Evaluation of the usefulness of six commercial agglutination assays for serologic diagnosis of toxoplasmosis

Odile Villard a,⁎, Bernard CIMON b, Jacqueline Franck c, Hélène Fricker-Hidalgo d, Nadine Godineau e, Sandrine Houze f, Luc Paris g, Hervé Pelloux d, Isabelle Villena h, Ermanno Candolfi i, and

The network from the French National Reference Center for Toxoplasmosis

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1 Members of the National Reference Centre for Toxoplasmosis are listed at the end of the article.

a Institut de Parasitologie et de Pathologie Tropicale de Strasbourg, Université de Strasbourg, Hôpitaux universitaires de Strasbourg, 3 rue Koeberlé, 67000 Strasbourg, France
b Laboratoire de Parasitologie-Mycologie, Institut de Biologie en Santé, Centre Hospitalier Universitaire, 4 rue Larrey, 49933 Angers cedex 9, France
c Laboratoire de Parasitologie-Mycologie, Hôpital de la Timone, 264 rue Saint Pierre, 13385 Marseille cedex 05, France
d Laboratoire de Parasitologie-Mycologie, Université Joseph Fourier, Grenoble 1 et Centre Hospitalier Universitaire A Michallon, BP 217, 38043 Grenoble cedex, France
e Laboratoire de Parasitologie-Mycologie, Centre Hospitalier de Saint-Denis, 2 rue du Docteur Pierre Delafontaine, BP 279, 93205 Saint-Denis cedex, France
f Laboratoire de Parasitologie-Mycologie, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris cedex 18, France
g AP-HP, groupe hospitalier Pitié-Salpêtrière, laboratoire de Parasitologie-Mycologie, 47-83 bd de l'hôpital, 75651 Paris cedex 13, France
h Laboratoire de Parasitologie-Mycologie, Hôpital Maisonneuve-Rosemont, Centre Hospitalier Universitaire, 45 rue Cognacq-Jay, 51092 Reims cedex, France
i Institut de Parasitologie et de Pathologie Tropicale de Strasbourg, Université de Strasbourg, Hôpitaux universitaires de Strasbourg, 3 rue Koeberlé, 67000 Strasbourg, France

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ABSTRACT

Six agglutination tests for detecting Toxoplasma gondii-specific antibodies (immunoglobulin G or M) in serum were performed and compared. In total, 599 sera were examined using direct and indirect agglutination assays. Sensitivity varied from 93.7% to 100% and specificity from 97.1% to 99.2%. In a selected population with interfering diseases, the percentage of false positives ranged from 4.3% to 10.9%. Although an overall agreement of 100% was found for chronic toxoplasmosis, sensitivity for the detection of confirmed acute toxoplasmosis ranged from 86.4% to 97.3%. Regarding the large variability in terms of the performance of the 6 assays, tests based on the hemagglutination principle were found to be better than the other agglutination tests for all the panels evaluated, meaning that they could be used as qualitative or semiquantitative low-cost screening assays.

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1. Introduction

Toxoplasmosis is often an asymptomatic infection in immunocompetent subjects occurring worldwide (Montoya and Liesenfeld, 2004). Infection during pregnancy may lead to transplacental transmission of the parasite, resulting in congenital toxoplasmosis (Garcia-Meric et al., 2010). In 2003, in France, the seroprevalence of toxoplasmosis in pregnant women was estimated at 44%, with the incidence of congenital toxoplasmosis being 2.9 per 10,000 live births (Villena et al., 2010). Among the available methods, those based on agglutination have attracted much interest, due to their lower cost. However, their performance is usually not evaluated and their specificity tends to be less than 95% (Evans and Ho-Yen, 2000; Fourlinnie et al., 1985; Robert et al., 1984).

