Evaluation of the VITROS ECiQ immunodiagnostic system for detection of anti-Toxoplasma Immunoglobulin G and Immunoglobulin M antibodies for confirmatory testing of acute Toxoplasma infection in pregnant women.

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ABSTRACT

Infection with Toxoplasma gondii is often asymptomatic and may lead to connatal Toxoplasmosis in offspring when acquired during pregnancy. The newly introduced VITROS anti-Toxoplasma IgG and IgM assays, designed for the VITROS® ECiQ Immunodiagnostic System, a fully automated system based on chemiluminescence, were evaluated as a screening method for serological detection of acute and chronic Toxoplasma infection in the sera of 719 pregnant women. The combination of both VITROS IgG and IgM assays demonstrated high sensitivity and specificity of 100% for the successful detection of all acute T. gondii infections when compared with the Sabin-Feldman dye test as reference test. The VITROS IgG assay parameter revealed a sensitivity of 95.0%, a specificity of 100.0%, a positive predictive value of 100.0%, a negative predictive value of 86.2% and an overall agreement of 96.2% when compared with the dye test. VITROS Toxoplasma IgM compared with the immunosorbent agglutination assay (ISAGA) resulted in following percentages: 77.1%, 99.0%, 97.7%, 88.5% and 91.1%, respectively. Subsequent ROC curve analysis for the discrimination of Toxoplasma IgM in acute (n = 90) and chronic infection (n = 461) demonstrated high sensitivity (92.2%) and specificity (81.6%). Combination of Toxoplasma-specific IgG assay with specific IgM antibody detection has improved the diagnosis of T. gondii infection by decreasing follow-up testing. Nonetheless, positive Toxoplasma IgM test results during pregnancy necessitate a confirmatory testing done by a reference laboratory to ensure fast and above all, accurate test results.

Keywords: Toxoplasma gondii; connatal Toxoplasmosis; VITROS ECiQ; anti-Toxoplasma-specific IgG IgM; Sabin-Feldman dye test; ISAGA IgM
INTRODUCTION

Infection with the protozoan Toxoplasma gondii in immunocompetent individuals is mostly asymptomatic (11). The incidence of gestational Toxoplasma infection in European countries ranges from 0.2 – 1.0 percent (7). Maternal infection during pregnancy may cause placental and fetal infection. Connatal Toxoplasmosis is associated with a wide spectrum of clinical symptoms, such as retinochorioiditis, intracerebral calcifications, and hydrocephalus. These symptoms may be present at birth or develop later in life, leading finally to blindness, psychomotor retardation and hearing difficulties (13, 21).

Austria and France are the only countries which have implemented a nationwide obligatory serological screening program for detection of gestational Toxoplasma infection. The system is providing systematic serologic assessment early in pregnancy and periodic follow-up of pregnant women at risk (7). Serological diagnosis of infection with T. gondii is performed indirectly by enzyme immunoassays, indirect immunofluorescence test and, more precisely, by the Sabin-Feldman dye test (18). The dye test is considered as the reference test for the detection of Toxoplasma infection (16).

Any serological test system has to meet several criteria of adequacy such as high sensitivity and specificity, easy handling and providing reproducible results under routine laboratory conditions. The present study investigated the newly introduced VITROS ECiQ Toxoplasma IgG and IgM assays (Ortho-Clinical Diagnostics, NJ, USA) as a screening method for the diagnosis of acute and chronic Toxoplasma infection in the sera of pregnant women. The VITROS test results were compared with the Sabin-Feldman dye test and the immunosorbent agglutination assay (ISAGA) for determination of anti-T. gondii-specific IgM (10). Diagnosis of maternal infection status was provided by routine serology of the Toxoplasmosis reference laboratory located at the
Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria. In addition, the technical precision of both VITROS Toxoplasma IgG and IgM assays were evaluated by serial specimen measurements.

MATERIALS AND METHODS

Sample and patients. Sera samples of 719 healthy pregnant women were collected according to the recommendations to the Austrian Toxoplasmosis screening program and submitted to the laboratory for routine analysis. Sabin-Feldman dye test and ISAGA IgM were performed within 24 – 48 hours of receiving the samples. Sera were stored at -20°C. For the evaluation of the VITROS Toxoplasma IgG and IgM assays an aliquot of sera was thawed and retrospectively analyzed in this study. The results were compared by in-house serology using the dye test and by determination of anti-\textit{T. gondii}-specific IgM with the immunosorbent agglutination assay (bioMérieux, France).

VITROS ECiQ system. This automated system is based on an immunometric technique.

