UNIVERSITÉ GRENOBLE ALPES

UFR DE PHARMACIE DE GRENOBLE

Année : 2022

Diagnostic sérologique de la toxoplasmose : évaluation du test commercial *recom*Line *Toxoplasma* IgG immunoblot (Mikrogen) sur 171 sérums humains caractérisés

MÉMOIRE DU DIPLÔME D'ÉTUDES SPÉCIALISÉES DE BIOLOGIE MÉDICALE

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Né le 13/01/1994 à Châtenay-Malabry (092)

Le 11/10/2022

Présenté devant la Commission locale du DES de Biologie Médicale - Subdivision de GRENOBLE

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I. Introduction

La toxoplasmose est une anthropozoonose parasitaire due à *Toxoplasma gondii*, un parasite protozoaire de la classe des Coccidies. *T. gondii* existe sous trois formes parasitaires qui diffèrent selon le stade de l'infection. Les tachyzoïtes sont retrouvés durant la phase active de l'infection, les sporozoïtes contenus dans les oocystes résultent de la reproduction sexuée chez les félidés et sont à l'origine de la contamination des hôtes intermédiaires (oiseaux, mammifères dont l'Homme…) par les végétaux ou la terre souillée. Enfin, les bradyzoïtes sont regroupés au sein de kystes toxoplasmiques présents principalement dans les muscles et le cerveau et sont responsables de la contamination par l'ingestion de viande insuffisamment cuite. Ces kystes sont inaccessibles aux défenses immunitaires et aux traitements actuels et persistent toute la vie chez l'hôte infecté, on parle de toxoplasmose chronique. Ils correspondent ainsi à l'état de latence de l'infection et sont également impliqués dans la transmission par transplantation d'organe d'un donneur séropositif pour la toxoplasmose (D+) vers un receveur négatif avant la greffe (R-). Ces kystes sont aussi largement impliqués dans la réactivation de la toxoplasmose en cas d'immunodépression (**Figure A**) [26]. Même si la prévalence de la toxoplasmose varie fortement entre les pays (de 10 à 80 %), on estime que 25 à 30 % de la population mondiale est infectée par *T. gondii* [26]. En France, la séroprévalence globale a été estimée à 31,3 % en 2016 [28].

Lors de la primo-infection, la toxoplasmose aiguë est généralement asymptomatique mais peut plus rarement être associée à de la fièvre, des adénopathies cervicales, des myalgies, de l'asthénie et d'autres signes cliniques non spécifiques chez les patients immunocompétents (IC). Lorsqu'une femme enceinte se contamine au cours de la grossesse, une transmission materno-fœtale pourra conduire à une toxoplasmose congénitale dans 30 % des cas [4,36]. Chez les sujets immunodéprimés (ID), la réactivation de kystes tissulaires explique les formes graves qui se manifestent sous la forme d'atteintes d'un organe (au niveau cérébral, pulmonaire, oculaire, cardiaque...) ou d'atteintes disséminées dont le pronostic est fonction de la précocité du traitement et de la profondeur de l'immunodépression.

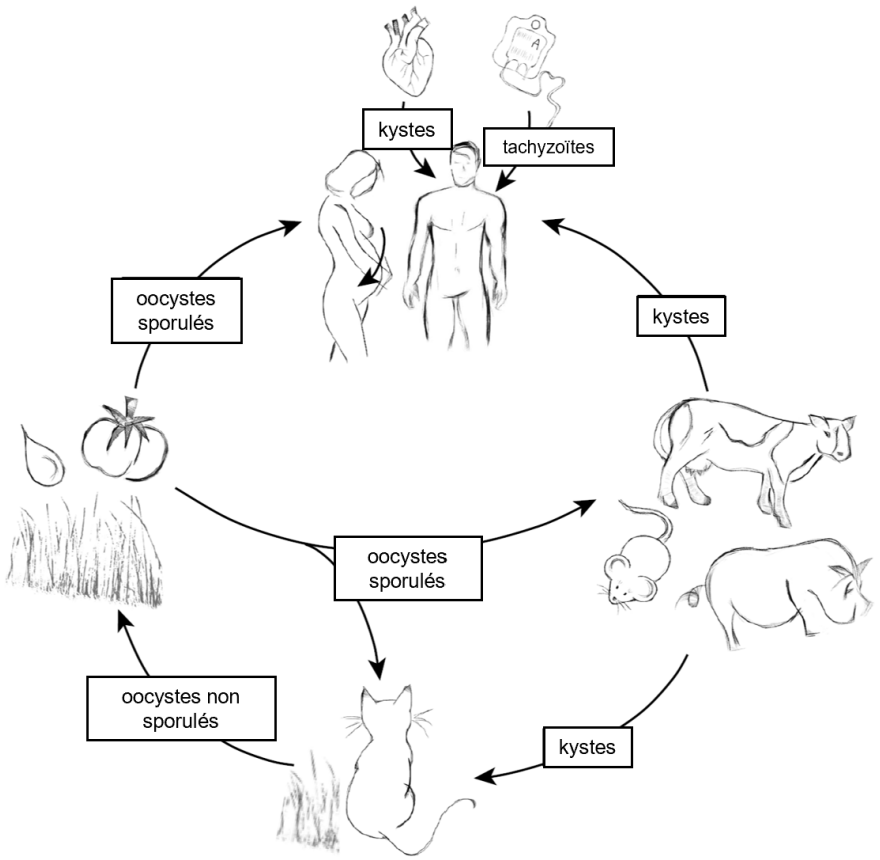

Figure A : Cycle de vie de *Toxoplasma gondii* (adapté de Dard *et al.*, [4])

Les signes cliniques de la toxoplasmose n'étant pas spécifiques, le diagnostic de cette infection repose essentiellement sur la biologie : la sérologie et la PCR ciblée. La sérologie, permet la détection indirecte d'anticorps de différents isotypes, principalement des IgM et des IgG anti-*T. gondii*, mais aussi des IgA anti-*T. gondii* et l'avidité des IgG (examen complémentaire aidant à dater une infection).

La détermination du statut immunitaire est essentielle dans trois situations principales : les femmes enceintes, les patients immunodéprimés et les receveurs ou donneurs de greffons [28]. Pour les femmes enceintes, la détection d'anticorps IgG spécifiques sans détection d'IgM en début de grossesse, indique une infection ancienne et écarte le risque d'une primo-infection et donc d'infection congénitale. Aucune précaution particulière ni aucun suivi ne sont alors nécessaires, en dehors des patientes immunodéprimées [22].

À l'inverse, pour éviter l'infection durant la grossesse, une sérologie IgG négative implique le respect des recommandations hygiéno-diététiques et un suivi sérologique mensuel selon les

recommandations nationales [12]. En France, un programme de prévention réservé aux femmes enceintes non immunisées impose le dépistage des IgG et des IgM anti-*T. gondii* tous les mois jusqu'à l'accouchement [25]. Une ultime sérologie est fortement recommandée un mois après l'accouchement pour vérifier l'absence d'infection du *per partum*. Lorsqu'une infection récente est suspectée au cours de la grossesse (en cas de détection d'IgM) ou lorsqu'une séroconversion est confirmée (apparition d'IgG spécifiques anti-*T. gondii*), la patiente doit être adressée à un centre de référence pour confirmation et datation précise de l'infection [4]. Tout l'enjeu est de disposer de techniques sérologiques suffisamment sensibles pour détecter précocement les IgG anti-*T. gondii* afin d'initier un traitement médicamenteux spécifique (Spiramycine, Pyriméthamine-Sulfadiazine ou Pyriméthanmine-Sulfadoxine) et proposer un diagnostic anténatal par recherche d'ADN toxoplasmique par PCR sur une ponction de liquide amniotique.

Chez les patients ID (patients transplantés, infectés par le VIH…), il est essentiel de diagnostiquer et de traiter une réactivation toxoplasmique ou plus rarement une primo-infection afin d'éviter une encéphalite, une myocardite, une pneumopathie ou une infection disséminée potentiellement mortelle [5].

Enfin, il est essentiel d'identifier le risque de mésappariement D+/R-. Les conséquences d'une sérologie faussement négative chez un D+ pourraient être fatales : le greffon du D+ (par exemple son cœur) risque de contenir des kystes responsables d'une toxoplasmose disséminée et mortelle chez le R-, en l'absence de chimioprophylaxie adaptée.

