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Comparison of a Commercial Enzyme Immunoassay and an Immunoblot Technique for Detection of Immunoglobulin A Antibodies to *Toxoplasma gondii*

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A commercial enzyme immunoassay (Platelia-Toxo IgA) and an immunoblot technique were compared with regard to their ability to detect IgA antibodies to the major surface protein P30(SAG1) of *Toxoplasma gondii* in 105 serum samples from patients with suspected or proven acquired toxoplasmosis. Comparison of the IgA-EIA with the immunoblot technique showed a concordance of 81.0 %, with a sensitivity of 92.6 % and a specificity of 78.4 %. Due to its high sensitivity the IgA-EIA might detect IgA antibodies against *Toxoplasma gondii* at an early stage of infection, although excessive sensitivity could lead to detection of IgA antibodies for an extended period of time following the onset of infection.

Since *Toxoplasma gondii* is difficult to isolate from clinical specimens, diagnosis of acute or reactivated toxoplasmosis is based on serological tests. Tests to detect IgG antibody are generally used to identify patients with past infection, whereas tests to detect IgM antibody are used to document recent infection (1–4). However, in some instances tests for detection of IgM may be of low diagnostic value (5, 6).

The presence of IgA antibodies in infants indicates congenital infection because IgA, like IgM, does not cross the placenta (7). However, it is not clear whether IgA antibodies persist as long as IgM antibodies. Some investigators have reported persistence of specific IgA several months after documented infection (8), whereas others detected specific IgA mainly in sera of patients with acute infection (7, 9–11) and rarely in chronically infected patients (11). It thus seems critical to establish the value of serological methods to detect IgA antibodies in the diagnosis of acute or reactivated toxoplasmosis.

Recent studies have shown that detection of IgA antibodies against the major surface protein P30 (SAG1) aided the early serological diagnosis of congenital and acute toxoplasmosis (9). We recently reported the development of an immunoblot technique for detection of antibodies to P30(SAG1) (12, 13). In the present study we evaluated a commercial enzyme immunoassay (EIA) for detection of IgA (14) in comparison to the immunoblot technique and conventional serological tests.

Materials and Methods. A total of 105 serum samples submitted to our laboratory for *Toxoplasma gondii* antibody studies were subjected to the dye test (1), indirect immunofluorescence assay (2), complement fixation test (3), IgM and IgA immunoblot (12), and IgM and IgA-EIA (Platelia-Toxo, Diagnostics Pasteur, Germany) (4, 14). The IgA-EIA was used as described in the supplier's manual. The 105 serum samples were from 82 patients who were classified as having no infection, latent or chronic infection or acute infection, as recently suggested (6). In addition, a fourth group of patients was established that was characterized by persistent IgM antibody titres with stable titres in the dye test, immunofluorescence assay and complement fixation test in at least two follow-up examinations (Table 1). The serum samples were further classified according to the diagnoses of the patients: pregnancy, suspected congenital infection, cervical lymphadenitis, diseases of the central nervous system (encephalitis, abscess, lymphoma, chorioretinitis), and AIDS (Table 1). Serum of these patients were analyzed either routinely due to pregnancy, or because the patients presented with

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Table 1: Classification of the 105 serum samples according to the status of toxoplasmosis infection and the diagnosis of the patient.

Diagnosis	No infection	Latent infection	Acute infection	Persistent IgM	Total
Pregnancy	1	10	22	17	50
Suspected congenital infection	4	0	1	0	5
Lymphadenitis	1	6	21	4	32
CNS disease	1	2	6	0	9
AIDS	1	4	4*	0	9
Total	8	22	54	21	105

*Reactivation of a latent infection likely.
CNS = Central nervous system.

Table 2: Results of testing 105 serum samples for specific IgA antibodies to *Toxoplasma gondii* by the EIA and immunoblot techniques.

Patient group	IgA-EIA		IgA-immunoblot	
	Positive	Negative	Positive	Negative
Uninfected patients (n = 8)	1	7	0	8
Patients with latent infection (n = 22)	3	19	1	21
Patients with acute infection (n = 54)	50	4	45	9
Patients with persistant IgM (n = 21)	7	14	3	18
Total	61	44	49	56

clinical signs that might be the result of an infection with *Toxoplasma gondii*, such as enlarged nuchal lymph nodes.

The serum samples were tested by both the IgA-immunoblot and the IgA-EIA. The results obtained were compared with the results of conventional serological tests including the dye test, immunofluorescence assay, complement fixation test, IgM-EIA and IgM-immunoblot, also in follow-up sera.

Results and Discussion. In 20 sera (19.0 %) there was discordance between results of the IgA-EIA and the IgA-immunoblot. The highest concordance was found in non-infected and acutely infected patients (87.5 % and 87.0 %, respectively), whereas the lowest concordance was found in patients with chronic infection and persistence of IgM (61.9 %). Comparison of the IgA-EIA with conventional serological tests showed the highest concordance for the dye test (78.7 %) (data not shown). Whereas the positive predictive values for the IgA-EIA and the IgA-immunoblot were 82.0 % and 91.8 % respectively, the negative predictive values were 90.9 % and 83.9 % respectively.

