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Evaluation of DPC Immulite 2000® *Toxoplasma* Quantitative IgG/IgM Kits for Automated Toxoplasmosis Serology with Immulite 2000®

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In this study, we evaluated a new immunoassay for the automated detection of anti-*toxoplasma* IgG and IgM with Immulite 2000® (DPC-Siemens, La Garenne-Colombes, France). We tested 280 sera from 112 patients with past infection (PI), 40 PI with residual IgM, 75 seronegatives, 16 infants (31 sera) monitored for neonatal screening of congenital toxoplasmosis, and 13 patients with recent seroconversion (SC) (22 sequential sera). Detection sensitivity and specificity for IgG were 99 and 100%, respectively. IgG titers obtained with Immulite 2000® were higher than with Vidas® (BioMérieux, Marcy l'Etoile, France) and Access® (Beckman-Coulter, Villepinte, France) (paired Wilcoxon test $z = 4.44$ and $z = 3.67$, respectively, $P < 0.001$). IgM specificity was 100%. Detection sensitivity for IgM was 100% in the SC group, 86% in congenitally infected infants, and 75% in PI with persistent IgM. IgM detection seemed less prolonged in time than with the IgM Access® and ISAGA® IgM techniques. J. Clin. Lab. Anal. 23:336–340, 2009.

Key words: toxoplasmosis; serological diagnosis; ELISA; antibody; TORCH screening

INTRODUCTION

Toxoplasmosis serology is one of the most frequent serologies prescribed in France, due to the primary prevention program for seronegative (SN) pregnant women. Infection by the *Toxoplasma gondii* parasite is most often asymptomatic, and diagnosis is primarily serologic, at least with immunocompetent subjects. However, this infection can be transmitted to the fetus by transplacental passage when it is acquired by a SN pregnant woman, and can result in congenital toxoplasmosis, in varying degrees of severity depending on the term in pregnancy at the time of infection (1).

In France, primary prevention is based on serological screening for all pregnant women at the beginning of pregnancy, and subsequent monthly monitoring of those who are SN to screen a possible seroconversion (SC) early. The detection of IgM in the first serum tested at the beginning of pregnancy must often be confirmed by an IgG-avidity study, making it possible to eliminate any risk of recent infection if it is high (2,3). However, avidity may remain low in patients with past-acquired infection, leading to unnecessary further follow-up of the pregnancy. It is therefore necessary to have reliable and specific serological techniques for assaying IgG and IgM, as definitive diagnosis of *Toxoplasma* acute infection is critical for the clinical management of the mother and her fetus (4). All the SC must be accompanied with early treatment for the mother and lead to screening for the infection in the infant, whether prenatal or postnatal (5–9). Postnatal screening is based (i) on examination of the placenta, (ii) on a serological examination of the newborn, requiring techniques that

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are particularly sensitive and specific, for which the results will condition the treatment of the infant to limit later sequelae (10–12).

So we see the great interest here, for diagnosis laboratories, to have reliable techniques for *Toxoplasma* serology, allowing for comfort and safety in serological interpretation. There are many kits for assaying anti-toxoplasmic IgG and IgM on the market, with varying characteristics and performance levels. In this study, we evaluated the performance of the DPC Immulite 2000® *Toxoplasma* quantitative IgG and IgM kits (DPC-Siemens), for automated assay with the Immulite 2000® or Immulite 2500® devices, recently placed on the market. These assays are based on the chemiluminescence principle with enzymatic amplification. This is an immunocapture technique in solid phase on beads, initially developed for immunochemical assays, and then applied to TORCH serology.

MATERIALS AND METHODS

Sera and Patients

Two hundred and eighty sera were selected in two Laboratories (Laboratory of Medical Biology in the Hospital of Saint-Malo [site 1] and Department of Medical Parasitology and Mycology of the University Hospital of Rennes [site 2]). This study involved sera analyzed prospectively, in a routine hospital context (April–June 2006) and sera from the serum bank. Different banked sera were selected, corresponding to varied clinical categories (defined on the basis of serological results using the techniques described below) to best assess test performances in diverse situations. The series consisted of 112 sera from patients with past infection (PI), 40 sera with PI with residual IgM, 75 sera from SN pregnant women, including 4 with the presence of nonspecific IgM, 31 sequential sera from 16 infants monitored for neonatal screening of congenital toxoplasmosis (7 infected infants [17 sera] and 9 noninfected infants [14 sera]), and 22 sequential sera from 13 patients with SC during pregnancy.

