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Antenatal Screening for Hepatitis B and Antibodies to *Toxoplasma gondii* and Rubella Virus: Evaluation of Two Commercial Immunoassay Systems

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A comparative evaluation of the Abbott AxSYM and DPC Immulite random-access analyzers was performed using 497 prospectively collected serum samples. These samples were sent to the laboratory for routine antenatal screening for hepatitis B surface antigen and immunoglobulin G (IgG) and IgM antibodies to *Toxoplasma gondii* and rubella virus. The overall agreement between the two assay systems ranged from 97.4 to 100%. After discrepancy analysis, the outcome in terms of sensitivity and specificity varied from 98.2 to 100% for all but one of the assays tested. The AxSYM rubella virus IgG assay tended to report protective or indeterminate antibody levels in 1% of the samples. This shortcoming might be overcome by raising the cutoff of the microparticle enzyme immunoassay system.

*Toxoplasma gondii* and rubella virus (RV) infection both cause a generally benign disease in immunocompetent individuals (3, 9) but may lead to congenital infection with serious sequelae for the newborn after primary infection in pregnant women.

Primary maternal RV infection during the first trimester of pregnancy carries a high risk for the development of the congenital rubella syndrome with characteristic malformations of the heart, eye, and ear. Although rubella vaccination has reduced the incidence of RV infection substantially, maternal infection in industrialized countries is still estimated to occur in 1 out of 6,000 to 10,000 pregnancies (1).

A high proportion of pregnant women are seronegative for *T. gondii* and therefore at risk for primary *T. gondii* infection and, subsequently, for the development of congenital toxoplasmosis in their offspring (6). Symptoms, like chorioretinitis and delay in development, can be prevented if timely treatment with spiramycin is initiated (4).

It is therefore of utmost importance to identify susceptible women in order to offer early treatment. Screening programs for pregnant woman are now available in various Western countries (7, 14). Most recently, hepatitis B has been added to these screening programs, because hepatitis B vaccination of the newborn can actually prevent transmission from an HBsAg-positive mother to her child (12).

Antenatal screening programs produce a substantial workload for the microbiological laboratory. Testing of large numbers of serum samples has shifted in recent years from batch-processing enzyme immunoassays to sophisticated random-access systems capable of processing a variety of tests simultaneously.

In this study, we compared the results obtained from the Abbott AxSYM and DPC Immulite systems during routine antenatal screening for HBsAg and for immunoglobulin G (IgG) and IgM antibodies to *T. gondii* and RV.

MATERIALS AND METHODS

The evaluation, which ran from August to November 1999, included blood specimens referred to the medical microbiology laboratory for antenatal screening. Blood samples were clotted and centrifuged prior to testing. All sera were then tested for the presence of HBsAg and for IgG and IgM antibodies to RV and *T. gondii* with the Abbott AxSYM and DPC Immulite systems. An aliquot of 2 ml of each serum sample was frozen at −20°C for retesting and/or confirmatory procedures.

The AxSYM immunoassay system (Abbott Laboratories, Abbott Park, Ill.) is based on the microparticle enzyme immunoassay technology (2). The AxSYM is a fully automated system, with a random-access menu, that utilizes primary tube sampling. The DPC Immulite (Diagnostic Products Corporation, Los Angeles, Calif.) is a bench top immunoassay analyzer, with continuous random-access capabilities, that uses enzyme-amplified chemiluminescence chemistry for antibody or antigen detection (13). Analysis by both immunoassay systems was performed according to the manufacturers’ protocols.

If an HBsAg-reactive serum sample was identified, the test was repeated in duplicate on both systems. If still positive, they were confirmed by a confirmatory or neutralization assay provided by the respective manufacturers (H. Ypelaar, D. Havanna-Barns, C. Cervantes, M. Ghadessi, and A. S. El Sham, Abstr. 90th Gen. Meet. Am. Soc. Microbiol., abstr. A-56, 1998). Samples that yielded discordant results in the *T. gondii* and RV IgG and IgM assays were also retested in duplicate on both systems.

Repeatedly discordant serum samples were shipped to a reference laboratory for IgG or IgM and avidity testing with the VIDAS system (bioMerieux, Marcy l’Etoile, France). RV IgG concentrations of ≤5 IU/ml were considered negative, those between 5 and 10 IU/ml were considered indeterminate or equivocal, and those of ≥10 IU/ml were considered positive (i.e., protective in case of exposure to RV). Discrepant *T. gondii* and RV IgG results were resolved by testing with the VIDAS system (11). Repeatedly discordant RV IgM results were retested with an immunofluorescence assay (Virgo, Colombia, Md.) (5).

RESULTS

Serum samples from 497 pregnant women were collected for analysis with both assay systems. The overall agreement between the two systems ranged from 97.4 to 100%. A comparison of the respective results is presented in Table 1.

Approximately 0.8% of all specimens were found positive
for HBsAg by both Immulite and AxSYM. One sample that tested repeatedly positive in the Immulite system was not available for confirmation and was therefore excluded from further analysis. Four samples were found to be positive for HBsAg in both the AxSYM and Immulite systems. Of these, three were identified as true positives, and one was determined to be negative after confirmation testing.

For *T. gondii* IgG a seroprevalence of approximately 31% was demonstrated. One confirmed *T. gondii* IgG-positive sample scored negative with the Immulite system. Five *T. gondii* IgG samples found to be positive with AxSYM and negative by Immulite could not be confirmed positive by the reference laboratory. For the *T. gondii* IgM, after avidity testing, all but one of the samples that scored positive or indeterminate in either of the assays were considered not indicative of recent infection. The resolved sensitivities and specificities after discrepancy analysis are shown in Table 2.