(I) VITROS Toxoplasma IgG assay: This involves the reaction of anti-Toxoplasma IgG present in the sample with Toxoplasma antigen coated onto the reaction wells. After a wash step a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-human IgG) is added which complexes with bound anti-Toxoplasma IgG.

(II) VITROS Toxoplasma IgM assay: An antibody class capture technique is used. This involves an automatic dilution of the sample and the simultaneous reaction of human IgM in the diluted sample with a biotinylated antibody (mouse monoclonal anti-human IgM). The immune complex
is captured by streptavidin on the wells, unbound materials are removed by washing. A HRP-
labeled mouse monoclonal anti-Toxoplasma antibody (F(ab)$_2$ fragment) which complexes with
inactive Toxoplasma antigen (conjugate) is captured by anti-Toxoplasma specific IgM bound to
the wells.

(III) Finally, unbound material is removed by washing. The bound HRP conjugate is measured by
a luminescent reaction (20). A reagent containing luminogenic substrates (a luminal derivative
and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound
conjugate catalyzes the oxidation of the luminol derivative thus producing light. The electron
transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its
emission. The light signals are read by the VITROS Immunodiagnostic System. The amount of
HRP conjugate bound is directly proportional to the concentration of anti-Toxoplasma IgG/ IgM
present in the sample.

Results are expressed as international units per milliliter (IU/ mL) in the IgG assay and as a ratio
in the IgM assay. This ratio is derived by dividing the signal for test sample by the signal at the
cutoff (cutoff value). Interpretation of VITROS results were based on the manufacturer’s criteria
as follows: ≤ 3.99 IU/ mL negative, 4.00 to 7.99 IU/ mL borderline, and ≥ 8.00 IU/ mL positive
for IgG antibodies. For IgM antibodies the ratio was classified: < 0.80 as negative, ≥ 0.80 to <
1.20 as borderline, and ≥ 1.20 as positive.

The VITROS test kits were used according to the manufacturer’s protocol. Sera with an IgG level
higher than > 500 IU/ mL were automated diluted by the ECiQ system and re-analyzed.

**Serological tests.** The Sabin-Feldman dye test and the ISAGA IgM test were performed as
previously described (4). The final discrimination of acute and chronic infection status was
performed according to the Lebech criteria (8). For the definite diagnosis, the patients were further investigated by subsequent serum sample analysis (by Sabin-Feldman dye test and ISAGA IgM) and/or IgG avidity. The ISAGA IgM was described to be more sensitive than double sandwich enzyme-linked immunosorbent assay (5, 10). The ISAGA IgM is suitable for the diagnosis and screening of acute Toxoplasma infection in pregnant women, as well as for detection of IgM in blood samples of the child to identify congenital infection (2, 12).

**Technical performance.** The technical precision of the VITROS system was evaluated by intra- and inter-assay testing of sera, negative and positive controls, respectively. Intra-assay precision was determined by running eight consecutive runs of two sera for IgG and six consecutive runs of four sera for IgM antibodies. Inter-assay precision was determined by testing negative and positive controls, each in triplicates, over a period of fifteen days (Liquicheck™ ToRCH Plus Positive Control (Bio-Rad, USA) for Toxoplasma IgG and Liquicheck™ ToRCH Plus IgM Control Positive and Negative for Toxoplasma IgM).

**Statistics.** Calculations were performed using Excel 2007 (Microsoft Inc., USA), SPSS 16.0 (SPSS science, USA) and MedCalc 9.0 (MedCalc Software, Belgium) for receiver operating characteristic curve (ROC) analysis. The coefficient of correlation (Spearman's rank correlation coefficient r) and Cohen’s Kappa (kappa) were used to determine the statistical agreement between the comparison assays (22). The coefficient of variation was used to determine intra- and inter-assay precision.
RESULTS

Performance of VITROS IgG and IgM. The analysis of 719 sera samples determined by the VITROS IgG assay resulted in a sensitivity of 95.0%, specificity of 100.0%, a positive predictive value of 100.0%, a negative predictive value of 86.2%, and overall agreement of 96.2% (r = 0.905; kappa = 0.900) compared with the Sabin-Feldman dye test. Table 1 shows the comparison of the ISAGA IgM with the VITROS IgM test. Of the 719 serum samples tested, 259 (36.0%) were positive in the ISAGA IgM. Of the latter sera, 172 (23.9%) were positive, 36 (5.0%) borderline and 51 (7.1%) negative in the VITROS IgM. Of the 407 (56.6%) of the total serum samples that were negative in the ISAGA IgM, 391 (54.4%) were negative and 12 borderline (1.7%) and 4 (0.6%) were positive in the VITROS IgM. Results in the ISAGA IgM were equivocal in 53 (7.4%) of the total serum samples. Results in the VITROS IgM were equivocal in 61 (8.5%) serum samples. Thirteen (1.8%) serum samples gave equivocal results in both tests. Sera with equivocal results were excluded from final analysis. When compared with the ISAGA IgM test, the VITROS IgM assay had a sensitivity of 77.1%, specificity of 99.0%, positive predictive value of 97.7%, negative predictive value of 88.5%, and overall agreement of 91.1% (r = 0.768; kappa = 0.626).