Serological Techniques

These sera were analyzed with routine serological techniques used in each laboratory, i.e., Access® Toxo IgG and Access® Toxo IgM (Beckman-Coulter) and/or Vidas Toxo IgG II® and Vidas Toxo IgM® (BioMérieux) (site 1), and EIA Enzygnost® Behring IgG and IgM (Behring-Siemens, Paris-La Defense, France), and/or Vidas Toxo IgG II® and Vidas Toxo IgM® (BioMérieux) (site 2). In case of positive or questionable IgM detection (discrepant techniques or IgM positivity within the gray zone), IgM specificity was confirmed in both laboratories by the ISAGA® IgM technique (BioMérieux) (positivity threshold >6). For the cases of clearly positive IgM, an avidity determination of the anti-*Toxoplasma* IgG (Vidas Toxo IgG II avidity® BioMérieux) was carried out, so as to estimate how old the SC was and to classify the sera as follows: (i) sera with an avidity index >0.300 (SC dating over 4 months) were classified as "PI with persistent IgM," (ii) sera with an avidity index <0.300 (suspected SC dating of less than 4 months) and/or increasing IgG titers detected at 2 weeks apart were classified as "SC".

Statistical Analysis

Owing to the recruitment, not all of the sera were analyzed with all of the techniques of the two laboratories. Only those that were able to cause interpretation problems (questionable IgG or IgM) were analyzed retrospectively with the seven routine techniques of the two laboratories, in order to obtain the most reliable expert report possible. Results obtained with the DPC Immulite 2000® kits for the various groups of patients were expressed in terms of sensitivity and specificity. IgG titers obtained with the DPC Immulite 2000® device were compared statistically two by two with each technique. The statistics test used was the paired Wilcoxon test. For IgM detection, results were compared using the concordance test and the kappa coefficient.

RESULTS

Assaying IgG

Of the 280 sera tested on Immulite 2000®, 87 were tested with the EIA Behring®, 173 with Access®, and 135 with Vidas®.

The positivity threshold with the Immulite 2000® technique is 8 UI/ml, with an uncertain area from 6 to 8 UI/ml. Only the positivity threshold was taken into account for the comparison among the different immunoassays. The reproducibility of the Immulite 2000® technique was not evaluated in our study, as it has previously been shown to be excellent (data not shown). Detection specificity for IgG with Immulite 2000® assessed with the SN cohort was 100%.

The sensitivity of IgG detection was estimated on the groups of patients with unquestionable infection (PI with or without persistent IgM and SC groups). The overall sensitivity was 99%; it was 100% in PI without IgM detection (112/112 sera) and PI with residual IgM (40/40 sera), and 95% in SC (20/21 sera) (Table 1). In one case where serum was taken very early after SC, no IgG was detected with any technique; this serum was therefore withdrawn for IgG analysis. In another case of

TABLE 1. Detection Sensitivity and Specificity for Anti-*Toxoplasma* IgG and IgM with Immulite 2000®

	All categories (%)	Non-infected (seronegatives) (%)	Infected patients			
			PI without IgM (%)	PI with residual IgM (%)	Infected infants (%)	SC (%)
IgG sensitivity	99	na	100	100	na	95
IgG specificity	100	100	100	100	100	100
IgM sensitivity	na	na	na	75	86	100
IgM specificity	100	100	100	100	100	100

na: not applicable; PI: past injection; SC: seroconversion.

TABLE 2. IgG Titers Statistical Comparative Tests

	Comparison of IgG titers of				
	Immulite 2000 vs. Access		Immulite 2000 vs. Vidas		Immulite 2000 vs. Behring
Serum categories	All seropositive sera	Excluding evolutive toxoplasmosis[a]	All seropositive sera	Excluding evolutive toxoplasmosis[a]	All seropositive sera
$N =$	115	99	97	66	76
Paired Wilcoxon test (z)	3.67	4.10	4.44	1.49	1.13
P	<0.001	<0.001	<0.001	NS	NS

[a]Seroconversions and infected infants excluded, NS: non significant.

early SC, the DPC-Immulite 2000® technique did not reveal any IgG, although the Access technique was slightly positive (IgG = 8 UI/ml) and both Vidas® and Behring® techniques were negative too.

