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Detection of Anti-Toxoplasma Immunoglobulin A Antibodies by Platelia-Toxo IgA Directed against P30 and by IMx Toxo IgA for Diagnosis of Acquired and Congenital Toxoplasmosis

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Platelia-Toxo IgA and IMx Toxo IgA assays were used with 260 serum samples, of which 93 were from seroconverted patients, 58 were from 21 congenitally infected children, and 109 were from uninfected patients, to detect anti-P30 immunoglobulin A antibodies. Because of its enhanced sensitivity, Platelia-Toxo IgA is more efficient in diagnosing acute or congenital toxoplasmosis. IMx Toxo IgA must not be used to diagnose congenital toxoplasmosis.

A new immunoassay, IMx Toxo IgA (Abbott Diagnostics, Rungis, France), was recently developed for the measurement of toxoplasma-specific immunoglobulin A (IgA) (1). We have compared the findings of this test with those of Platelia-Toxo IgA (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France), a double-sandwich enzyme-linked immunosorbent assay (ELISA) aimed at detecting IgA antibodies directed against P30, the major *Toxoplasma gondii* membrane protein, which we have shown to be of interest in the early diagnosis of seroconversion and of congenital infection in children whose mothers had seroconverted during pregnancy (4).

Patients and methods. Two hundred sixty serum samples were evaluated: 93 from women infected during pregnancy, 30 from pregnant women without toxoplasma infection, 30 from pregnant women with chronic toxoplasma infection, 58 from congenitally infected children, 30 from healthy newborns, and 19 from patients whose sera contained rheumatoid factor, antinuclear antibodies, circulating immune complexes, anti-human immunodeficiency virus (HIV) antibodies, anti-cytomegalovirus IgM antibodies, or anti-Epstein-Barr virus IgM antibodies.

Serum samples from 21 congenitally infected children were collected at birth ($n = 13$), in the first month of life ($n = 7$), in the second month ($n = 7$), in the third month ($n = 5$), in the fourth month ($n = 7$), in the fifth month ($n = 9$), in the sixth month ($n = 6$), and after the sixth month of life ($n = 4$). Four samples were collected from a child with both toxoplasma and HIV infection.

Platelia-Toxo IgA and IMx Toxo IgA assays were carried out by following the manufacturer's instructions.

Acquired toxoplasmosis. No false-positive reactions were recorded by either Platelia-Toxo IgA or IMx Toxo IgA, despite the inclusion of 19 specimen types which have been associated with nonspecific reactions in other serological assays. In particular, no false-positive reactions were observed in sera collected from 60 pregnant women, of whom 30 were seronegative and 30 were in the course of chronic toxoplasmosis (they were sampled more than 3 years after the onset of the infection). We did not find a significant difference in the specificities of the two IgA assays.

Ninety-seven serum samples collected from 29 seroconverted patients and 4 samples from a patient infected with HIV were tested. In the 75 serum samples collected in the first months after seroconversion, anti-P30 IgA antibodies were present simultaneously with antitoxoplasma IgA antibodies detected by IMx Toxo IgA. In the 14 serum samples collected in the subacute phase of toxoplasmosis, no anti-P30 antibodies were detected by Platelia-Toxo IgA but specific IgA antibodies were detected by IMx Toxo IgA. IgA antibodies detected by IMx Toxo IgA disappeared before anti-P30 IgA antibodies disappeared (at 2 to 11 months). In eight samples, no specific IgA antibodies were detected; four samples were collected in the chronic phase of toxoplasmosis (14 months, 18 months, 2 years, and 3 years after seroconversion), and four were collected from a patient infected with HIV with clinical signs of cerebral toxoplasmosis.

The correlation coefficient of the values obtained for these 97 samples is 0.674, and 14.5% of the results are discrepant.

Congenital toxoplasmosis. The levels of detection of antitoxoplasma IgA antibodies in 88 samples collected from 21 congenitally infected children and 30 uninfected neonates have been compared.