In 2006, the French National Reference Center for Toxoplasmosis (NRCT) conducted an investigation on the methods used for the serologic diagnosis of toxoplasmosis, showing that agglutination-based assays were still used in several laboratories in France. Although 75% of the network of NRCT laboratories (n = 30) were using at least 2 methods, which were generally used as a second technique combined with an enzyme immunoassay. Another investigation conducted in private medical biology laboratories (n = 58) in eastern France revealed that only 34% were using 2 methods, but for 94%, the second method was an agglutination-based reagent.

Certainly, agglutination methods can be used for rapid screening or confirmation in the case of pregnant women, as they are easy to

while it also confirms the absence of any previous exposure (Liesenfeld et al., 1997). In clinical situations requiring the dating of the Toxoplasma infection, Toxoplasma IgG avidity may be measured (Candolfi et al., 2007; Lachaud et al., 2009; Lecolier and Pucheu, 1993).
perform, rapid, and inexpensive. Some assays detect either IgG and/or IgM, but they cannot be used alone, as French legislation requires IgM and IgG to be determined using a titration of IgG \cite{HAS, 2009}. Moreover, other assays detect only IgG, with IgM being eliminated during sample pretreatment, although such methods are not able to diagnose early infection characterized by positive IgM and negative IgG results.

The aim of our study was therefore to evaluate the 6 most commercially used reagents based on agglutination of sensitized polystyrene latex beads, red blood cells, or *Toxoplasma* in order to determine their specificity, sensitivity, as well as positive and negative predictive values (PPV and NPV, respectively) in various clinical settings.

2. Materials and methods

2.1. Panel sera

The sera \((n = 589)\) were supplied by 30 members of the NRCT network. The presence of IgG or IgM was confirmed using at least 2 different methods for each antibody isotype, namely, the indirect immunofluorescence assay (IFAT-G) \cite{Walton, 1966} and the dye test \cite{Desmonts, 1963, Reiter-Owona, 1999, Sabin, 1948} for IgG, and IFAT-M and immunosorbent agglutination assay \cite{ISAGA, 1990, Sabin, 1974} for IgM. The reference methods used to confirm the presence of IgG and/or IgM are historical and consensual methods most commonly used in expert laboratories to qualify sera in the serologic diagnosis of toxoplasmosis \cite{Montoya, 2002}. The sera were consequently divided into 4 panels.

Panel 1 containing 452 sera from routine testing was used to estimate sensitivity, specificity, PPV, and NPV. This panel comprised 199 negative sera, 174 sera positive for IgG and negative for IgM, and 79 positive for both IgG and IgM.

Panel 2 was used to evaluate clinical specificity. This panel consisted of 46 sera taken from patients with potentially interfering diseases, namely, autoimmune diseases (scleroderma, antinuclear antibodies [ANA]), viral infections (hepatitis A, herpes, and cytomegalovirus diseases), and bacterial infections (lyme and syphilis diseases).

Panel 3 was aimed at assessing the detectability and specificity of reagents, with 33 sera taken from patients with low amounts of IgG, without IgM, and classified in the grey zone by at least one of the methods utilized by a member of the network.

Panel 4 was used to evaluate the methods in clinical situations, such as acute and chronic infections based on patient follow-up \cite{Cozon, 1998}. This panel comprised 58 sera, with 37 sera taken from 16 patients with acute toxoplasmosis (seroconversion confirmed by a previously negative serum) and 21 sera from chronic infection (more than 1 year confirmed by computer data anteriorities) patients with or without persistent positive IgM. Among the 16 patients with acute toxoplasmosis, 16 sera were IgG and IgM negative at the initial serologic test during pregnancy, while 5 were IgM positive with equivocal IgG and 16 were positive for both antibodies \cite{FDA, 2007}.

