of serum samples with a variety of characteristics has confirmed the value and reliability of indirect ELISA for the detection of IgG anti-toxoplasma antibodies and of the double-sandwich ELISA for the detection of antibodies directed against p30, the major *T. gondii* surface protein. This enabled us to use the Platelia Toxo IgM and IgG assays as reference methods (2, 17, 21). Indeed, detailed studies have shown that the early immune response, of which IgM and IgG are components, is primarily directed against membrane antigens of the parasite, whereas antibody-recognizing cytoplasmic antigens are formed as the immune response matures (12). Consequently, methods that use membrane antigens, such as the Platelia or Access Toxo IgM and IgG assays, are recommended for use in the investigation of acute acquired toxoplasmosis because of the enhanced sensitivities of these assays compared with those of methods which focus on the detection of antibodies against the various toxoplasma antigens.

Evaluation of clinical specimens for IgM- and IgG-specific antibodies to *T. gondii* was performed by using the Access Toxo IgM and IgG antibody assays. The assays proved to be highly reproducible both within runs and between runs. Compared with the Platelia Toxo IgM and IgG assays, the Access Toxo IgM antibody assay was 97.7% sensitive and 99.5% specific and the Access Toxo IgG antibody assay was 98.6% sensitive and

TABLE 4. Comparison of performances of Access and IMx Toxo IgG assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated results			% Agreement
		IMx Toxo IgG		IMx Toxo IgG positive, Access positive	
		Access negative	Access positive		
1	302	302			100
2	309	6	17	286	94.5
3	61	20	3	38	95
4	25	5	4	16	84
5	40	11	1	28	97.5
Total	737	344	25	368	96.6

[a] $r = 0.81$ between the two assays.

TABLE 6. Comparison of performances of IMx and Platelia Toxo IgM assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated result			% Agreement
		Platelia Toxo IgM negative, IMx negative	Platelia Toxo IgM positive		
			IMx negative	IMx positive	
6	302	302			100
7	56		5	51	91
3	61	20		41	100
4	25	25			100
5	37	36		1	100
Total	481	383	5	93	98.8

[a] $r = 0.91$ between the two assays.

TABLE 7. Comparison of performances of Access and IMx Toxo IgM assays[a]

Patient group	No. of serum samples	No. of serum samples with indicated results				% Agreement
		IMx Toxo IgM negative		IMx Toxo IgM positive		
		Access negative	Access positive	Access negative	Access positive	
6	302	301	1			99.7
7	56		5	1	50	89.3
3	61	20		1	40	98.4
4	25	25				100
5	37	35	1	1		94.6
Total	481	381	7	3	90	98

[a] $r = 0.81$ between the two assays.

100% specific for the detection of *T. gondii* in the population of blood donors, pregnant women, patients demonstrating seroconversion to *T. gondii* infection, and individuals with serum containing agents that may interfere or cross-react with the assay. With sera from the same population, the IMx Toxo IgM antibody assay was 91% sensitive and 100% specific and the IMx Toxo IgG antibody assay was 92.5% sensitive and 100% specific. The Access Toxo IgM and IgG assays were more sensitive than the IMx Toxo IgM and IgG assays. The level of agreement between the various assays was greater than 95%. The correlation between the different assays was good ($r >$ 0.81) except for that between the Access and Platelia Toxo IgG assays ($r = 0.74$) and particularly that between the IMx and Platelia Toxo IgG assays ($r = 0.47$). Good separation of the negative samples from the assay cutoff was demonstrated for the Access Toxo IgM and IgG antibody assays. In addition, the ability of the Access Toxo IgM and IgG assays to be used for the diagnosis of early acute primary toxoplasmosis as well as the greater range of titers that can be measured by the Access Toxo IgG assay (0 to 500 IU/ml) are worth noting.

The Access Toxo IgM and IgG antibody assays, performed on the Access analyzer, were proven to be fast, fully automated procedures for the sensitive, specific, and reproducible measurement of IgM and IgG antibodies to *T. gondii*. This method, used for the determination of maternal anti-*T. gondii* IgM and IgG antibodies, is of clinical value for the physician as an indicator of maternal infection. These assays must now be evaluated in the context of congenital toxoplasmosis.

TABLE 8. Comparison of performances of Access, Platelia, and IMx Toxo IgM and IgG assays

Assay	Specificity (%)	Sensitivity (%)	% Agreement	*r*
Toxo IgM				
Access vs Platelia	99.5	97.7	98.4	0.82
IMx vs Platelia	100	91	98.8	0.91
Access vs IMx			98	0.81
Toxo IgG				
Access vs Platelia	100	98.6	99.3	0.74
IMx vs Platelia	100	92.5	95.8	0.47
Access vs IMx			96.6	0.81

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