In the case of RV, only nine women (1.8%) were consistently found to have unprotective IgG antibody levels in both assays. One RV IgG sample that scored indeterminate with the Immulite was found to have protective antibody levels by the reference laboratory. Three RV IgG samples that were initially found to have protective levels by AxSYM were reported as indeterminate by the reference method. Two RV IgG samples initially reported as indeterminate with AxSYM were scored as unprotective by the reference method. Consequently, the specificity of AxSYM was somewhat lower than that of Immulite (81.5 versus 100%).

Only one of five RV IgM results positive by AxSYM and one of six IgM results positive by Immulite were confirmed positive by the immunofluorescence assay.

The sensitivities and specificities of the various assays in the two systems ranged from 98.2 to 100%, with the exception of the RV IgG (Table 2).

**DISCUSSION**

In this study, we evaluated the Abbott AxSYM and DPC Immulite analyzers using 497 prospectively collected serum samples obtained during routine antenatal screening.

Only four samples (0.8%) tested positive for HBsAg in both assay systems (overall agreement, 100%) (Table 1), as was to be expected considering the low incidence of hepatitis B infection in The Netherlands.

We found that approximately 31% of our specimens were confirmed to be positive for *T. gondii* IgG antibodies. This value is similar to seroprevalence data from some other Western countries but substantially higher than values reported from Scandinavian countries (7). Our data indicate that almost 70% of pregnant women are seronegative and therefore at risk of acquiring primary *T. gondii* infection. Due to a lack of IgG present in most IgM screen positives, only one case within our population was confirmed as IgM positive by the IgG avidity test. In most instances, IgM positivity without clinical symptoms will not indicate recent infection and has to be subsequently confirmed by a confirmatory assay such as avidity testing (11). Any positive *T. gondii* IgM result usually will lead to additional diagnostic procedures and in some cases will even lead to therapeutic interventions like antiprotozoal therapy. For RV we also found a substantial proportion of IgM-positive specimens (approximately 1%). As with *T. gondii*, only one recent RV infection could be demonstrated by a second method. However, the seroprevalence of RV IgG in our population was over 90%, which is typical of countries that have instituted a rubella vaccination program. Although the specificity of the Immulite RV IgG assay was 90%, after discrepancy analysis, the specificity of the Immulite assay improved to 100%. In comparison, the AxSYM assay resulted in an 81.5% specificity after resolution testing. This is similar to the report by O'Shea et al. (10), who noted that the AxSYM tended to report protective or indeterminate RV IgG antibody levels in approximately 1% of the assayed samples which could not be confirmed by the reference laboratory.

Considering the high rate of protection against RV infection and the low predictive value of a positive RV IgM test, routine antenatal testing of RV IgM antibodies seems questionable.

The performances of the Abbott AxSYM and DPC Immulite systems were found to be equivalent, with the overall agreement for the five assays evaluated ranging from 97.4% for RV IgG to 100% for HBsAg. These results are comparable to those of previously reported comparative studies (8, 12). In addition, the sensitivity and specificity of these assays were over 98%, with the exception of the RV IgG assay, as men-

### TABLE 1. Agreement and discrepancies between the AxSYM and Immulite assay systems

<table>
<thead>
<tr>
<th>Assay</th>
<th>Nonreactive by both methods</th>
<th>Reactive by both methods</th>
<th>Indeterminate by both methods</th>
<th>No. (%) of specimens with overall agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>492 (99.2)</td>
<td>0 (0.0)</td>
<td>4 (0.8)</td>
<td>496 (100)</td>
</tr>
<tr>
<td><em>T. gondii</em> IgG</td>
<td>333 (67.0)</td>
<td>154 (31.0)</td>
<td>3 (0.6)</td>
<td>487 (98.0)</td>
</tr>
<tr>
<td><em>T. gondii</em> IgM</td>
<td>483 (97.2)</td>
<td>467 (93.9)</td>
<td>2 (0.4)</td>
<td>484 (97.4)</td>
</tr>
<tr>
<td>RV IgG</td>
<td>9 (1.8)</td>
<td>467 (93.9)</td>
<td>8 (1.6)</td>
<td>484 (97.4)</td>
</tr>
<tr>
<td>RV IgM</td>
<td>487 (98.0)</td>
<td>487 (98.0)</td>
<td>1 (0.2)</td>
<td>490 (98.6)</td>
</tr>
</tbody>
</table>

### TABLE 2. Resolved sensitivities and specificities of the AxSYM and Immulite assay systems after discrepancy analysis

<table>
<thead>
<tr>
<th>Assay</th>
<th>Immulite</th>
<th>AxSYM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>T. gondii</em> IgG</td>
<td>99.4</td>
<td>100</td>
</tr>
<tr>
<td><em>T. gondii</em> IgM</td>
<td>100</td>
<td>98.4</td>
</tr>
<tr>
<td>RV IgG</td>
<td>99.8</td>
<td>100</td>
</tr>
<tr>
<td>RV IgM</td>
<td>100</td>
<td>99.0</td>
</tr>
</tbody>
</table>
tioned above. Therefore, the results of this study indicate that the Immulite random-access analyzer is a sensitive and specific tool that can be used in the laboratory for routine antenatal screening for detection of HBsAg, Toxoplasma IgM, Toxoplasma IgG, RV IgG, and RV IgM.

REFERENCES