The levels of IgG titers obtained with the various techniques were also compared. Generally, IgG titers obtained with Immulite 2000® did not differ from those obtained with EIA Behring (paired Wilcoxon test, $z = 1.13$, NS) ($N = 76$ serum pairs) (Table 2). However, IgG titers obtained with Immulite 2000® were higher than those obtained with Vidas® and Access®, all patient categories included (paired Wilcoxon test, $z = 4.44$ [$N = 97$ serum pairs], and $z = 3.67$ [$N = 115$], respectively, $P < 0.001$). When the sera for SC and infected infants were not taken into account, the statistical test was always significant for Access ($z = 4.10$, [$N = 99$], $P < 0.001$), but became nonsignificant for Vidas® ($z = 1.49$, NS, [$N = 66$]). These data suggested that the IgG titers obtained with the Vidas® and Immulite 2000® techniques were similar, outside the context of evolutive toxoplasmosis (Table 2).

Assaying IgM

IgM specificity with Immulite 2000® was estimated in the PI without IgM, SN, and noninfected infant groups. It was 100% in the three groups (Table 1). A positive (index >1) or questionable (index between 0.9 and 1) IgM index was never observed when ISAGA® considered to be the reference technique was ≤ 6. In this same series, we were able to observe false-positive results with the other immunoassays used: four for Behring®, three for Vidas®, two for Access®. In three cases, these false-positive results occurred in SN women and in three other cases, in patients with PI without IgM (confirmed with a negative ISAGA® test). In one infant serum, IgM antibody was detected only by Immulite 2000® (index of 1.5), although the Vidas® and Behring® techniques were negative, as well as ISAGA®. However, we did not consider it as a genuine false positive, as it involved an infant (at 10 months), otherwise, proven congenitally infected by *Toxoplasma*, on the basis of (i) IgM detection at birth and on later sequential sera until 7 months, and (ii) a serology still positive at 1 year of life. Taken together, these data seem to point to a better specificity of the Immulite 2000® technique in comparison with the other immunoassays used in this study, though statistically nonsignificant.

The IgM sensitivity evaluation is especially pertinent in the groups of infected infants and SCs. IgM sensitivity with Immulite 2000® was 86% in infected infants, with IgM detection in 6/7 infants within the first few months of life (vs. one case not detected with Vidas®, three cases with Behring®, in the same neonatal period) (Table 3). The sera tested were taken between 1 day and 15 months (mean: 3.5 months). The infant that

TABLE 3. IgM Detection Sensitivity in Congenitally Infected Infants

	ISAGA®	Vidas®	Immulite 2000®	Enzygnost Behring®	Access®
No. of patients screened positive	6/7	6/7	6/7	1/5	nd
No. of positive sera	12/16	6/11	12/17	1/11	nd
Average age of infants at time sample taken (months)	3.9	3.2	3.6	3.5	nd

nd: not done.

was not detected with Immulite 2000® had the serum sampled at birth, for which ISAGA® was positive (index 9), ELISA Enzygnost-Behring® was negative and the other immunoassays were not performed due to an insufficient quantity of serum available. In one case of serological rebound at the end of the 1-year treatment in an infected infant, IgM antibody was detected with Vidas® but not with Immulite 2000®.

Detection sensitivity for IgM was 100% for the SC group, including at an early stage phase, when no IgG were yet detected (vs. two false-negative results observed with Behring®).

Residual IgM, confirmed by an ISAGA® index >6, were detected with Immulite 2000® in 75% of the cases (30/40 PI with persistent IgM) compared with 70% for Vidas® (26/37) and 96% for Access® (22/23) (Table 1). All the sera of this group had an avidity index >0.300. The sampling of tested sera did not allow us to accurately estimate the average length of time after SC, for IgM detection.

The mean of the indices for IgM detection was, respectively, 3.68 (± 2.79) in past-acquired toxoplasmosis (residual IgM) and 8.23 (± 7.46) in the SC cohort. The concordance test for IgM detection with Immulite 2000® vs. Access® Vidas® or Enzygnost-Behring® immunoassays was 93, 86, and 75%, respectively, with kappa coefficients of 82, 67, and 28%, respectively.

