Use of a Single Serum Sample for Diagnosis of Acute Toxoplasmosis in Pregnant Women and Other Adults

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Using a single serum sample for testing for immunoglobulin G (IgG) Toxoplasma antibodies, differences in sensitivity of the dye test (which measures primarily IgG antibodies) and an IgG enzyme immunoassay were found useful for very early diagnosis of acute Toxoplasma gondii infection.

Women at greatest risk of giving birth to offspring with congenital toxoplasmosis are those who acquire the infection during gestation. Diagnosis of the acute infection during pregnancy is complicated by the fact that a significant percentage of pregnant women in the United States have preexisting immunoglobulin G (IgG) antibodies to Toxoplasma gondii, reflecting infection acquired in the distant past rather than during the present pregnancy (9). A relatively significant number of these women have long-lasting IgM Toxoplasma antibodies as well. Thus, the presence of IgG and/or IgM Toxoplasma antibodies in a single serum sample drawn during gestation cannot be used to define whether the infection was recently acquired or chronic (9).

Demonstration of seroconversion or a significant rise in IgG Toxoplasma antibodies usually establishes recently acquired infection. (In the United States, there is no systematic serologic screening of pregnant women to detect acute toxoplasmosis; physicians most often submit only a single serum sample, generally rather late in gestation, from which a diagnosis is expected but usually is not possible in most laboratories [9]. Serologic test results in the first serum sample submitted may suggest, but not clarify, whether the infection was recently acquired.

In some of these patients in their early months of gestation, a high avidity test has proven invaluable to exclude that the patient was infected in the prior 3 to 5 months (the exclusion period depends on the diagnostic kit employed) (6, 7). The utility of the avidity test is based on the observation that toxoplasma IgG antibodies from patients with a recently acquired T. gondii infection bind antigens weakly (i.e., have low avidity), whereas IgG antibodies from chronically infected patients have stronger binding capacity (high avidity) (4). Thus, avidity of toxoplasma-specific IgG antibodies for toxoplasma antigens gradually rises over time following acute T. gondii infection and continues to rise as the acute infection evolves into a chronic infection. The time of conversion from low to high avidity is variable among individuals (4, 5, 7).

Evidence has been presented suggesting infection of the fetus occurs relatively rapidly following acute T. gondii infection in the mother (9). Thus, early diagnosis of acute infection in the pregnant woman is critical to determine if treatment of the mother is indicated to attempt to prevent transmission of T. gondii to the fetus (9). If initial serologic testing suggests the possibility of a recently acquired infection, obtaining a second serum for confirmatory testing is recommended (9) but can delay treatment and expose the fetus to increased risk of infection. What is needed is a serologic approach that allows the rapid diagnosis of recently acquired infection in a single sample of serum.

The results described here provide a sensitive means for the early diagnosis of the infection in a variety of clinical case scenarios and illustrate the value of performing a combination of tests to diagnose toxoplasmosis. During routine testing of pregnant women in our Toxoplasma Serology Laboratory (7), we were unable to perform the IgG avidity test (bioMerieux, Lyon, France) for some patients with a positive dye test (which measures primarily IgG Toxoplasma antibodies) and IgM antibody test titer in their initial serum submitted to our laboratory. This was due to the fact that the VIDAS IgG (bioMerieux, Lyon, France) test result, the first step in performing the avidity test, was too low and in most cases negative. After this was observed with several patients, it became apparent that these results reflected very recently acquired infection, not only in pregnant women but in other clinical situations as well. The purpose of this communication is to bring these observations to the attention of laboratory technicians who perform Toxoplasma serology and to physicians who must evaluate the results.

The sera described in this study were all received for routine testing by our Toxoplasma Serology Laboratory; they were not chosen retrospectively. The serologic tests initially performed on each serum depended on the request that was submitted with the serum. However, the Sabin Feldman dye test (3, 10) was performed on all sera, and if results were positive, at least an IgM enzyme-linked immunosorbent assay (ELISA) (8, 12) was performed as well. Additional tests performed were the AC/HS (a differential agglutination test) (1), direct agglutination (2), IgA ELISA (11, 13), IgE ELISA (15), and the VIDAS IgG avidity test (7). A dye test result of ≥1:16 (2 IU/ml) is considered positive. The following titers were considered positive or negative, respectively, in the other tests: IgM ELISA,
As can be seen in Table 1, the initial serum sample from each of the patients had a VIDAS IgG titer of <15 IU/ml despite a significant IgG titer in the dye test. Thus, the VIDAS IgG avidity test could not be performed. On testing of follow-up sera, it became apparent that each of the patients was recently infected. Thus, a significant titer in the dye test (which primarily measures IgG antibodies) and IgM ELISA in the presence of a negative titer in the VIDAS IgG test reflected a recently acquired infection. When such results are noted for pregnant patients, we recommend immediate treatment in an attempt to prevent transmission to the fetus rather than delaying treatment until subsequent follow-up sera can be obtained and tested. Data in Table 1 also reveal that the requested second serum samples for pregnant patients often arrive many weeks later, thereby placing the fetus at increasing risk of infection in the untreated mother.