Les tests sérologiques se sont améliorés et diversifiés au fil des années. Le dye test de Sabin-Feldman mis au point en 1948, repose sur la lyse du parasite par les anticorps sériques en présence de complément et représente la technique de référence depuis de nombreuses années même s'il n'est aujourd'hui réalisé que par très peu de laboratoires spécialisés [24]. Si de nombreuses méthodes ont été développées, la plupart des laboratoires d'analyses médicales utilisent aujourd'hui des techniques quantitatives et automatisées comme les tests ELISA (enzyme linked immunosorbent assay) pour le dépistage de routine des IgG et IgM spécifiques anti-*T. gondii* [33]. Au laboratoire de Parasitologie-Mycologie du CHU de Grenoble, les tests ELISA utilisés pour le diagnostic de la toxoplasmose sont l'ARCHITECT (Abbott, Wiesbaden, Allemagne) +/- le VIDAS (bioMérieux, Marcy l'Étoile, France). Bien que dans la plupart des cas, la seule utilisation de ces tests ELISA est suffisante pour déterminer le statut immunitaire des patients, certaines situations biologiques nécessitent le recours à des tests de confirmation pour compléter l'interprétation sérologique. Des situations courantes consistent à devoir interpréter de faibles taux d'IgG, des taux équivoques d'IgG ou des discordances entre les deux

tests ELISA [32]. Pour les IgG, la technique complémentaire de confirmation utilisée actuellement en France est le Western blot LDBIO-Toxo II IgG (WB-LDBIO) (LDBio Diagnostics, Lyon, France). Le WB-LDBIO est une technique qualitative, manuelle ou semi-automatisée qui révèle des réponses d'IgG spécifiques à plusieurs antigènes naturels de *T. gondii* et qui présente une sensibilité de 99,2 % et une spécificité de 100 % par comparaison au dye test [6]. Par rapport au WB-LDBIO, l'immunoblot *recom*Line *Toxoplasma* IgG (IB-*recom*Line) (Mikrogen Diagnostik, Neuried, Allemagne) est une technique qualitative et manuelle plus récente, basée sur des antigènes recombinants de *T. gondii*. L'IB-*recom*Line présenterait l'avantage d'une lecture et d'une interprétation digitale des bandes à l'aide d'un logiciel permettant une évaluation plus objective par rapport au WB-LDBIO.

Dans ce mémoire, nous avons évalué les performances diagnostiques de l'IB-*recom*Line pour la détection d'IgG dans 171 sérums humains sélectionnés et caractérisés et provenant de trois contextes cliniques et biologiques différents :

- des sérums dont les taux d'IgG conduisaient à une interprétation biologique univoque sans ambiguïté (patients anciennement infectés ou non infectés, groupe 1),
- des sérums dont les taux d'IgG étaient à l'origine de difficultés d'interprétation biologique (taux équivoques et/ou discordants entre les deux techniques ELISA) nécessitant la réalisation d'une technique de confirmation (WB-LDBIO) (groupe 2),
- et enfin des sérums séquentiels provenant de femmes enceintes ayant présenté une séroconversion au cours de la grossesse (groupe 3).

Ainsi, l'objectif principal de notre étude était d'évaluer l'IB-*recom*Line en tant que technique alternative au WB-LDBIO, actuellement utilisé comme test de confirmation de l'infection à *T. gondii*.

II. Article scientifique

Parasite

Research article

Serological diagnosis of toxoplasmosis: evaluation of the commercial test *recom*Line *Toxoplasma* IgG immunoblot (Mikrogen) based on recombinant antigens on 171 characterized human sera.

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Abstract: 202 words
Manuscript: 3807 words
Figures: 3
Tables: 3

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[1] Enzyme linked immunosorbent assay (ELISA); negative recipient (R-); positive donor (D+); *recom*Line *Toxoplasma* IgG immunoblot (IB-*recom*Line); sensitivity (Se); specificity (Sp); LDBIO-Toxo II IgG Western blot (WB-LDBIO).

Abstract:

Background: The IgG detection for determining immune status to *Toxoplasma gondii* infection and seroconversion mainly relies on ELISA techniques and, if necessary, on a confirmatory test: Western blot. This study evaluated the performance of the *recom*Line *Toxoplasma* IgG immunoblot (IB-*recom*Line) (Mikrogen) as a confirmatory test. The 171 selected sera (113 patients) were previously analyzed by two ELISA tests, ARCHITECT (Abbott) and VIDAS (bioMérieux) +/- LDBIO-Toxo II IgG Western blot (WB-LDBIO) (LDBio) and were classified into three groups. Group 1 included 50 sera without difficulty in interpreting the IgG results (patients with documented past infection or uninfected); group 2 included 47 sera with difficulty in interpreting the ELISA results; group 3 included 74 sequential sera from 25 pregnant women with seroconversion. **Results:** In group 1, overall IgG agreements were 94% and 90% with ARCHITECT and VIDAS respectively. In group 2, low agreement was observed between IB-*recom*Line and WB-LDBIO: eight false positive and 13 false negative results. In group 3, 4/13 seroconversions were detected earlier with IB-*recom*Line compared to other tests. **Conclusions:** IB-*recom*Line allowed an earlier diagnosis of toxoplasmic seroconversion compared to both ELISA tests and WB-LDBIO but led to insufficient performance to confirm the immune status when ELISA results were discordant or equivocal.

Key words:

Toxoplasmosis, Serology, Immunoblot, Western blot, Seroconversion

Résumé :

Contexte : La détection des IgG anti-*Toxoplasma gondii* pour déterminer le statut immunitaire et diagnostiquer une séroconversion toxoplasmique repose principalement sur des techniques ELISA et, si nécessaire, sur un test de confirmation : le Western blot. Cette étude a évalué la performance de l'immunoblot *recom*Line *Toxoplasma* IgG (IB-*recom*Line) (Mikrogen) comme test de confirmation. Les 171 sérums sélectionnés (113 patients) ont été préalablement analysés par deux tests ELISA, ARCHITECT (Abbott) et VIDAS (bioMérieux) +/- LDBIO-Toxo II IgG Western blot (WB-LDBIO) (LDBio) et ont été classés en trois groupes. Le groupe 1 comprenait 50 sérums sans difficulté d'interprétation des résultats d'IgG (patients anciennement infectés ou non infectés) ; le groupe 2 comprenait 47 sérums avec des difficultés d'interprétation des résultats des tests ELISA ; le groupe 3 comprenait 74 sérums séquentiels provenant de 25 femmes enceintes avec séroconversion. **Résultats :** Dans le groupe 1, la concordance globale des IgG était de 94 % et 90 % avec ARCHITECT et VIDAS respectivement. Dans le groupe 2, une faible concordance a été observée entre IB-*recom*Line et WB-LDBIO : huit résultats faux positifs et 13 faux négatifs. Dans le groupe 3, 4/13 séroconversions ont été détectées plus tôt avec IB-*recom*Line par rapport aux autres tests. **Conclusions :** IB-*recom*Line a permis un diagnostic plus précoce de la séroconversion toxoplasmique par rapport aux tests ELISA et au WB-LDBIO, mais ses performances ont été insuffisantes pour confirmer le statut immunitaire lorsque les résultats des tests ELISA étaient discordants ou équivoques.

Introduction

Toxoplasmosis is a widespread parasitic zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite that infects approximately 25 to 30% of the world's human population [26]. This infection is usually asymptomatic or accompanied by self-limited signs in immunocompetent patients but can result in severe forms in immunocompromised patients and complications in newborns when a mother acquires her first *T. gondii* infection during pregnancy (congenital toxoplasmosis) [4,36]. Since the clinical signs of toxoplasmosis are non-specific, the diagnosis of this infection is primarily based on serology which relies on the detection of both specific anti-*T. gondii* IgM and IgG antibodies [8,34].

Determining the immune status is essential in three main situations: pregnant women, immunocompromised patients and transplant recipients or donors [27]. For pregnant women, detection of specific IgG antibodies without IgM detection at the beginning of pregnancy indicates a past infection and rules out the risk of a congenital primary infection [21]. Conversely, negative IgG serology implies compliance with hygiene recommendations +/- follow-up of pregnant women according to national guidelines [12]. For immunocompromised patients (HIV infection, immunosuppressive treatments, organ and hematopoietic stem cell transplantations etc.), it is essential to diagnose and treat primary infection or toxoplasmic reactivation to avoid life-threatening encephalitis, myocarditis, pneumonitis or disseminated infection [5]. Finally, it is essential to identify the risk of mismatch of a positive organ donor (D+) to a negative organ recipient (R-) and prevent disseminated toxoplasmosis in the recipient.

