Latex Agglutination Test for Detection of Antibodies to Toxoplasma gondii

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Received 28 April 1988/Accepted 20 July 1988

A resurgence of interest in Toxoplasma gondii has occurred because this coccidian parasite causes lethal infections in immunologically compromised hosts and is responsible for at least 3,000 congenitally infected infants in the United States annually. Thus, rapid, specific, and inexpensive serologic tests are required for routine screening of patients, especially pregnant women. We have developed a latex agglutination test for antibodies to T. gondii which utilizes covalently coupled T. gondii antigens. When compared with an indirect immunofluorescence assay, the latex test had a sensitivity of 94% and specificity of 100%. Compared with an enzyme-linked immunosorbent assay, the latex test had 86% sensitivity and 100% specificity. When testing samples which exhibited nonspecific polar staining by the immunofluorescence assay, the enzyme-linked immunosorbent assay had a 50% false-negative rate, whereas the latex agglutination test yielded no false-positive results. Thus, the latex agglutination test provided an efficacious method for routine serological screening for antibodies to T. gondii.

Toxoplasma gondii is a coccidian parasite that causes infections in birds, mammals, and reptiles worldwide. Serological surveys of humans indicate that as much as one-third of the world’s population has been infected by T. gondii (3). In the United States alone, 50% of the population has antibodies to T. gondii, and infections of pregnant women account for at least 3,000 congenitally infected infants annually (6).

Although of little concern in normal, immunologically competent hosts, T. gondii infections can be fatal when the patient’s immune system is unable to control the infection. Such is the case in congenital toxoplasmosis when T. gondii crosses the placenta during a primary maternal infection and causes disseminated infection of the fetus. In adults, primary and reactivated T. gondii infections are frequently fatal in patients suffering from the acquired immune deficiency syndrome (7, 10, 14). In addition, encysted T. gondii in transplanted hearts or other organs may cause serious infections in immunosuppressed, toxoplasma-negative transplant recipients (8).

Toxoplasma infections can be detected by histological examinations of biopsy specimens or by isolation of T. gondii from body fluids or tissues (4), but clinical diagnosis of toxoplasmosis is most commonly supported by serological testing (1). Recent concern about Toxoplasma infections in neonates and immunocompromised patients has increased the demand for test methods for Toxoplasma that are rapid, specific, and inexpensive. To this end, we have developed a latex agglutination test for detecting antibodies to T. gondii.

The antigen for the latex agglutination test was derived from whole, unfixed T. gondii (RH strain) tachyzoites which were passaged twice weekly in primary human foreskin fibroblast (HFF) cultures. For Toxoplasma antigen production, HFF cultures were infected at a multiplicity of infection of 5 for 72 h at 37°C in Eagle minimum essential medium containing 5% fetal bovine serum. The culture medium was decanted after 24 h, and fresh minimal essential medium–3% fetal bovine serum was added. The culture medium, containing 1 × 10^3 to 2 × 10^3 organisms per ml, was collected, and the tachyzoites were filtered through a 3.0-μm polycarbonate filter into a polypropylene tube containing 50 μl of sodium heparin (5,000 U/ml). The tachyzoites were washed three times in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) containing 50 U of sodium heparin per ml. Tachyzoites were suspended in PBS at 5 × 10^9 organisms per ml and sonicated at 125 W/cm^2 for 10 min at 4°C. The sonicated material was centrifuged at 15,000 × g for 5 min, and the supernatant fluid (Toxoplasma antigen), containing 1.6 mg of total protein per ml, was used to sensitize the latex particles.

The Toxoplasma antigen was covalently coupled to 1.10-μm carboxylated latex particles (Seragen, Indianapolis, Ind.). Carboxylated latex particles were brought to 1% (vol/vol) and activated overnight at 4°C in 0.1 M PBS (pH 7.6) containing 2 mg of 1-ethyl-3-(3 dimethylamino-propyl)carbodiimide hydrochloride per ml. The activated latex particles were centrifuged at 6,000 × g for 15 min and washed once with 0.1 M PBS (pH 7.6). The activated latex was suspended to 1% (vol/vol) in 0.1 M PBS (pH 7.6) containing 50 μl of Toxoplasma antigen per ml. The mixture was allowed to react for 2 h at room temperature on a shaker (Ames Aliquot Mixer; Miles Laboratories, Elkhart, Ind.) at 40 to 50 oscillations per min. Bovine serum albumin was then added to a 1% final concentration to block unreacted binding sites on the latex. The toxoplasma-sensitized latex reagent was stored at 2 to 8°C.