The greater sensitivity of the dye test in the cases described above, when compared with the IgG VIDAS test, likely reflects differences in affinity/avidity of the IgG antibodies for the different antigen preparations used in the AC test, when compared with the IgG VIDAS test, likely reflects differences in affinity/avidity of the IgG antibodies for the different antigen preparations used in the AC test.

### Table 1. Results for recently infected patients with low VIDAS IgG results

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specimen</th>
<th>Titer (IU/ml)</th>
<th>AC/HS result</th>
<th>Agglutination</th>
<th>IgG avidity result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VIDAS IgG</td>
<td>IgM</td>
<td>IgA</td>
<td>IgE</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Baseline</td>
<td>32</td>
<td>1</td>
<td>5.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Baseline</td>
<td>32</td>
<td>7</td>
<td>9.7</td>
<td>1.1</td>
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<tr>
<td>3</td>
<td>Baseline</td>
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<td>3</td>
<td>8.8</td>
<td>3.2</td>
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<tr>
<td>4</td>
<td>Baseline</td>
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<td>4</td>
<td>6.9</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>Baseline</td>
<td>64</td>
<td>2</td>
<td>5.8</td>
<td>0.9</td>
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<tr>
<td>6</td>
<td>Baseline</td>
<td>96</td>
<td>2</td>
<td>6.1</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>Baseline</td>
<td>256</td>
<td>10</td>
<td>7.8</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Baseline</td>
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<td>4</td>
<td>6.5</td>
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<tr>
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<td>7.9</td>
<td>0.2</td>
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<td>7.8</td>
<td>3.7</td>
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<td>12</td>
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<td>5.5</td>
<td>1.1</td>
</tr>
<tr>
<td>13</td>
<td>Baseline</td>
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<td>8.0</td>
<td>0.6</td>
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<tr>
<td>14</td>
<td>Baseline</td>
<td>2,048</td>
<td>0</td>
<td>8.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Notes:**
- Serum were tested in parallel unless otherwise indicated.
- Interpreted criteria: for VIDAS IgG, positive, ≥8; equivocal, 4 to <8; negative, <4; for IgG avidity, low, <0.200; equivocal, 0.200 to 0.300; high, ≥0.300.
- Reciprocal of titer.
- Baseline: first serum drawn on each patient.
- Interval in days between baseline and follow-up serum.
- Baseline: first serum drawn on each patient.
- Not tested in parallel.
- ND, not determined.
- *Not tested; IgM must be ≥15 IU/ml to perform avidity.

The greater sensitivity of the dye test in the cases described above, when compared with the IgG VIDAS test, likely reflects differences in affinity/avidity of the IgG antibodies for the different antigen preparations used in the two methods. The greater sensitivity (when compared with the dye test) of certain commercial kits for demonstration of IgG antibodies has been observed previously and is not unique to the IgG VIDAS test. In the present study, these differences proved to be highly useful for the early diagnosis of acute acquired infection with *T. gondii* in a number of different clinical situations. When results as described above were obtained with pregnant women, they allowed more-rapid initiation of treatment to attempt to prevent congenital transmission.

The results described above clearly demonstrate the clinical importance of the sensitivity of IgG *Toxoplasma* antibody tests used for diagnosis of the acute infection in pregnant women. If the sensitivity of an IgG toxoplasma antibody test is insufficient to detect IgG antibodies very early following seroconversion (i.e., as early as can be accomplished with the dye test), a test for IgM antibodies should be performed in toxoplasma sero-
logic screening programs for pregnant women. It should be noted that IgM antibodies were demonstrable for each of the patients shown in Table 1. A positive IgM antibody test in the absence of demonstrable IgG antibody should lead to follow-up testing in every case.

REFERENCES