Although IgM antibodies are the first serological markers to become positive following *T. gondii* infection, the detection of IgM alone is not conclusive [33]. Confirmation of a primary infection is indicated by the appearance of anti-*T. gondii*-specific IgG in a previously non-immune patient (seroconversion). Usually, specific IgG appear one to three weeks after IgM and are mostly detected with enzyme linked immunosorbent assays (ELISA). These indirect immunoenzymatic tests use a mixture of antigens and are performed on automated analyzers [7]. However, interpreting low or equivocal ELISA IgG titers or discordance between ELISA tests can be difficult and requires further testing [33]. The Sabin-Feldman dye test is the historical gold standard method using live *T. gondii* [24]. However, due to its high price and its fastidious use, the dye test is not commercially available and only used today in a few specialized laboratories [14]. The LDBIO-Toxo II IgG Western blot (WB-LDBIO) (LDBio Diagnostics, Lyon, France), based on natural *T. gondii* antigens, is currently recommended as a confirmatory test [33]. The *recom*Line *Toxoplasma* IgG immunoblot (IB-*recom*Line) (Mikrogen Diagnostik, Neuried, Germany), based on recombinant *T. gondii* antigens, is another qualitative assay that is still little used [22,32].

The objective of this work was to assess if the IB-*recom*Line could be used as a complementary test to confirm the absence or presence of anti-*T. gondii*-specific IgG when the results of the ELISA tests do not allow a clear biological interpretation, as the WB-LDBIO. To that purpose, we evaluated the diagnostic performance of the IB-*recom*Line for the detection of IgG in 171 selected and characterized human sera from three different clinical and biological settings: sera without difficulty of biological interpretation, sera that lead to difficulties in biological interpretation and sequential sera from infected mothers with proven *T. gondii* seroconversion.

Material and Methods

Ethics

This non-interventional, monocentric, retrospective study involving data and samples from human participants has been carried out in the Grenoble Alpes University Hospital according to the current French regulation. The principal investigator (Dr Marie-Pierre Brenier-Pinchart, MD, PhD) has signed a commitment to comply with Reference Methodology n°MR004 issued by French Authorities (CNIL). All subjects were informed and were not opposed; written consent for participation was not required for this study in accordance with national legislation and institutional requirements. The raw data supporting the conclusions of this article will be made available by the authors in respect of General Data Protection Regulation, without undue reservation.

Patients and sera

A total of 171 sera collected from 113 patients were included and were stored at -20°C for a few months up to five years (between May 2016 to April 2021) [3]. The study population included 92 females (81%) and 21 males (19%). The mean age was 42 years [21-92].

These sera were classified into three groups based on previous results obtained in routine diagnosis and were retrospectively analyzed by the IB-*recom*Line to examine the performance of the test in various diagnostic conditions. Group 1 included 50 sera without difficulty of biological interpretation with ELISA tests in routine diagnosis. Among these sera: 16 had a serological profile indicating a non-immune status to *T. gondii* (negative results with ELISA techniques) and 34 corresponded to a past infection and were immunized against *T. gondii* (positive or equivocal results with ELISA techniques). Among these 34 sera, three were equivocal in ELISA but were considered as well-defined sera based on previous positive sera for each of these three patients.

Group 1 was designed to assess the IgG agreement between the IB-*recom*Line and ELISA assays in unambiguous cases. In contrast, group 2 included 47 sera with equivocal or discordant IgG results with ELISA techniques. Sera were considered discordant in the following situations: serum negative in one ELISA technique and positive in the second one; serum equivocal in one ELISA technique and positive (or negative) in the second one. Group 2 was designed to assess the IgG agreement between IB-*recom*Line and WB-LDBIO. The objective was to evaluate the IB-*recom*Line capacity to detect low IgG titers in past infection and to resolve discrepant results between ELISA assays to unambiguously determine the immune status of the selected patients. Finally, group 3 included 74 sequential sera from 25 pregnant women with proven *T. gondii* seroconversion (one to five consecutive sera for each patient). Group 3 was designed to compare the capability for early detection of a toxoplasmic seroconversion between IB-*recom*Line and WB-LDBIO in pregnant women (**Figure 1**).

IgG serological tests

All commercial tests were performed in the Grenoble Alpes University Hospital (France) Parasitology Mycology unit and interpreted according to the manufacturer's instructions.

Automated immunoassays

The ARCHITECT® Toxo IgG test (ARCHITECT-IgG) (Abbott Laboratories, Wiesbaden, Germany) is a fully automated chemiluminescent microparticle immunoassay for the quantitative determination of IgG antibodies against *T. gondii* in human serum using recombinant antigens [SAG1 (= p30), GRA8 (= p35)]-coated microparticles [31]. The VIDAS® Toxo IgG II test (VIDAS-IgG) (bioMérieux, Marcy l'Étoile, France) is a fully automated enzyme linked fluorescent assay for the quantitative determination of IgG antibodies against *T. gondii* in human serum using native antigen SAG1 (= p30)-coated microparticles [29].

Both automated ELISA tests were previously performed on the 171 selected sera (**Figure 1**). According to the manufacturer, ARCHITECT-IgG results were positive if ≥3 IU/mL, negative if <1.6 IU/mL, and equivocal if ≥1.6 and <3 IU/mL. VIDAS-IgG results were positive if ≥8 IU/mL, negative if <4 IU/mL, and equivocal if ≥4 and <8 IU/mL; ARCHITECT-IgM results were positive if ≥0.6, negative if <0.5, and equivocal if ≥0.5 and <0.6. VIDAS-IgM results were positive if ≥0.65, negative if <0.55, and equivocal if ≥0.55 and <0.65.

LDBIO-Toxo II IgG Western blot (WB-LDBIO)

The WB-LDBIO (LDBio Diagnostics, Lyon, France) is a qualitative *in vitro* test for the detection of IgG antibodies against *T. gondii* in human serum. Once separated by electrophoresis, natural *T. gondii* antigens of different molecular weights 30 kDa (= p30), 31 kDa, 33 kDa, 40 kDa and 45 kDa, are bound by electroblotting to the surface of a nitrocellulose membrane [17]. According to the manufacturer, the WB-LDBIO was considered as positive when at least three specific bands including p30 are present on the strip [6,18].

Since the WB-LDBIO showed perfect agreement with the dye test gold standard technique, it was used as the confirmatory test in this study [15]. For group 1, when sera gave equivocal results, the WB-LDBIO were not performed to confirm immune status because the IgG titers were positive in a previous serum for these patients. For group 2, all serologies were prospectively confirmed with the WB-LDBIO to interpret serological profiles. For group 3, WB-LDBIO was retrospectively performed in each serum from seroconversion in the case of qualitative discordance between ELISA techniques (ARCHITECT-IgG and VIDAS-IgG) (**Figure 1**).

***Recom*Line *Toxoplasma* IgG immunoblot (IB-*recom*Line)**

The IB-r*ecom*Line (Mikrogen Diagnostik, Neuried, Germany) is another qualitative *in vitro* test for detecting IgG antibodies against *T. gondii* in human serum. Highly purified recombinant *T. gondii* antigens [ROP1c (= p66), GRA7 (= p29), GRA8 (= p35), SAG1 (= p30), MAG1 (= p65, p68), GRA1 (= p24), rSAG1 (= p30, low concentration)] are fixed on nitro-cellulose membrane test strips. The intensity of the cut-off band allowed the reactivity of each antigen band to be assessed. Only the bands for which the intensity was higher than or equal to the cut-off band, were considered as positive. ROP1c counted for 1 point, MAG1 counted for 2 points, GRA1, GRA7, GRA8 and rSAG1 counted for 4 points each, p30 counted for 6 points. According to the manufacturer, the IB-*recom*Line was considered as positive when the total number of points reported by the positive bands (total)

was ≥6, as negative when total was ≤3 and as equivocal when total was 4 or 5 [22]. Analysis of the test strips was computer-assisted using the test strip analysis software *recom*Scan.

Data analysis

IB-*recom*Line sensitivity (Se) and specificity (Sp) were estimated by comparing the IB-*recom*Line qualitative results (negative, equivocal or positive) with the global biological interpretation given by ELISA assays (ARCHITECT and VIDAS) +/- WB-LDBIO results +/- patient serological anteriority.

The value of each parameter was calculated twice: with IB-*recom*Line equivocal results considered as negative or as positive results (**Table 2**).