DISCUSSION

The performance levels of the anti-*Toxoplasma* IgG and IgM detection kits with Immulite 2000® were comparable to the other immunoassays used in this study. IgG detection had similar sensitivity and specificity, but the level of positivity seemed higher, in particular when the toxoplasmic infection had a pattern of evolutive or recent infection (greater titers observed in SC sera and in congenitally infected infants). These IgG titers, higher with Immulite 2000, did not affect serological interpretation, but once again illustrate the need to compare IgG titers over successive sera using the same assay technique.

Nevertheless, the value of a serologic technique for *Toxoplasma* serodiagnosis resides primarily in the detection of IgM and more precisely in the serological interpretation that could stem from IgM result. The expected performance for an "ideal test" for IgM detection would be (i) to be very sensitive for detecting a SC early and for neonatal screening of congenitally infected infants, (ii) to have a specificity of 100%, (iii) not to detect residual IgM too long over time (reduces the risk of incorrectly suspecting a SC in a pregnant woman). Of course, it is very difficult for a single technique to satisfy all these requirements.

We evaluated these different aspects, according to the miscellaneous clinical categories that we had selected. Thus, the *Toxoplasma* IgM DPC-Immulite 2000 test had a sensitivity and specificity of 100% on the SC cohort, while one of the three routinely used techniques showed a lack of sensitivity with two sera from the same patient, suggesting that the Immulite 2000® technique is capable of detecting a SC early. As for residual IgM detection, the sequential sera that we had available for this study did not allow us to correctly delimit the average period of detection over time, which is highly variable depending on the patient, but is obviously longer than 6 months. However, we can draw from the results obtained with the PI with persistent IgM cohort that kinetics of IgM detection and sensitivity using Vidas® and Immulite 2000® are similar (around 70%), though imperfectly correlated (concordance test of 86%). These results are in agreement with the study of Owen et al. (13). By contrast, results obtained with Access II showed a higher detection of residual IgM (96%), most results being highly correlated to Immulite 2000® (concordance test of 93%). However, too frequent detection of residual IgM can cause difficulty in dating the infection and contribute to pregnant woman's anxiety associated with further testing or antiparasitic treatment, not to mention the fact that it may also lead to unnecessary amniocentesis or abortion (14). In our study, the DPC test seemed to be in favor of limiting additional investigations (ISAGA® IgG avidity), by detecting residual IgM less often. Another anti-*Toxoplasma* IgM immunoassay was recently described and showed a preferential detection of IgM antibody produced by recent infection, but is not yet commercialized (15).

Finally, the last evaluation criteria for anti-*Toxoplasma* IgM concerned the ability of the kit to detect specific anti-*Toxoplasma* IgM for the neonatal diagnosis of

congenital toxoplasmosis, as early diagnosis and treatment are essential for further outcome (16). The weakness in our sampling did not allow us to provide a definitive answer to this question. However, observed sensitivity was correct, and even greater than other immunoassays used in this study. The false-negative result observed with a congenitally infected infant also occurred with Vidas® and corresponded to a serum sampled at birth. The postnatal diagnosis of congenital toxoplasmosis is rather a difficult task and relies, along with standard serology, on different approaches including detection of parasites in the placenta and cord blood by PCR and mouse inoculations (9,17), and comparison of mother and neonate antibodies using Western blot or enzyme-linked immunofiltration assay, allowing to discriminate between maternal transmitted antibodies or infant's antibodies (18–20). Overall, the DPC Immulite 2000® technique could constitute a quick and convenient screening technique, due to its low sample volume required (10 µl for assaying IgG and IgM and 50 µl of dead volume, i.e., a total of 60 µl) and acceptable sensitivity, without, however, questioning the leader position of the ISAGA® test as reference technique for IgM detection in neonates in terms of sensitivity and blood gain (10 µl sample volume).

In conclusion, this automated toxoplasmosis serologic technique with DPC Immulite 2000® appears in our hands to be very satisfactory for routine diagnosis on a high-speed machine, especially adapted for polyvalent biological laboratories. Of course, these results should be comforted by other studies on larger serum samples, especially on neonates. We underline that we used sera separated from cell pellets in a secondary tube, thus limiting the possible interference of fibrine that may lead to false-positive results with some automated immunoassays.

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