2.2. Serologic tests

Six agglutination registered kits with European Conformity (CE) marking were evaluated. Among the 6 kits, 3 were based on indirect agglutination of sensitized latex or polystyrene particles \cite{Dunford, 1991, Senet, 1974} of human or sheep red blood cells sensitized with *Toxoplasma* antigens \cite{Fourlinnie, 1985, Seguela, 1976}, and 1 on direct agglutination of formalin-fixed tachyzoites of *T. gondii* \cite{Johnson, 1989}. Study evaluations were conducted under “blinded conditions,” with each agglutination being assessed by 2 independent readers. The results were expressed using qualitative analysis strictly in accordance with the manufacturer's instructions.

To summarize the 6 kits, Toxolatex (Fumouze Diagnostics, Le Malesherbes, Levallois Perret, France) employed an indirect agglutination of polystyrene particles, sensitized with *Toxoplasma* antigens and membrane-enriched fraction of *Toxoplasma* antigen, allowing for the detection of IgG and IgM. The detection limit was 10 IU/mL.

Pastorex Toxo (Bio-Rad, Marnes-la-Coquette, France) involved an indirect agglutination of latex particles, sensitized with *Toxoplasma* soluble antigen, thus permitting the detection of IgG and IgM. The use of a green dye facilitated the reading when positive, showing a green background with red agglutinates. The detection limit was not provided by the manufacturer.

Toxocell Latex (Biokit, Llíria d’Amunt, Barcelona, Spain) was based on an indirect agglutination of latex particles sensitized with *Toxoplasma* antigen. This technique allowed for the detection of IgG and IgM, with a limit of 10 IU/mL.

Toxo-HAI (Fumouze Diagnostics) was an indirect hemagglutination assay using sheep red blood cells (RBCs) sensitized with *Toxoplasma* antigen. The detection and differentiation of IgG and IgM were possible using 2-mercaptoethanol (2-ME), with the detection limit being 8 IU/mL.

Toxoplasmosis Celloagnost (Siemens Healthcare Diagnostics, Deerfield, IL, USA) involved an indirect hemagglutination of human RBCs sensitized with *Toxoplasma* antigen. This technique permitted the detection of IgG and IgM, with a limit of 5 IU/mL.

Toxoscreen DA (bioMérieux) utilized a direct agglutination test with formalin-fixed tachyzoites of *T. gondii*. Dilution buffer containing 2-ME denaturing IgM allowed for the detection of IgG alone. The detection limit was 4 IU/mL.

A summary of the characteristics of the different reagents is provided in Table 1, including the sensitivity and specificity of the tests as supplied by the manufacturers. All sensitivities, specificities, overall agreement, NPV, and PPV were calculated following FDA recommendations \cite{FDA, 2007}.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sensitivity threshold</th>
<th>Announced sensitivity</th>
<th>Announced specificity</th>
<th>Clinical sensitivity</th>
<th>Clinical specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxolatex® (Fumouze)</td>
<td>5 UI</td>
<td>98.8%</td>
<td>97.2%</td>
<td>93.7%</td>
<td>97.1%</td>
<td>97.1%</td>
<td>93.7%</td>
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<td>Toxocell® (Biokit)</td>
<td>10 UI</td>
<td>98.7%</td>
<td>95.8%</td>
<td>96.8%</td>
<td>97.6%</td>
<td>97.6%</td>
<td>96.8%</td>
</tr>
<tr>
<td>Pastorex® (BioRad)</td>
<td>ND</td>
<td>94.3%</td>
<td>100%</td>
<td>98.8%</td>
<td>98.8%</td>
<td>98.8%</td>
<td>98.8%</td>
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<tr>
<td>Celloagnost® (Siemens)</td>
<td>5 UI</td>
<td>100%</td>
<td>97.3%</td>
<td>100%</td>
<td>98.8%</td>
<td>98.8%</td>
<td>100%</td>
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<tr>
<td>HAI® (Fumouze)</td>
<td>8 UI</td>
<td>99.2%</td>
<td>97.7%</td>
<td>100%</td>
<td>99.2%</td>
<td>99.2%</td>
<td>100%</td>
</tr>
<tr>
<td>Toxoscreen® (bioMérieux)</td>
<td>4 UI</td>
<td>96.2%</td>
<td>98.8%</td>
<td>100%</td>
<td>99.2%</td>
<td>99.2%</td>
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</tbody>
</table>

ND = Not done.