At birth, the serum from one uninfected child was weakly positive for anti-P30 IgA antibodies (fixation index, 1.3) but negative for specific IgA detected by IMx Toxo IgA. These false-positive data are likely due to contamination by maternal blood, since sera from the children were negative at day 7 for anti-P30 IgA antibodies. For 29 of the 30 uninfected children, neither Platelia-Toxo IgA nor IMx Toxo IgA gave positive results with samples taken at birth or in the months following birth.

Among the 21 infected children, anti-P30 IgA antibodies were present in 20 infants, whereas specific IgA antibodies detected by IMx Toxo IgA were present in 5 of them, either at birth (only 13 children were tested at birth [Table 1]) or in the following weeks (Table 2). Among the 58 samples tested, 37 were positive by Platelia-Toxo IgA and 15 were positive by Abbott's IMx Toxo IgA. All the sera positive by Abbott's IMx Toxo IgA were positive by Platelia-Toxo IgA.

One child was congenitally coinfected with toxoplasma and HIV; specific IgA antibodies were present at birth and were still detectable by Platelia-Toxo IgA but not by IMx Toxo IgA after 4 years of life. No specific IgM antibodies were ever detected in sequential samples by Platelia-Toxo IgA.

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TABLE 1. Detection of specific anti-P30 IgM and IgA antibodies obtained in samples from 13 congenitally infected children tested at birth

Patient	Level of IgG (IU/ml)	Detection by Platelia-Toxo IgA of:			Detection by IMx Toxo IgA of IgA (FI)[c]
		Anti-P30 IgM (FI)[a]	Anti-P30 IgA (FI) at dilution[b]		
			1/100	1/20	
1	300	17	OL[d]	OL	1.112
2	950	0	22	OL	1.011
3	100	17	OL	OL	0.722
4	700	12	9	14	0.689
5	950	0	OL	OL	0.569
6	250	15	9	14	0.402
7	1,500	0	14	OL	0.261
8	100	9	OL	OL	0.206
9	250	19	5.2	20	0.119
10	1,700	0	10	14.8	0.048
11	0	16	3	4.7	0.045
12	2,300	0	3	5.6	0.029
13	150	8	2	4.7	0.025

[a] FI, fixation index.
[b] Two serum dilutions were recommended by the manufacturer for Platelia-Toxo IgA.
[c] Cutoff for detection, 0.500.
[d] OL, over limits.

A comparison of the results described below for the detection by Platelia-Toxo IgA and IMx Toxo IgA of IgA antibodies in sera of children tested at birth was performed. The correlation coefficient was 0.517. For the two methods performed on samples taken at birth, the specificities were similar (30 of 30 samples tested positive, i.e., 100% for IMx Toxo IgA, versus 29 of 30 samples, i.e., 97% for Platelia-Toxo IgA), but the sensitivity of Platelia-Toxo IgA for the detection of anti-P30 IgA antibodies was better (5 of 13 samples, i.e., 38% for IMx Toxo IgA, versus 13 of 13 samples, i.e., 100% for Platelia-Toxo IgA). Differences among the diagnostic sensitivities are statistically highly significant for a risk factor (α) of 5% (χ^2 test).

Discussion. The clinical interest in the detection of specific antitoxoplasma IgA antibodies for the early diagnosis of acute acquired and congenital toxoplasma infection has been evaluated during the past few years and is now well established (2–4, 6, 7, 10–13, 17).

This report presents a comparison between Platelia-Toxo IgA, used to detect IgA antibody response directed against P30, the major surface antigen of *T. gondii* (5, 9), and IMx Toxo IgA, a new commercial kit for the early diagnosis of acquired and congenital toxoplasmosis (1).