A minor discrepancy was defined as an equivocal result in IB-*recom*Line while the biological interpretation was either negative or positive. A major discrepancy was defined as a negative result in IB-*recom*Line while the biological interpretation was positive and *vice versa*.

Overall IgG agreements (and Cohen's kappa values) between IB-*recom*Line and ELISA assays in group 1, and between IB-*recom*Line and WB-LDBIO in group 2, were calculated as follows: agreement = (number of concordant samples / number of tested samples on both assays) x 100%. Agreement with kappa values of 0.21 to 0.40 was considered low, of 0.41 to 0.60 was considered moderate, of 0.61 to 0.80 was considered substantial and of 0.81 to 1.00 was considered almost perfect [16].

In group 3, the positivity rate for each technique (ARCHITECT, VIDAS, WB-LDBIO, IB-*recom*Line) was the percentage of equivocal and positive IgG results among the first sera in which IgG was detected by at least one test.

Results

High agreement between IB-*recom*Line and ELISA assays in group 1

Results of IgG assays for group 1 are provided in **Table 1**. Group 1 included unambiguous cases without difficulty of biological interpretation: 16 sera without IgG (no immunization) and 34 sera with low or high titers of IgG (past infection) in ELISA assays. Among the 16 sera IgG negative with both ARCHITECT and VIDAS: 15 were also negative with IB-*recom*Line and one sample was equivocal and represented a minor discrepancy. Among the 34 sera with low or high titers of IgG: a) three were equivocal in VIDAS +/- ARCHITECT (with previous positive sera) and were positive with IB-*recom*Line and b) 31 were IgG positive with both ARCHITECT + VIDAS, 30 were also positive with IB-*recom*Line while one was equivocal and represented a minor discrepancy.

In total, among the 33 IB-*recom*Line-positive cases, the p30 band was consistently present. The GRA7 and GRA8 bands were frequently present (64% and 70% of cases respectively), followed by the MAG1 band (45%), the GRA1 and rSAG1 bands (36% each) and the ROP1c that appeared less frequently (24%).

Overall IgG agreement was good with almost perfect agreement between IB-*recom*Line and ARCHITECT: 94% (kappa = 0.87), and substantial agreement between IB-*recom*Line and VIDAS: 90% (kappa = 0.8). Depending on

whether equivocal results of IB-*recom*Line were considered as negative or positive, Se were 97.1% [84.7-99.9] and 100% [89.7-100] and Sp were 100% [79.4-100] and 93.8% [69.8-99.8], respectively (**Table 2**).

Low agreement between IB-*recom*Line and WB-LDBIO in group 2

Results of IgG assays for group 2 are provided in **Table 1**. Group 2 included 47 sera with equivocal results and/or qualitative discordances between ELISA assays therefore a confirmatory technique was needed to conclude and to propose a biological interpretation. IB-*recom*Line results were compared to those obtained with the WB-LDBIO currently used as reference confirmatory test in our laboratory and both overall IgG and kappa agreements were low: 55.3% (kappa = 0.27). Depending on whether equivocal results of IB-*recom*Line were considered as negative or positive, Se were 58.1% [39.1-75.5] and 77.4% [58.9-90.4], and Sp were 87.5% [61.7-98.5] and 50% [24.7-75.4] respectively (**Table 2**).

In total, there were 12 minor discrepancies and nine major discrepancies. Among the eight IB-*recom*Line-false positive cases the GRA8 band was present in six cases (75%), the GRA7 band was present twice and the ROP1c, p30 and GRA1 bands were present only once. Furthermore, among these eight sera, six were also false positive in ARCHITECT and negative in VIDAS; one was equivocal in both ARCHITECT and VIDAS and one other was equivocal in VIDAS and negative in ARCHITECT.

There were also 13 IB-*recom*Line-false negative results that correspond to sera from patients with chronic *T. gondii* infection with IgG that persist at residual titers not detected with IB-*recom*Line. Among them, we visually identified the bands whose intensity appeared slightly lower than the cut-off band and were considered as negative by the *recom*Scan software. The p30 and GRA1 bands were detectable with visual reading but not quantifiable with *recom*Scan software in nine (69%) and seven cases (54%) respectively followed by the GRA8 (38%), GRA7 (31%) and MAG1 (23%) bands. An example of a false-negative result with the IB-*recom*Line is described in **Figure 2**. In this figure, the WB-LDBIO was positive with all specific IgG bands. However, the IB-*recom*Line was negative because the intensity of the p30 band was not sufficient to be considered as positive by the *recom*Scan software and the total score of the bands was 0.

Precocity of seroconversion detection with IB-*recom*Line compared to other techniques (group 3)

The WB-LDBIO was retrospectively performed when at least one serum for each seroconversion showed a discordance between ARCHITECT-IgG and VIDAS-IgG results (13/25 seroconversion sequences). Antibody kinetics (IgM and IgG) in these 13 seroconversions are provided in **Table 3**. We examined the positivity rate (equivocal or positive results) of serological techniques for the first sera in which IgG was detected by at least one assay. We showed that IB-*recom*Line was positive in 92.3% of the cases (12/13), followed by ARCHITECT-IgG (61.5%, 8/13), WB-LDBIO (46.2%, 6/13) and VIDAS-IgG (15.4%, 2/13). The positivity of IB-*recom*Line preceded all other tests in 4/13 cases while ARCHITECT-IgG, WB-LDBIO and VIDAS-IgG were still negative. In contrast, VIDAS-IgG was the last assay to become positive in 7/13 cases while all other tests were either equivocal or positive. A total of 7/13 seroconversions were detected earlier with IB-*recom*Line compared to WB-

LDBIO (four positive and three equivocal with IB-*recom*Line). A total of 2/13 seroconversions were detected later with IB-*recom*Line than with WB-LDBIO and 4/13 seroconversions were detected simultaneously by both techniques.

Among the seven IB-*recom*Line results allowing earlier seroconversion diagnosis compared to WB-LDBIO, the GRA8 and GRA7 bands were present in four cases each and the p30 and MAG1 bands were present once each. **Figure 3** provides an example of toxoplasmic seroconversion with IgG detected earlier with the IB-*recom*Line compared to the WB-LDBIO. Serum 1 was considered IgG negative with the WB-LDBIO technique because only the p30 and p40 bands were slightly positive. However, the same serum was considered IgG positive with the IB-*recom*Line technique because both GRA7 and GRA8 bands were positive and reported a total of 8 points to the strip.

In addition, when ARCHITECT-IgG and VIDAS-IgG were concordant, WB-LDBIO was not performed and IB-*recom*Line was consistently concordant with ELISA tests (12/25 seroconversion sequences) (data not shown).

Discussion

The diagnosis of toxoplasmosis in immunocompetent patients relies mainly on the detection of anti-*T. gondii* antibodies. While in most cases, the use of ELISA tests is sufficient to determine the immune status of patients, the clinical interpretation of the results of first-line techniques may require the use of a confirmatory technique [33]. The WB-LDBIO, based on natural *T. gondii* antigens, is currently recommended as a reference confirmatory test thanks to its high sensitivity (99.2%) and its perfect specificity (100%) [6]. Unlike the WB-LDBIO, the IB-*recom*Line, based on recombinant antigens, remains seldom described in the literature. To our knowledge, this study is the first that has evaluated the diagnostic performance of the IB-*recom*Line as a confirmatory serological test for the detection of IgG. We challenged the IB-*recom*Line with several groups of characterized sera to determine its ability to respond to different clinical and biological settings in which it could be used.

The IB-*recom*Line was evaluated for its ability to detect low levels of formerly synthesized IgG. In our study, the IB-*recom*Line exhibited unsatisfactory analytical performance with too many false negative results (13/47 including seven major discrepancies). In a large majority of these misdiagnosed sera, the intensity of the p30 (SAG1) band was close to being interpreted as positive. As this band alone rates for 6 points, it would have made the IB-*recomLine* positive. This apparent lack of sensitivity in detecting residual IgG titers compared to WB-LDBIO could be explained by the nature of p30 antigen that differs between these two assays. Natural p30 antigen of WB-LDBIO might enable a more sensitive diagnosis of chronic *T. gondii* infection than recombinant p30 antigen of IB-*recom*Line. Therefore, the use of IB-*recom*Line as a confirmatory test could result in an unnecessary and expensive serological follow-up in pregnant woman in the French context for instance [30]. It could also misdiagnose seropositive organ donors that could lead to life-threatening consequences with transmission of toxoplasmosis to seronegative transplant recipients (D+/R-) [7].