* Information from the box insert provided by the manufacturers.

** Tests assay results are in comparison to the reference methods for IgG and IgM tests.
showed poor sensitivity for both Toxolatex and Pastorex Toxo kits with chronic toxoplasmosis. The best sensitivity was 97% based on hemagglutination, Toxo-HAI and Cellognost, achieved the reagents based on red blood cells or susceptible to syphilis antibodies, although they did not have the Latex- and polystyrene-based reagents were susceptible to viral results were accounted for by positive ANA in the evaluated assays. revealed false-positive results. The majority of false-positive antibodies in patients with potentially interfering diseases were consistent with the performances publicized by the manufacturers. The overall agreement observed for panel 4, the group of patients to exhibit 100% sensitivity (Table 1). However, specificity varied, being 98.8% for the IHA kits and 99.2% for Toxoscreen. These results were consistent with the performances published by the manufacturers (Table 1). NPV was 100% for the 3 reagents, while PPV approached 99%, being 99.8% for the IHA kits and 99.2% for Toxoscreen. These results were consistent with the performances published by the manufacturers (Table 1). NPV was 100% for the 3 reagents, while PPV approached 99%.

The reagent kit Pastorex Toxo, based on the indirect agglutination of latex particles, revealed a sensitivity and specificity of 98.8%, with similar results for PPV and NPV. Although the sensitivity was better than the figure provided by the manufacturer (98.8% versus 94.3%), the specificity was found to be lower (98.8% versus 100%).

The sensitivity of the final reagents, Toxolatex and Toxocell Latex, also based on indirect agglutination, was 93.7% and 96.8%, respectively. The performance was lower than that advertised by the manufacturer (98.8% and 98.7%, respectively). Furthermore, the specificity of these 2 reagents was 97.1% and 97.6%, respectively, which was consistent with the performances supplied by the manufacturers. PPV approached 97% for the 2 reagents, while NPV was 93.7% for Toxolatex and 96.8% for Toxocell Latex.

The analysis of data relating to the detection of Toxoplasma antibodies in patients with potentially interfering diseases revealed false-positive results (Table 2). The majority of false-positive results were accounted for by positive ANA in the evaluated assays. Latex- and polystyrene-based reagents were susceptible to viral antibodies (IgM anti-hepatitis A virus or IgM anti-cytomegalovirus). Reagents based on red blood cells or Toxoplasma agglutination were susceptible to syphilis antibodies, although they did not have the highest specificity, noted to be 95.7%.

The ability of reagents to detect low amounts of IgG showed poor sensitivity for both Toxolatex and Pastorex Toxo kits with 51.5% and for Toxocell Latex with 66.7% (Table 2). Better sensitivity was observed with Toxoscreen (84.8%), while the reagents based on hemagglutination, Toxo-HAI and Cellognost, achieved the best sensitivity with 97%.

The overall agreement observed for panel 4, the group of patients with chronic toxoplasmosis (>1 year after the primary infection), was recorded to be 100% for each kit (Table 3).

When analyzing the data for patients with confirmed acute toxoplasmosis (panel 4) (Table 3), overall agreement approached 98% for Toxocell Latex, Pastorex Toxo, and Toxo-HAI. For the 3 remaining kits, overall agreement was 86.4% (Toxoplasmosis Cellognost), 91.8% (Toxoscreen), and 94.8% (Toxolatex).

### 3. Results

Regarding the performance of reagents on routine sera (panel 1), the IHA kits of Toxo-HAI and Toxoplasmosis Cellognost in addition to the direct agglutination T. gondii kit of Toxoscreen DA were all shown to exhibiting 100% sensitivity (Table 1). However, specificity varied, being 98.8% for the IHA kits and 99.2% for Toxoscreen. These results were consistent with the performances published by the manufacturers (Table 1). NPV was 100% for the 3 reagents, while PPV approached 99%.