Covalently coupled Toxoplasma antigens were used because this procedure yielded significantly lower background levels of agglutination than did the passive absorption methodology. In addition, covalent coupling prevented antigen desorption problems, which limited the shelf life of the reagents (data not shown). A 1:4 dilution of the patient sample was chosen for testing to minimize nonspecific agglutination, which occurred at higher serum concentrations.

For the latex agglutination procedure, serum and plasma samples were diluted 1:4 with 0.1 M PBS (pH 7.4) containing 5% bovine serum albumin. The diluted serum sample (25 μl) was mixed on a glass slide with an equal volume of the toxoplasma-sensitized latex by using a plastic spatula. The slide was rotated by hand for 3 to 5 min, and the agglutina-

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Table 1. Performance of the latex agglutination test compared with the IFA and ELISA

<table>
<thead>
<tr>
<th>Comparison with:</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
<th>Agreement (%)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>IFA</td>
<td>94</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>ELISA</td>
<td>86</td>
<td>100</td>
<td>100</td>
<td>91</td>
<td>94</td>
</tr>
</tbody>
</table>

Table 2. Summary of discordant IFA, latex agglutination, and ELISA results for Toxoplasma IgG antibodies

<table>
<thead>
<tr>
<th>Sample</th>
<th>IFA titer</th>
<th>Latex agglutination</th>
<th>ELISA result</th>
</tr>
</thead>
<tbody>
<tr>
<td>58-12</td>
<td>16</td>
<td>-</td>
<td>ND*</td>
</tr>
<tr>
<td>76-0005</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>38020</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>38228</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>71-488</td>
<td>&lt;16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>77-572</td>
<td>&lt;16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>54-332</td>
<td>&lt;16</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* ND, Not done due to insufficient quantity of serum.

** Specimen was negative for Toxoplasma antibodies but exhibited nonspecific polar staining.

The clinical relevance of nonspecific polar staining has not been clearly established. Van Renterghem and van Nimmen reported that 691 (18.7%) of 3,694 sera exhibited polar staining at ≥1:50 dilutions (13). Using a second set of sera, they observed nonspecific polar staining in 50% of the 200 specimens tested. Our testing of 785 sera indicated that 2% of sera exhibit nonspecific polar staining by IFA. A 50% false-positive rate when testing specimens exhibiting nonspecific polar staining suggests that the ELISA may have an overall false-positive rate of 1 to 25%.

A qualitative screening test for Toxoplasma antibodies should have high predictive value for a positive result while maintaining high specificity levels. This latex agglutination test fulfills these criteria as it is very specific and exhibits 100% predictive value for a positive result compared with the IFA. Patients with past Toxoplasma infections routinely have IFA IgG titers of 1:32 to 1:256, and patients with current or recent infections almost always have antibody titers in excess of 1:1,000 (6). Thus, the latex test exhibits a higher level of sensitivity suitable for routine antibody testing. The latex agglutination test has an additional advantage in that it is not subject to false-positive results when testing samples exhibiting nonspecific polar staining as seen by IFA.

With six independently produced lots of Toxoplasma-sensitized latex, the latex agglutination procedure exhibited perfect within-run and day-to-day reproducibility while also exhibiting 98.3% agreement in lot-to-lot testing. However, the ability to produce the sensitized latex can vary with the latex manufacturer and the lot of latex used. Once a suitable latex lot was identified, the manufacturing reproducibility of this test was quite good. Time and materials estimates indicate that the production costs for the toxoplasma-sensitized latex were about $0.05 per test. This compares favorably with retail costs of $0.80 for a qualitative IFA test and $3.00 for this ELISA.

The latex agglutination test is not a panacea for Toxoplasma antibody testing. This method cannot distinguish immunoglobulin classes and is not helpful in cases of suspected congenital infection where the clinician must distinguish maternal IgG from the infant’s IgM response. However, this latex agglutination test provides an excellent format for routine serological screening because of its high specificity and low cost and because it is not subject to false-positive results with samples exhibiting nonspecific polar staining.

We thank Thomas L. Park and Fred W. Richford for their help in the development of the Toxoplasma latex agglutination test and Dina Hinrichsen for preparing the manuscript.

LITERATURE CITED