The lack of sensitivity of IB-*recom*Line to detect low IgG titers in patients with past infection contrasted sharply with its ability to detect low IgG titers early after primary infection. In our study, IB-*recom*Line was the most sensitive technique for detecting the appearance of newly synthesized IgG in pregnant women followed by ARCHITECT-IgG, WB-LDBIO, and VIDAS-IgG. Indeed, among the 13 sera where seroconversion was detected, the IB-*recom*Line was positive (or equivocal) 12 times. Until now, WB-LDBIO was considered as the most

sensitive commercial technique [19]. Armengol *et al.* showed that the median durations before positive IgG detection was shortened by 13 days and more than 20 days with the use of WB-LDBIO compared to ARCHITECT-IgG and VIDAS-IgG respectively [1]. As shown by Armengol *et al.* and many previous reports, we found that IgG detection with VIDAS-IgG was delayed in 7/13 seroconversions, being the last technique allowing detection of IgG in pregnant women in our study [1,9,20]. To date, there is only one study in the literature that has focused on the IB-*recom*Line and more particularly on the value of the different recombinant antigens it uses to improve the diagnosis of acute toxoplasmosis during pregnancy [22]. Pfrepper *et al.* showed that IgG antibodies against GRA7 and GRA8 were exclusively present at the beginning of the IgG response and those against GRA7 were the most present in patients with recent seroconversion [22]. Our study strengthened these findings showing that most of the time, GRA7 and GRA8 were the bands that appeared first following an early seroconversion contrary to p30 band that appeared only once. Therefore, GRA8 and GRA7 bands would allow earlier detection of newly synthetized IgG antibodies. These observations could explain the precocity of IB-*recom*Line compared to other techniques that use neither GRA7 nor GRA8, as VIDAS-IgG. Providing a *T. gondii* seroconversion diagnosis before other tests, the IB-*recom*Line would enable an earlier therapeutic intervention that may reduce the risk of brain lesions in infected newborns even if it would have little or no impact on the fetomaternal transmission rate [10,11,13,35].

Interestingly, in most studies, p30 is known to be one of the most immunogenic antigens following an acute seroconversion, possibly due to its position on the surface membrane of the tachyzoite stage of *T. gondii* [2,23]. However, our study showed a lack of reactivity of IgG antibodies against p30 in the early stages of infection as already described by Pfrepper *et al.* [22]. This last result reinforces the hypothesis that the formulation of recombinant p30 antigen of IB-*recom*Line is probably not appropriate.

Equivocal IgG results and/or discordances between ELISA tests may correspond either to low titers of IgG in case of past infection or to the beginning of seroconversion but may also correspond to false positive IgG results. We showed that 7/8 false positive results obtained with the ARCHITECT-IgG are also false positive with the IB-*recom*Line. Similarly to the IB-*recom*Line and unlike the VIDAS-IgG and the WB-LDBIO, the ARCHITECT-IgG uses the GRA8 recombinant antigen to detect anti-*T. gondii*-specific IgG. Thus, as already shown by Simon *et al.*, we observed that reactivity against the GRA8 antigen led to false positive results [32]. Simon *et al.* hypothesize that past contact with *Neospora caninum* and *Hammondia hammondi*, two parasites close to *T. gondii*, could explain false positive results with ARCHITECT-IgG and IB-*recom*Line mostly due to cross-reaction with GRA8 (used by both ARCHITECT and IB-*recom*Line) and also GRA7 to a lesser extent (only used by IB-*recom*Line) [27]. Therefore, IB-*recom*Line could result in false positive IgG detections and bias the determination of immune status. In total, GRA7 and GRA8 bands were the most frequently detected at the onset of seroconversions, making IB-*recom*Line highly sensitive (group 3), but detection of GRA7 and especially GRA8 bands was also responsible for false positive results (group 2). Thus, the presumed high sensitivity or earliness of IB-*recom*Line should be interpreted with caution since detection of GRA7 or GRA8 bands may also correspond to false positive results.

The IB-*recom*Line is a manual technique not designed for large-scale screening of toxoplasmosis. Therefore, our study explored its performance as a confirmatory test that has never been done before. The groups selected in this study were composed of few sera but all these sera were fully characterized by our reference laboratory in toxoplasmosis in Grenoble (France). The digital reading of the bands appeared to be an advantage of the IB-

*recom*Line over the WB-LDBIO allowing an objective evaluation of the bands. However, IB-*recom*Line appeared to have difficulty distinguishing between low positive and negative sera for patients with chronic infection and failed to invalidate false positive results obtained with ELISA tests with a risk of serious consequences for the patients. Besides, the presence of a gray zone in the IB-*recom*Line assay, unlike the WB-LDBIO, made it impossible to conclude on the immune status for some patients. Its use could then be limited to early confirmation of the onset of seroconversion in pregnant women in case of equivocal or discordant results with ELISA tests. In conclusion, although IB-*recom*Line was associated with the quickest detection of seroconversion, it showed insufficient performance to confirm the immune status when ELISA results were discordant or equivocal.

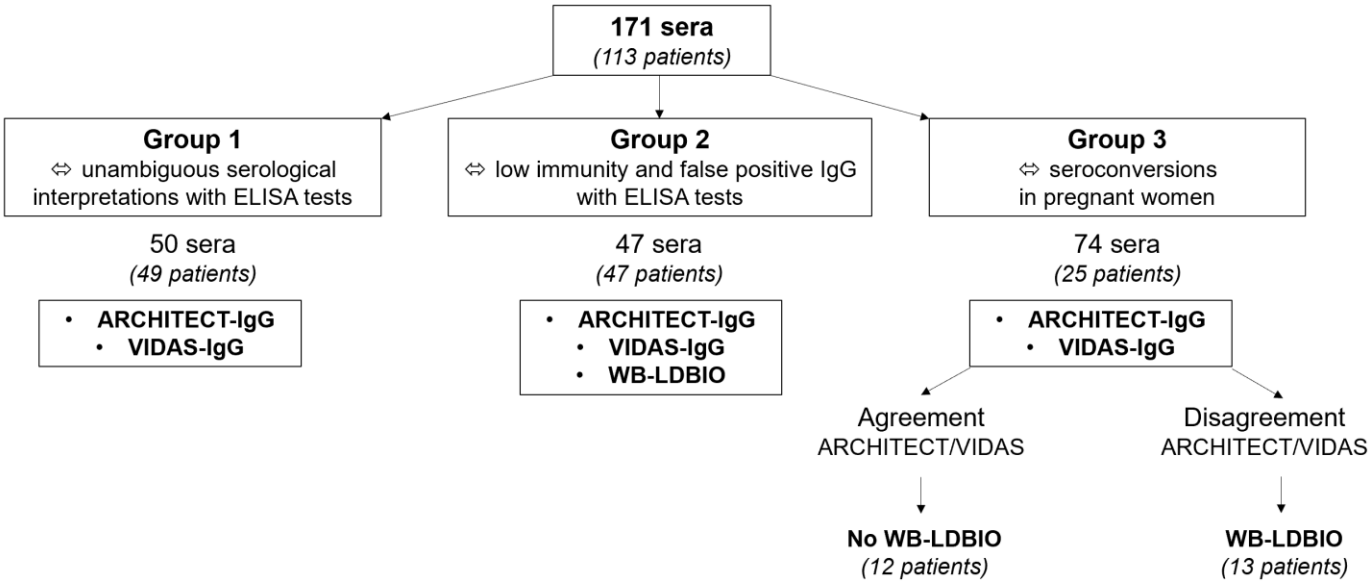
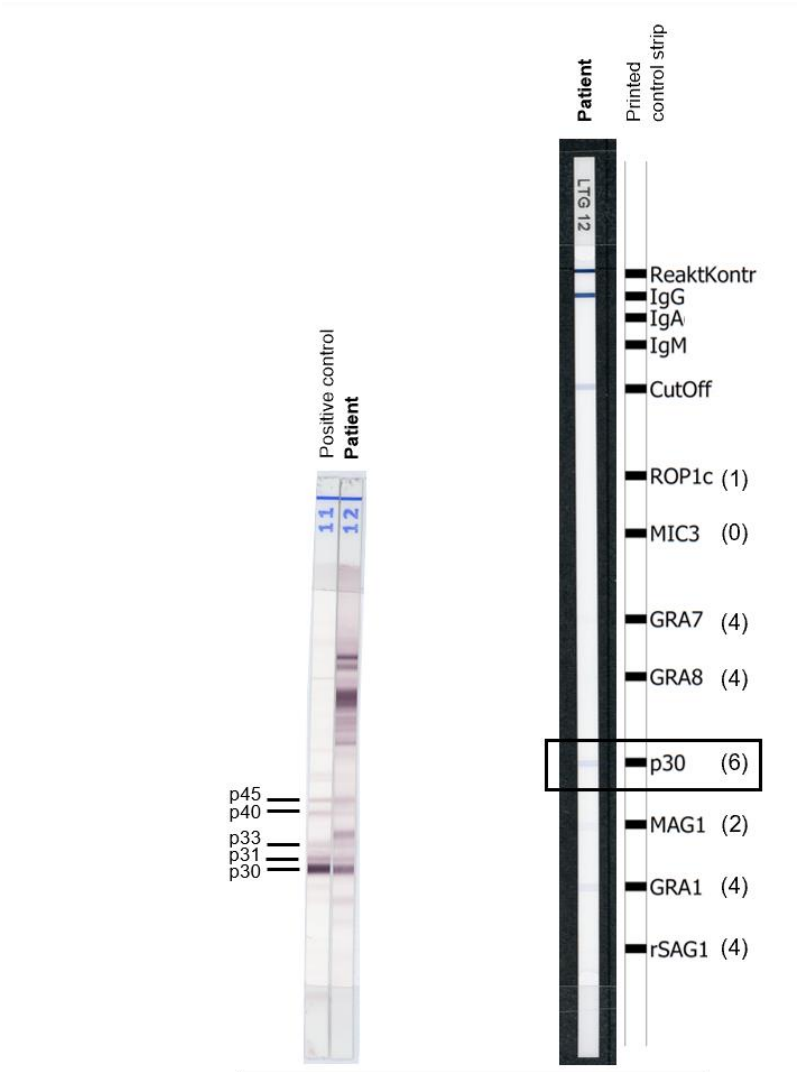