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### 4. Discussion

A survey of medical biology laboratories in eastern France was conducted in 2006–2007 in order to assess the practices relating to the biological diagnosis of toxoplasmosis, with the large use of agglutination reagents being confirmed. Comparing this recent survey to an earlier one conducted in 56 laboratories 10 years prior (Le Pape et al., 1996), we observed that 66% of medical biology laboratories were exclusively using an enzyme-linked immunosorbent assay (ELISA) method in 2006–2007 compared to only 29.7% in 1996. This trend points to the increased performance of such assays as well as to the general desire to reduce costs. However, when a second method was used in the remaining 34% of medical biology laboratories, a latex or polystyrene agglutination assay was employed in 94% of the investigated laboratories. Our investigation thus sought to comprehend the current position of these agglutination-based reagents in the serologic diagnosis of toxoplasmosis by assessing 6 assays in the same biobank in order to determine their sensitivity and specificity. The kits were also stress tested with specific panels involving interfering antibodies, low levels of Toxoplasma antibodies, and cases of acute infection.

A central issue currently under discussion is the potential value of screening in order to prevent congenital toxoplasmosis, mainly related to the high costs of serologic tools in terms of their cost-benefit ratio. Maternal serologic screening to prevent congenital toxoplasmosis is cost saving a test cost of US$12 for rates of congenital toxoplasmosis greater than 0.0001. If tests costs are reduced, screening becomes cost saving even in a population with rates of congenital infection below 0.0001 (Ancelle et al., 2009; Stillwaggon et al. 2011). Low-cost tools should therefore be investigated, although the major purpose of reagents should not be forgotten, that is, the detection of toxoplasmosis during pregnancy via serologic screening.

Firstly, a high degree of specificity is essential, as false positives lead to discontinued testing, meaning that these risky samples are discarded from follow-up and pregnant women are wrongly
reassured into believing that they were immune. Agglutination assays are renowned for being susceptible to the presence of autoantibodies, which generate false-positive results (Holliman et al., 1989). We found that these assays were indeed susceptible to autoantibodies (Gray et al., 1990), such as ANA antibodies, as well as to acute viral infections, although our results proved to be better than the findings of Johnson et al. (1989). The major bias in our study was of course the limited number of sera analyzed from potentially interfering diseases.

A high degree of sensitivity is desirable, although in cases of false negatives, pregnant women are inappropriately followed up with economic consequences. In the case of acute infection, a false-negative result leads to delayed treatment of the pregnant woman, thus resulting in an increased risk of serious sequelae (Cortina-Borja et al., 2010; Gilbert and Gras, 2003). In our study, the tests based on hemagglutination or Toxoplasma agglutination were found to be the most sensitive.

The methods based on the agglutination of polystyrene or latex particles (i.e., Toxolatex, Toxocell, and Pastorex Toxo) were not able to differentiate between IgG and IgM antibodies. In routine samples, their sensitivity ranged from 93.7 to 98.8% and specificity from 97.1 to 98.8%. However, such screening in susceptible pregnant women must aim to achieve high specificity (99.2%), which was reached with the IHA-based methods and Toxoscreen DA in our study. Consequently, the easy-to-use and low-cost methods based on the agglutination of polystyrene or latex beads should be systematically used along with more conventional methods, which are able to detect low levels of IgG and IgM antibodies, such as ELISA- or chemiluminescent-based methods.

The ability of the kits to detect Toxoplasma antibodies in patients with chronic infections was found to be perfect for all reagents. However, when sera containing low levels of IgG antibodies were tested and confirmed by 2 reference methods, such as the dye test or IFAT, performance severely declined. An exception was the 2 IHA-based assays, which were still highly sensitive. Therefore, in the case of equivocal IgG, we recommend the use of other methods, such as the immunoblot or dye test, which have very low detectability available at a reference laboratory (Fiori et al., 2009; Franck et al., 2008).

