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Brief communication

Analytical validation of anti-toxoplasma IgG immunoassays

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ABSTRACT

There are often discrepancies when using different methods to measure anti-*Toxoplasma gondii* IgG levels in patient samples. The diagnostic performance of a chemiluminescent immunoassay (CLIA) and an enzyme-linked fluorescent assay (ELFA) used as confirmatory tests for samples identified as positive or equivocal by an electrochemiluminescent immunoassay (ECLIA) were examined. Cut-off values were those stated by the manufacturer, and Western blot was used to confirm the results of all methods. All samples identified as positive by ECLIA (n = 93) were confirmed as positive by Western blot, as were 14 of the 28 samples identified as equivocal. When these 121 samples were retested, the sensitivities of CLIA and ELFA were 64.4% and 73.8%, respectively. Both methods exhibited a specificity of 100%. This study confirms that the results obtained from the different immunoassays are not comparable, and neither CLIA nor ELFA should be used to confirm ECLIA results, which should instead be confirmed by methods such as Western blot or Sabin-Feldman dye test.

Infection with *Toxoplasma gondii* is very common: previous studies report infection rates of 49% to 71% in Brazilian women,[1,2] and 44% in French women of childbearing age.[3] Toxoplasmosis exhibits a wide spectrum of clinical presentation ranging from asymptomatic to the severe forms observed in fetal infections and in immunosuppressed patients.[4] The Sabin-Feldman dye test is considered the diagnostic gold standard for the detection of toxoplasmosis, but its use is complicated by the need for live parasites. Diagnostic results obtained by Western blot show excellent correlation with the Sabin-Feldman dye test: the presence of a 30 kDa band in combination with at least two other bands at 31, 33, 40, or 45 kDa are indicative of samples containing anti-*T. gondii* IgG.[5] Automated testing methods, such as enzyme-linked fluorescent assay (ELFA), chemiluminescent immunoassay (CLIA), and electrochemiluminescent immunoassay (ECLIA), are often preferred for routine screening. These assays identify infection by detecting IgG antibodies specific for the parasite. Additional detection of IgM antibodies can be used to differentiate whether infection has occurred recently or not, although the persistence of IgM for several months can necessitate the use of IgG avidity testing in order to determine time of infection.[6]

A low correlation between equivocal results obtained using the different automated assays has recently been reported; it is suggested to be due to a lack of standardization in the antigens used.[7] The authors have also previously observed a low correlation between assays regarding equivocal results.

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Table 1 – Diagnostic performance of alternative immunoassays used to confirm anti-*T. gondii* IgG results from ECLIA testing of patient serum samples.

Assay	Result	Total	Western blot results		Sensitivity*	Specificity*
			Positive	Negative		
ECLIA	Positive	93	93	0	N/A	N/A
	Equivocal	28	14	14		
	Negative	N/A	N/A	N/A		
CLIA	Positive	63	63	0	64.4%	100.0%
	Equivocal	6	6	0		
	Negative	52	38	14		
ELFA	Positive	61	61	0	73.8%	100.0%
	Equivocal	18	18	0		
	Negative	42	28	14		

* Equivocal results were considered as positive results for the calculation of sensitivity and specificity. The manufacturers' recommended cut-off values were used: ECLIA (negative, < 1 IU/mL; equivocal, ≥ 1 IU/mL and ≤ 30 IU/mL; positive, > 30 IU/mL); CLIA (negative, < 7.2 IU/mL; equivocal, ≥ 7.2 IU/mL and < 8.8 IU/mL; positive, ≥ 8.8 IU/mL); ELFA (negative, < 4 IU/mL; equivocal, ≥ 4 IU/mL and < 8 IU/mL; positive, ≥ 8 IU/mL). There were no equivocal results from Western blot. N/A, not applicable.

The aim of the present study was to evaluate the performance of CLIA and ELFA in the confirmation of results obtained by ECLIA.

Serum samples sent to the Division of Immunology of the Instituto Hermes Pardini in Belo Horizonte, Brazil, were analyzed using the MODULAR® ECLIA (Roche Diagnostics – Mannheim, Germany). A total of 121 samples identified as positive or equivocal (combined anti-*T. gondii* IgG titer > 1 IU/mL) were subsequently analyzed using two different automated assays: CLIA (LIAISON®, DiaSorin - Saluggia, Italy) and ELFA (VIDAS®, bioMérieux – Marcy-l'Étoile, France). In addition, all samples were cryopreserved below − 20 °C for one month and sent to the Laboratoire de Parasitologie in Marseille, France, for confirmatory testing by Western blot (LDBIO TOXO II IgG, LDBIO DIAGNOSTICS – Lyon, France). All assays (including cut-off values) were performed according to the manufacturer's specifications; equivocal results were considered positive for the purpose of calculating assay sensitivities and specificities. No clinical or socio-demographic characteristics of patients were obtained.

All samples identified as positive (n = 93) by ECLIA were confirmed as positive by Western blot. Half of the 28 samples identified as equivocal by ECLIA were confirmed as positive by Western blot. Therefore, there were 107 true positive samples in the 121 samples identified as either positive or equivocal by ECLIA (Table 1). When these 121 samples were retested using the other assays, CLIA correctly identified 69 of the 107 positive samples (sensitivity = 64.4%), and ELFA correctly identified 79 of the 107 positive samples (sensitivity = 73.8%). All true negative samples (samples tested negative by Western blot) were identified as negative by CLIA and ELFA, i.e. the specificity of both assays was 100%. All samples that had equivocal results during retesting by CLIA or ELFA were shown to be positive by Western blot.

It is noteworthy that the high cut-off value used for a positive result recommended by the manufacturer of the ECLIA test is quite different from the other methods. This cut-off value (≥ 30 IU/mL) proved appropriate for diagnostic accuracy in the present study, which confirms the results reported by Leslé et al.[7] Western blot was positive in 50% of samples identified as equivocal by ECLIA, which is also in agreement with the results of a previous study.[7] In practice, any equivocal IgG results should be retested, initially using the same samples and then, if still equivocal, using fresh samples taken within a few weeks of the initial sample. Samples continuing to yield equivocal IgG results should be tested by other methods, such as Western blot or the Sabin-Feldman dye test. If equivocal results are obtained by ECLIA, the use of other automated assays for confirmation is not appropriate, because their relatively low sensitivity may lead clinicians to incorrectly consider many patients to be uninfected. ELFA is widely used in Brazil and is often recommended by laboratories as the most accurate test, even though ECLIA has demonstrated and confirmed higher sensitivity.[5,8] Further studies are clearly warranted in order to support this recommendation.

Unfortunately, this study was not able to determine the performance of tests in detecting seroconversion, as clinical information of the patients was not obtained; however, the superiority of Western blot for such determinations has been reported.[7] The results presented here cannot be applied to the general population due to the small sample size and to the absence of clinical data. Such generalization would require a much larger clinical study involving additional markers, such as IgM or IgG avidity, and other patient factors, such as co-infections or pregnancy.

Serum anti-*T. gondii* IgG results obtained with different automated assays are not comparable, despite the fact that assays are standardized to World Health Organization's International Reference Standards. It is recommended that serological monitoring be performed consistently using the same manufacturer's test, and that confirmation of results from ECLIA should rely on methods such as Western blot or Sabin-Feldman dye test rather than on alternative automated assays.

Conflict of interest

All authors declare to have no conflict of interest.

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