Figure 1: Classification of sera into three groups and IgG detection techniques performed on each category of sera



Type of test	« WB-LDBIO »	« IB-*recom*Line »
Test interpretation (+)	Presence of a minimum of **3 specific bands including p30**	Total **≥ 6** of values of bands with **stain intensity = or > than the cut-off** band
Positive band(s)	p30 p31 p33 p40 p45	/
Score	/	0
Strip assessment	positive	negative

Figure 2: Example of a false negative result with the IB-*recom*Line compared to the WB-LDBIO

The number of points reported by positive bands with IB-recomLine is specified in brackets behind each antigen.

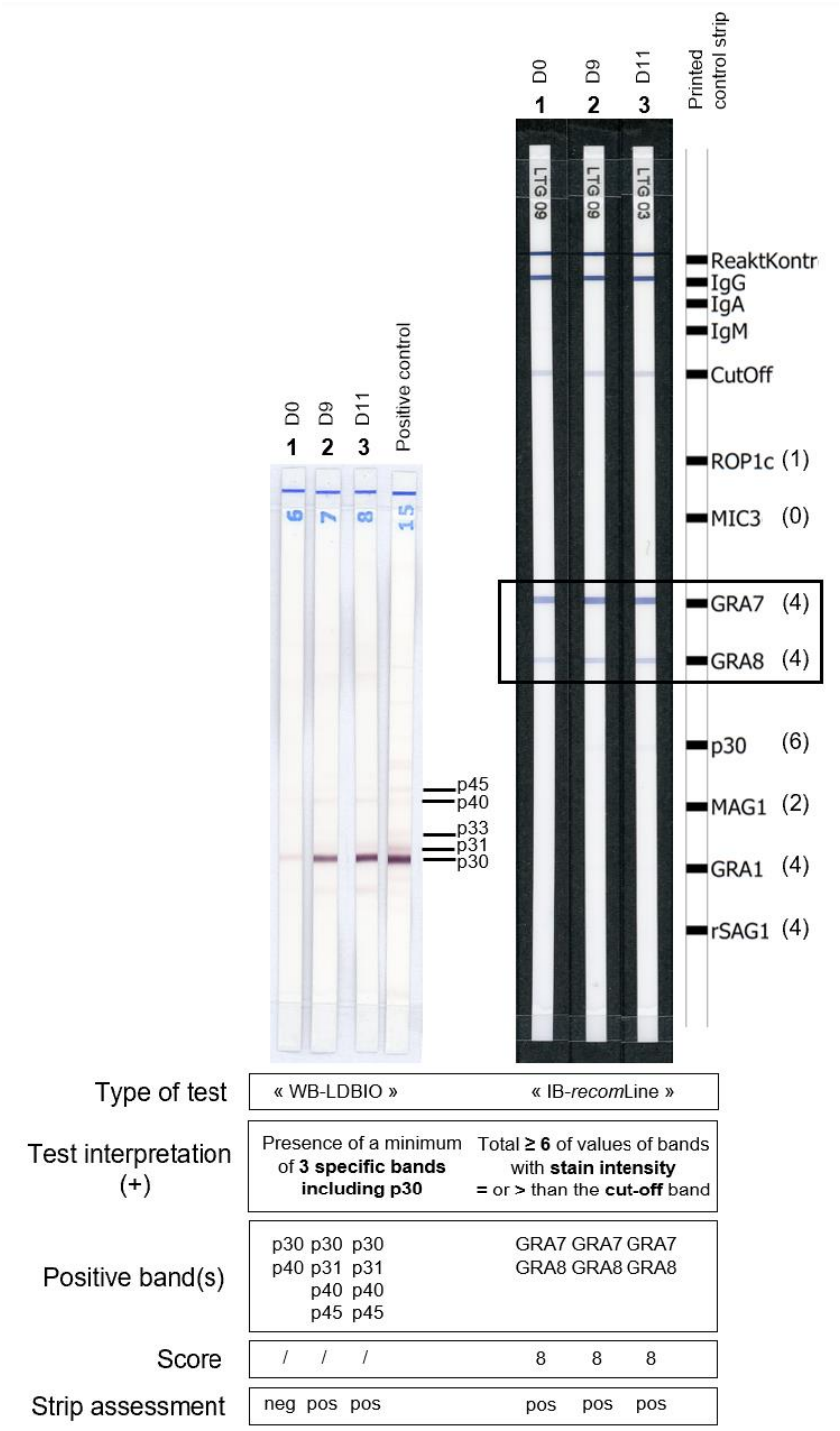


Figure 3: Example of toxoplasmic seroconversion with IgG detected earlier with the IB-*recom*Line compared to the WB-LDBIO

In this sequence of three sera belonging to the same patient: serum 1 corresponded to "day 0", serum 2, to "day 9" and serum 3, to "day 11". The number of points reported by positive bands with IB-recomLine is specified in brackets. Neg = negative result; pos = positive result.

Table 1: IB-*recom*Line IgG results of the 97 characterized sera of groups 1 and 2

	Number of sera	Biological interpretation	IB-*recom*Line result (number of samples)			WB-LDBIO result	ARCHITECT-IgG result	VIDAS-IgG result
			-	eq	+			
Group 1 *(50 sera)*	16	Negative	15	1*	0	NP	-	-
	34	Positive	0	0	1	NP	eq	eq
			0	0	2	NP	+	eq
			0	1*	30	NP	+	+
Group 2 *(47 sera)*	16	Negative	4	1*	0	-	-	eq
			2	2*	**1****	-	eq	-
			1	2*	**1****	-	+	-
			1	1*	0	-	eq	eq
	31	Positive	0	0	1	+	-	eq
			2**	1*	0	+	eq	-
			1**	0	0	+	-	+
			1**	0	1	+	+	-
			1**	3*	12	+	eq	eq
			1**	1*	3	+	eq	+
			1**	1*	1	+	+	eq

*+: positive result; -: negative result; eq: equivocal result; eq: equivocal result with previous positive sera; NP: test not performed; *: minor discrepancy with biological interpretation ; **: major discrepancy with biological interpretation.*

Table 2: IgG titers, IB-*recom*Line sensitivity, specificity and overall agreement between IB-*recom*Line and other assays