Of the 20 human sera with discordant results in the IgA tests, 16 were positive in the IgA-EIA, whereas only four showed positive results in the IgA-immunoblot (Table 2). Taking into account results of follow-up tests, ten of these positive results in the IgA-EIA were assumed to be due to excessive sensitivity or to be false-positive. As an example, the positive result of the IgA-EIA in case no. 1 (Table 3) was proven to be false-positive by negative titres in follow-up tests, as well as by the inability to demonstrate *Toxoplasma gondii* by means of mouse inoculation and polymerase chain reaction (PCR) in the cerebrospinal fluid of this patient (15). This patient was tested for *Toxoplasma gondii*-specific antibodies because he presented with clinical signs of cerebral infection. However, further investigations revealed a noninfectious cause of his disease. Case no. 2 represented a pregnant women who later gave birth to an uninfected infant. Demonstrating stable titres in the dye test, immunofluorescence assay and complement fixation test, and persistent IgM antibodies in the EIA and immunoblot in more than three follow-up sera within eight weeks, this patient was shown to have a chronic infection with persistent IgM antibody titres.

Table 3: Serological analysis of sera with discordant results in the IgA-EIA and IgA-immunoblot.

Case no.	CFT	DT	IFA	IgM-EIA	IgM-Blot	IgA-EIA	IgA-Blot
1	o	o	o	o	o	+	o
2	1:20	1:256	1:1,024	+	+	+	o
3	1:5	1:256	NT	o	+	o	+

NT = not tested; CFT = complement fixation test; DT = dye test; IFA = immunofluorescence assay.

Whereas the positive result in the IgA-EIA in case no. 1 was clearly false-positive, it is possible that the positive IgA-EIA result in case no. 2 was due to higher sensitivity of this test compared to the IgA-immunoblot. The fact that four of the IgA-EIA positive/IgA-immunoblot negative patients also had positive results in IgA-immunoblots in previously drawn sera indicated that increased sensitivity of the IgA-EIA might lead to prolonged demonstration of IgA antibodies following acute infection (data not shown). On the other hand, the sensitivity of the IgA-EIA was insufficient in case no. 3: this patient suffered from AIDS with biopsy-proven cerebral toxoplasmosis. Parasites were demonstrated in his cerebrospinal fluid using the mouse-inoculation method and PCR. IgM and IgA antibodies in this patient were detected by immunoblot but not by the EIA.

This study also included nine sera from seven patients suffering from AIDS (Table 1). In the two patients with biopsy- or therapy-proven toxoplasmosis, tests of consecutive blood samples by immunoblot demonstrated the presence of IgA antibodies, indicating the possible value of this serological marker in cerebral toxoplasmosis. In contrast, the IgA-EIA detected IgA antibodies in only one of these two patients.

One reason for discordance of results between the EIA and the immunoblot in some patients might be the method used for preparation of the P30 (SAG1) antigen used in these tests. In the immunoblot technique the antigen is denatured by Nonidet-P40, and SDS-PAGE is carried out under reduced conditions. The IgA-EIA also utilizes P30 (SAG1) that is denatured by Nonidet-P40, but no mercaptoethanol is used for antigen preparation. Huskinson et al. (8) detected IgA antibodies in patients with chronic infection by immunoblotting under non-reduced conditions. It is thus possible that treatment of the antigen with mercaptoethanol influences the sensitivity of these tests. Another reason for increased sensitivity of the IgA-EIA might be the use of a monoclonal antibody for detection of antibody-bound P30 (SAG1). Different concentrations of the antigen in both test systems might also influence the sensitivity. Finally, sensitivity of the EIA might also be influenced by the fact that EIA results are read automatically providing objective data, whereas immunoblot results are read by technicians which introduces a subjective element. To minimize subjectivity, the immunoblot results were read by at least two independent technicians.

The IgA-EIA achieved an overall sensitivity and specificity of 92.6 % and 78.4 %, respectively, compared with the other serological tests in the same sera as well as in follow-up sera. Due to its high sensitivity, the IgA-EIA may detect IgA antibodies against *Toxoplasma gondii* at an early stage of infection. On the other hand, excessive sensitivity could lead to detection of IgA antibodies for an extended period of time after the onset of infection. To determine the kinetics of the IgA immune response, studies using sera of patients with a known date of infection (i.e. laboratory accidents) is necessary. So far, most investigators agree that detection of IgA antibodies to *Toxoplasma gondii* is useful in establishing a diagnosis (7, 9, 10). As was shown in most of the 105 sera tested, demonstration of the presence or absence of IgA antibodies to *Toxoplasma gondii* by either the IgA-EIA or IgA-immunoblot could lead to improved serodiagnosis of this parasitic disease. Especially in congenital toxoplasmosis and cerebral toxoplasmosis in immunocompromised patients, IgA antibodies seem to be a valuable additional serological marker of the disease. However, for reliable interpretation of test results, the sensitivity and specificity of serological test systems for detection of IgA have to be taken into consideration.

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