In acute infections, sensitivity ranged from 86.4% to 97.3%. The performance of the IHA-based assay, Cellognost, and Toxoscreen DA was poor due to their inability to detect low levels of IgG or, in the case of Toxoscreen DA, to detect IgM when using 2-ME in its dilution buffer. For Toxolatex, 2 false-positive results were recorded at the start of infection, with the sera testing IgG- and IgM negative with all the reference methods. Consequently, these false-positive results reminded us that this assay had the poorest specificity among the evaluated kits. On the other hand, Toxocell, Pastorex, and Toxo-HAI showed a very good performance in the panel of acute infection cases.

Agglutination assays are still widely used in laboratories, although their individual performances vary considerably. The majority of these assays should be used in tandem with another method as a confirmatory assay. However, the use of 2 or 3 tests appears to be appropriate for the situation in France, where a serologic survey is mandatory for women at risk during pregnancy. The serologic prevalence in France was 43.8% (Berger et al., 2008), while the risk of seroconversion was estimated to be between 5.4 and 13.2 per 1000 pregnant seronegative women (Ancelle et al., 1996). In response to our data, other countries may find it worthwhile to introduce a low-cost method, such as the hemagglutination assay kits of Toxo-HAI and Toxoplasmosis Cellognost, for the screening of congenital toxoplasmosis according to their estimated rate of congenital toxoplasmosis.

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Appendix A

Members of the National Reference Centre for Toxoplasmosis and Toxosurv network in alphabetical order

A Totet (Hospital and University Centre Amiens); B Cimon (Hospital and University Centre Angers); E Scherer (Hospital and University Centre Besançon); I Accocheberry (Hospital and University Centre Bordeaux); G Nevez and D Quinio (Hospital and University Centre Brest); J Bonhomme (Hospital and University Centre Caen); B Carme and M Demar (Hospital and University Centre Cayenne); A Bonnin, B Cuisenier, and F Dalle (Hospital and University Centre Dijon); N Desbois (Hospital and University Centre Fort de France); MP Brenier-Pinchart, H Fricker-Hidalgo, and H Pelloux (Hospital and University Centre Grenoble); L Delhaes (Hospital and University Centre Lille); D Ajzenberg and M L Dardé (Hospital and University Centre Limoges); J Franck and R Piarroux (Hospital and University Centre Marseille); P Bastien, Y Sterkers, and F Pratlong (Hospital and University Centre Montpellier); M Machouart (Hospital and University Centre Nancy); M Letterie and F Morio (Hospital and University Centre Nantes); N Ferret, C Pomares, and P Marty (Hospital and University Centre Nice); A Angoulvant (Hospital and University Centre Paris Bicêtre); S Houze (Hospital and University Centre Paris Bichat); T Ancelle and H Yera (Hospital and University Centre Paris Centre Cochon); F Derouin and J Menotti (Hospital and University Centre Paris St Louis); ME Bougnoux and N Hassouni (Hospital and University Centre Paris Necker Enfants Malades); F Touafek and L Paris (Hospital and University Centre Paris Salpetrière); N Godineau (Hospital and University Centre Paris St Denis); P Roux and C Hennequin (Hospital and University Centre Paris St Antoine); S Azı (Hospital and University Centre Pointe A Pitre); J Berthonneau (Hospital and University Centre Poitiers); D Aubert, C Chemla, and I Villena (Hospital and University Centre Reims); F Robert-Gangneux (Hospital and University Centre Rennes); L Favenneck and G Gargala (Hospital and University Centre Rouen); P Flori (Hospital and University Centre St Etienne); E Candolfi, D Filisetti, and O Villard (Hospital and University Centre Strasbourg); J Filiaux and S Cassaing (Hospital and University Centre Toulouse); N Vanlangendonck (Hospital and University Centre Tours).

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