Serum group *(number of sera)*		**ELISA mean IgG levels ± SD (IU/mL)**		**IB-*recom*Line sensitivity (%) [95% CI]**		**IB-*recom*Line specificity (%) [95% CI]**		**IgG overall agreement (%) (kappa value) between IB-*recom*Line and other assays**		
		ARCHITECT	**VIDAS**	**Equivocal IB results = negative**	**Equivocal IB results = positive**	**Equivocal IB results = negative**	**Equivocal IB results = positive**	**ARCHITECT**	**VIDAS**	**WB-LDBIO**
	negative *equivocal* *positive*	<1.6 [1.6-2.9] ≥3	<4 [4-7] ≥8							
1 *(n=50)*		51.4 ± 112.1	95.1 ± 102.9	97.1 [84.7-99.9]	100 [89.7-100]	100 [79.4-100]	93.8 [69.8-99.8]	94 (0.87)	90 (0.8)	NP
2 *(n=47)*		2.9 ± 3.5	6.7 ± 2.8	58.1 [39.1-75.5]	77.4 [58.9-90.4]	87.5 [61.7-98.5]	50 [24.7-75.4]	NP	NP	55.3 (0.27)

SD: standard deviation

CI: confidence interval

NP: not performed

Table 3: Antibody kinetics (IgM and IgG) in 13 cases of women with proven seroconversion

Case n°	Available sera (Days)	Toxo IgM assay results		Toxo IgG assay results (IU/mL)			
		ARCHITECT (index)	VIDAS (index)	ARCHITECT	VIDAS	WB-LDBIO	IB-*recom*Line
1	D0	0.05	0.04	0.1	0	negative	negative
	D32	**3.95**	**1.66**	**4.1**	1	**positive**	**positive**
	D40	**3.28**	**1.75**	**13.1**	**8**	**positive**	**positive**
2	D0	0.04	0.07	0.1	0	negative	negative
	D102	0.39	0.47	**5.8**	2	**positive**	**positive**
	D109	0.42	0.5	**9.2**	7	**positive**	**positive**
3	D0	**1.22**	**0.82**	2.4	0	negative	**positive**
	D9	**1.21**	**0.95**	**9.5**	4	**positive**	**positive**
	D11	**1.11**	**1**	**12.4**	7	**positive**	**positive**
4	D0	**5.31**	**3.36**	2	2	negative	**positive**
	D7	**4.02**	**3.06**	**5.2**	**8**	negative	**positive**
	D54	**1.99**	**1.66**	**75.6**	**123**	**positive**	**positive**
5	D0	**1.44**	**0.84**	0.4	0	negative	negative
	D7	**1.63**	**1.02**	1.1	2	negative	**positive**
	D18	**1.61**	**1.11**	2.9	**8**	**positive**	**positive**
6	D0	**3.92**	**2.15**	1.7	0	**positive**	negative
	D7	**3.51**	**2.16**	2.4	0	**positive**	equivocal
	D18	**3.5**	**2.05**	**5.8**	6	**positive**	**positive**
	D183	**0.71**	**11**	**6.2**	7	**positive**	equivocal
7	D0	0.07	0.14	0	0	negative	negative
	D41	**3.37**	**2.46**	**5**	3	negative	equivocal
	D44	**3.2**	**2.62**	**8**	6	**positive**	**positive**
	D87	**3.7**	**2.51**	**141.8**	**106**	**positive**	**positive**
8	D0	**7.18**	**4.5**	**6.9**	2	**positive**	**positive**
	D8	**8.35**	**4.83**	**23.9**	**14**	**positive**	**positive**
9	D0	**11.24**	**5.4**	**5**	6	**positive**	equivocal
	D144	**3.55**	**3.07**	1.9	2	**positive**	negative
10	D0	**1.38**	**0.92**	0.1	0	negative	**positive**
	D9	**2.55**	**1.92**	0.7	0	**positive**	**positive**
	D22	**2.22**	**1.52**	**3.5**	1	**positive**	**positive**
	D29	**1.83**	**1.36**	**6**	4	**positive**	**positive**
11	D0	0.59	0.48	1.4	0	negative	equivocal
	D35	**4.26**	**3.64**	**20.7**	0	**positive**	**positive**
	D43	**6.46**	**4.58**	**47.7**	2	**positive**	**positive**
	D85	**2.63**	**2.62**	**259.2**	**119**	**positive**	**positive**
12	D0	**6.62**	**4.79**	1.1	0	negative	equivocal
	D28	**4.75**	**3.9**	1.7	1	**positive**	**positive**
	D35	**4.62**	**3.98**	1.6	1	**positive**	**positive**
	D63	**3.65**	**3.05**	2.4	1	**positive**	equivocal
	D122	**2.53**	**2.17**	2.7	1	**positive**	equivocal
13	D0	**5.81**	**4.17**	**5.8**	4	**positive**	**positive**

D: day; shaded boxes indicate equivocal results; values in boldface represent positive results.

Acknowledgements:

The authors would like to thank V. Martin, M. Carbillet, M. Potinet and C. Perrin (Grenoble, France) for their technical support.

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

The authors received no specific funding for this work.

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III. Conclusion

La détection des IgG anti-*T. gondii* pour déterminer le statut immunitaire et diagnostiquer une séroconversion toxoplasmique repose principalement sur les techniques ELISA et, si nécessaire, sur un test de confirmation réalisé en seconde intention : le WB-LDBIO.

Nous avons ainsi évalué les performances diagnostiques de l'IB-*recom*Line pour la détection d'IgG avec 171 sérums humains sélectionnés et caractérisés, provenant de trois contextes cliniques et biologiques différents : des sérums sans difficulté d'interprétation biologique (groupe 1), des sérums entraînant des difficultés d'interprétation biologique (groupe 2) et des sérums séquentiels provenant de femmes enceintes avec une séroconversion prouvée (groupe 3). Nos résultats ont montré que les performances de l'IB-*recom*Line ne sont pas satisfaisantes pour détecter des taux résiduels d'IgG chez les patients anciennement infectés : l'utilisation de ce test a abouti à 13 résultats faussement négatifs (dont sept discordances majeures) principalement liés à une trop faible intensité de la bande p30 (SAG1) (groupe 2). En revanche, l'IB-*recom*Line s'est révélé très performant pour détecter, précocement, de faibles taux d'IgG à la suite d'une primo-infection (groupe 3). Dans notre étude, l'IB-*recom*Line était la technique la plus sensible pour détecter l'apparition d'IgG nouvellement synthétisés chez une femme enceinte : plus sensible que l'ARCHITECT, le WB-LDBIO et le VIDAS. La forte sensibilité de l'IB-*recom*Line serait liée aux bandes GRA8 et GRA7 qui sont les premières à apparaître à la suite d'une séroconversion. Enfin, pour huit sérums, l'IB-*recom*Line n'est pas parvenu à infirmer les résultats faussement positifs obtenus avec les tests ELISA contrairement à la technique de confirmation (WB-LDBIO) (groupe 2). Dans la majorité des cas de faux-positifs de l'IB-*recom*Line, les bandes GRA8 et/ou GRA7 ont été détectées.

Au total, GRA7 et GRA8 étaient les principaux antigènes détectés à la suite d'une séroconversion, rendant l'IB-*recom*Line fortement sensible (groupe 3) mais la détection de ces deux bandes était aussi responsable de résultats faussement positifs de l'IB-*recom*Line (groupe 2). Ainsi, la forte sensibilité présumée de l'IB-*recom*Line observée dans notre étude, se doit d'être interprétée avec précaution car la détection des bandes correspondant aux antigènes GRA7 et GRA8 peut également aboutir à des résultats faussement positifs.

Pour conclure, l'utilisation de l'IB-*recom*Line comme alternative au WB-LDBIO, actuellement utilisé comme test de confirmation de l'infection par *T. gondii*, permettrait, certes, une détection plus précoce des séroconversions toxoplasmiques mais il ne permettrait pas

d’infirmer les résultats faussement positifs ou faussement négatifs obtenus avec les tests ELISA pouvant entrainer des interprétations biologiques erronées et conduire à de graves conséquences pour les patients.
À notre connaissance, cette étude est la première à avoir évalué les performances diagnostiques de l’IB-*recom*Line en tant que test sérologique de confirmation pour la détection des IgG anti-*T. gondii*. Les résultats de nos expériences pourront être utiles et intéresser d'autres biologistes médicaux effectuant le diagnostic sérologique de la toxoplasmose.

À titre personnel, ce mémoire m’a donné l’occasion de présenter un poster au congrès de l’ECCMID (European Congress of Clinical Microbiology & Infectious Diseases) le 09 juillet 2021 et de soumettre, en premier auteur, un article original en cours de révision dans le journal scientifique *Parasite* (IF = 3,020).