TABLE 2. Detection of specific IgA antibodies by Platelia-Toxo IgA and IMx Toxo IgA in 58 serum samples from 21 congenitally infected children

Age (mo)	No. of children tested	No. of serum samples for:	
		Anti-P30 IgA (by Platelia-Toxo IgA)	Total specific IgA (by IMx Toxo Iga)
Neonate	13	13	5
0–1	7	4	2
1–2	7	6	2
2–3	5	3	1
3–4	7	5	1
4–5	9	2	1
5–6	6	1	1
>6	4	3	2
Total	58	37	21

As demonstrated by immunoblotting, IgA antibodies to a variety of antigens are present at the various stages of toxoplasmosis. Interestingly, only the antigens with molecular weights of 35,000 and 30,000 (P30) were recognized by the IgA antibodies from congenitally infected infants (8, 9, 11, 14, 17). In a previous work (4), we have shown that the detection of anti-P30 antibodies, present in infected children in either the antenatal, neonatal, or postnatal period, was very helpful, especially in the absence of clinical signs. This is of the utmost importance for better management of efficient antitoxoplasmic therapy.

In congenital toxoplasmosis, the sensitivity of IMx Toxo IgA is relatively low. The enhanced sensitivity of the Platelia-Toxo IgA compared with that of the IMx Toxo IgA is probably due to the use, in Platelia-Toxo IgA, of a monoclonal antibody directed against P30, the major toxoplasma membrane antigen, which is recognized to elicit a very early and intense antibody response (7, 15, 16). Detailed studies have shown that the early immune response, of which IgM and IgA are components, is primarily directed against membrane antigens of the parasite, whereas antibody-recognizing cytoplasmic antigens are formed as the immune response matures (8, 11). In addition, Platelia-Toxo IgA is a double-sandwich ELISA, which is recognized as more sensitive than the direct ELISA used by IMx Toxo IgA technology (18).

During the acute phase of toxoplasmosis, we detected IgA antibodies directed against P30 in the sera of all patients. In the majority of cases, when the level of IgG continued to rise and IgM antibodies persisted (residual IgM), IgA antibodies disappeared earlier (between 3 and 9 months) and were not detected in the chronic phase of toxoplasmosis, as described in other reports (1, 3, 7, 17).

We did not find a significant difference between the sensitivities of the two IgA assays. In particular, early diagnosis of acute toxoplasmosis can be obtained with sufficient efficacy by the two methods. Nevertheless, Platelia-Toxo IgA is more efficient in establishing the date of infection, because anti-P30 IgA antibodies disappeared earlier than other specific IgA antibodies.

As previously noticed (12, 17), patients with primary toxoplasmosis associated with AIDS were found consistently to produce specific IgM and IgA, whereas there were no significant differences in the frequencies of detectable IgA between AIDS patients with chronic toxoplasmosis and those with secondary reactivation resulting in cerebral disease.

No false-positive reactions were recorded, despite the evaluation of a large number of samples and the inclusion of specimen types which have been associated with nonspecific reactions in other serological assays. We did not find a significant difference in the specificities of the two IgA assays.

The data obtained with the Platelia-Toxo IgA kit extend and confirm the interest in the use of anti-P30 IgA in the early diagnosis of acquired and particularly of congenital toxoplasmosis; anti-P30 IgA antibodies were present more frequently than other IgA antitoxoplasma antibodies in congenitally infected children. Furthermore, it is clearly indicated by our study that the testing of IgA in newborns is essential for a more efficient diagnosis of infection and that Platelia-Toxo IgA is a simple and reliable method for early detection of IgA antibodies directed against P30, the major membrane antigen of *T. gondii.*

We have shown that toxoplasma-specific IgA can be mea-

sured precisely by IMx Toxo IgA and Platelia-Toxo IgA in seroconverted patients. We recommend that Platelia-Toxo IgA be used for estimating the duration of toxoplasma infection, because of the shorter antibody response, and for the investigation of congenital toxoplasmosis, because of the enhanced sensitivity of this assay.

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