ESCMID

Online
9 – 12 July 2021

31st ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Basel, 5th August 2021

To whom it may concern:

CONFIRMATION OF PRESENTATION AT ECCMID 2021

We hereby confirm that the following abstract was submitted, accepted and presented at the 31st ECCMID, the European Congress of Clinical Microbiology and Infectious Diseases, that took place online from 9 – 12 July 2021.

Title: Serological diagnosis of toxoplasmosis: evaluation of available commercial IgG immunoblot test based on recombinant antigens on 142 characterised human sera

Abstract Authors: Vincent JEAN-PIERRE, Julien MIOZZO, Hélène FRICKER-HIDALGO, Cécile GARNAUD, Marie Gladys ROBERT, Hervé PELLOUX, Marie-Pierre BRENIER-PINCHART - (1)Parasitology-Mycology, Grenoble Alpes University Hospital, France

Presenter: Vincent Jean-Pierre

Session Title: Contemporary issues in the diagnosis of parasitic infections

Presentation Type: 1-hour ePoster Review

Abstract / Presentation Number: 1473

Yours sincerely,

Jacob Moran-Gilad
ECCMID Programme Director

31st ECCMID Online 9 – 12 July 2021

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Abstract number: 1473

Serological diagnosis of toxoplasmosis: evaluation of available commercial IgG immunoblot test based on recombinant antigens on 172 characterized human sera

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CONTEXT

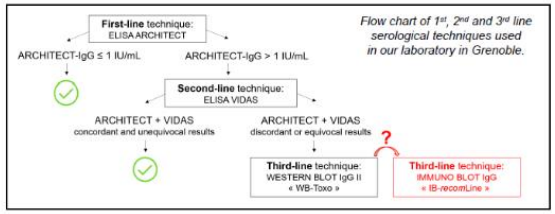
Detection of anti-*Toxoplasma gondii (Tg)* IgG is essential in two situations:

- determination of the immune status of pregnant women or immunodeficient patients
- confirmation of a seroconversion after IgM appearance.

Detection of *Tg*-specific IgG usually relies on ELISA techniques. However, interpretation of low or equivocal IgG ELISA titers can be difficult and requires further testing.

In France, the **Toxo II IgG Western blot** (WB-Toxo) (LDBio), based on **natural *T. gondii* antigens**, is currently recommended as a **reference confirmation technique**.

The ***recom*Line IgG immunoblot** (IB-*recom*Line) (Mikrogen), based on **recombinant *T. gondii* antigens**, is another qualitative assay commercially available for the detection of *Tg*-specific IgG.



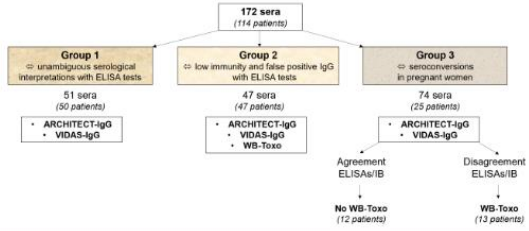
Flow chart of 1st, 2nd and 3rd line serological techniques used in our laboratory in Grenoble.

AIM

This study aimed at evaluating the **diagnostic performances** of the ***recom*Line IgG immunoblot** with digital reading (*recom*Scan) for the detection of IgG in different situations.

METHODS

The ***recom*Line IgG immunoblot** was retrospectively performed on 172 sera (114 patients), classified into three groups based on previous ELISA ARCHITECT-IgG, ELISA VIDAS-IgG +/- WB-Toxo (in case of equivocal IgG titers) results.



RESULTS

Group 1 *(51 sera)* ⇔ unambiguous serological interpretations with ELISA tests

Number of sera	ARCHITECT-IgG	VIDAS-IgG	Biological interpretation	IB-*recom*Line -	eq	+
17	-	-	-	16	1	0
1	eq	eq	+	0	0	1
2	+	eq	+	0	0	2
31	+	+	+	0	1	30

Sensitivity [95% CI]:
- Eq → -: 97% [84.7-99.9]
- Eq → +: 100%

Specificity [95% CI]:
- Eq → -: 100%
- Eq → +: 94% [71.3-99.9]

Minor discrepancies (= 2)
- - → eq (=1)
- + → eq (=1)

IgG overall agreement (Kappa coeff.):
- ARCHITECT/IB: 94.1% (0.88)
- VIDAS/IB: 90.2% (0.80)

Group 2 *(47 sera)* ⇔ low immunity and false positive IgG with ELISA tests

Number of sera	ARCHITECT-IgG	VIDAS-IgG	WB-Toxo	IB-*recom*Line -	eq	+
6	-	eq	-	5	4	1	0
			+	1	0	0	1
8	eq	-	-	5	2	2	1
			+	3	2	1	0
1	-	+	+	1	1	0	0
6	+	-	-	4	1	2	1
			+	2	1	0	1
18	eq	eq	-	2	1	1	0
			+	16	1	3	12
5	eq	+	+	5	1	1	3
3	+	eq	+	3	1	1	1

Sensitivity [95% CI]:
- Eq → -: 58.1% [39.1-75.5]
- Eq → +: 77.4% [58.9-90.4]

Specificity [95% CI]:
- Eq → -: 87.5% [61.7-98.5]
- Eq → +: 50% [24.7-75.4]

Minor discrepancies (= 12)
- - → eq (=6)
- + → eq (=6)

Major discrepancies (= 9)
- - → + (=2)
- + → - (=7)

Group 3 *(25 patients)* ⇔ seroconversions in pregnant women

Number of seroconversions	ARCHITECT-IgG	VIDAS-IgG	WB-Toxo	IB-*recom*Line
12	concordant	concordant	/	concordant
4	-	-	-	eq
			-	+
3	Eq	-	-	+
			+	-
4	+	-	-	eq
			+	+
2	+	eq	+	eq
			+	+

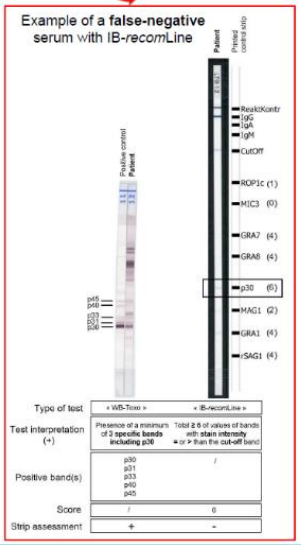
***Result of the first IgG-positive serum** whatever the techniques used*

IB-*recom*Line was positive **later** than WB-Toxo (= 2)

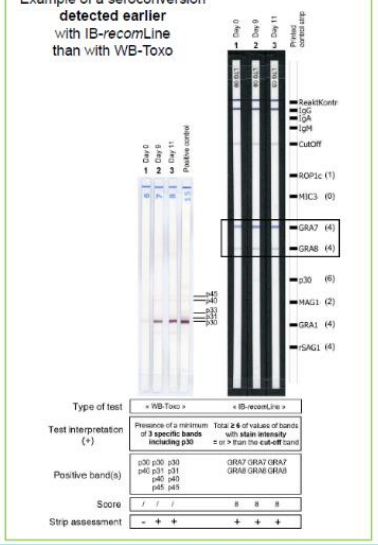
IB-*recom*Line was positive **at the same time** than WB-Toxo (= 4)

IB-*recom*Line was positive **earlier** than WB-Toxo (= 7)

Example of a **false-negative** serum with IB-*recom*Line



Example of a seroconversion **detected earlier** with IB-*recom*Line than with WB-Toxo



CONCLUSIONS

- When both IgG ELISA results (ARCHITECT-IgG and VIDAS-IgG) were **concordant**, IB-*recom*Line **performed well** with high overall agreements between ARCHITECT or VIDAS and IB-*recom*Line and with Kappa coefficients of 0.88 and 0.80, respectively (group 1).
- However, when both ARCHITECT-IgG and VIDAS-IgG results were **discordant or equivocal**, performances of IB-*recom*Line **were insufficient** to confirm toxoplasmosis immune status (group 2) because of many equivocal (= 12) or false-negative (= 7) results.
- Nevertheless, compared to WB-Toxo, IB-*recom*Line may allow an **earlier diagnosis** of toxoplasmosis during pregnancy (=7/25 seroconversions) (group 3). IB-*recom*Line was the most sensitive of the 4 techniques for detecting the appearance of IgG in pregnant women in 4 out of 25 seroconversions.

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