The Sabin-Feldman dye test is capable to detect Toxoplasma specific immunoglobulins of all classes (IgM, IgA and IgG antibodies). Consequently, dye test results should not be compared with the results of isolated IgG or IgM assays but only to combined IgG and IgM results (14). Table 2 shows a comparison of the results of the Sabin-Feldman dye test and the combined IgG and IgM results obtained by the VITROS system. All 34 samples which were found to be negative within the VITROS system were chronic T. gondii infections (positive Sabin-Feldman dye test results). Based on the dye test as reference standard, the VITROS system demonstrated a
sensitivity, specificity, a positive and negative predictive value of 100.0%, respectively, for the detection of acute Toxoplasma infections.

Receiver operating characteristic curve analysis for VITROS IgM values for potential discrimination of acute (n = 90) and chronic infection (n = 461) were performed (Figure 1). Table 3 displays the respective sensitivity, specificity, positive and negative predictive value for cut-off value ≥ 1.2 (92.2%, 77.0%, 43.9% and 98.0%; cut-off value according to the manufacturer’s recommendation) and for the optimal cut-off value of ≥ 1.47 (92.2%, 81.6%, 50.9% and 98.0%) calculated in this samples setup. An increase of the cut-off value resulted in an enhanced specificity for the discrimination of acute and chronic T. gondii infection in this study.

Technical Precision. The intra- and inter-assay coefficients of variation for the VITROS IgG and IgM assays for the positive controls and sera were below 9.6% and 4.6% for IgG and 1.9% and 6.6% for the IgM assay, respectively. The serial measurements of the Liquicheck\textsuperscript{TM} ToRCH Plus IgM negative control resulted in a maximum IgM ratio of 0.12 (< 0.80 negative test result).

DISCUSSION

VITROS ECiQ Immunodiagnostic System is a simple to use, fully automated laboratory test system. This is the first study to evaluate the VITROS system in comparison with the Sabin-Feldman dye test. Analysis of anti-Toxoplasma-specific IgG and IgM antibodies with the VITROS system demonstrated a good correlation with the results obtained by the dye test. The Sabin-Feldman dye test is considered as the “gold standard” and detects all classes of Toxoplasma-specific immunoglobulins, including IgG, IgM and IgA (15). However, the Sabin-
Feldman dye test is expensive, time-consuming and difficult to standardize. Due to its high sensitivity the dye test still serves as the recommended reference test for confirmation of acute infection in pregnant women and validation of commercial kits (16).

Using the VITROS IgG assay results in comparison with the Sabin-Feldman dye test, a high sensitivity and specificity of 95% and 100%, respectively, was achieved. A comparison of the VITROS IgM to the ISAGA IgM resulted in a high specificity (99%) but in a sensitivity of 77% indicating that the ISAGA IgM is more sensitive than the VITROS IgM. It is well known that the detection of anti-Toxoplasma-specific IgM antibodies by automated test system is a common problem in the diagnosis of T. gondii infection (17).

The combination of anti-Toxoplasma-specific IgG assays with specific IgM antibodies has improved the diagnosis of T. gondii infection (17). The good correlation between the dye test and the combination of both VITROS IgG and IgM assays indicates that the VITROS system is a valuable system for the detection of recent acquired infection of T. gondii. In acute infection there is an excellent correlation between the combination of both, VITROS IgG and IgM assays and the Sabin-Feldman dye test expressed by a sensitivity and specificity of 100%. Consequently, the VITROS system can be used as a screening test of seronegative women.

Subsequent ROC curve analysis for the discrimination of Toxoplasma IgM in acute and chronic infection demonstrated a high sensitivity (92.2%) and specificity (82.9%). However, chronic infection with T. gondii can lead to a long persistence of IgM antibodies (1, 3). IgM antibodies are detectable in the median of 12.8 months (interquartile range 6.9 - 24.9) and moreover, in a substantial minority of women IgM-positive test results can persist beyond two years (6). Since IgM can persist years after infection, Toxoplasma IgM-positivity alone is unable to discriminate acute from chronic infection (19).
Diagnosis of the infection status is achieved by interpretation of the immunoglobulin concentrations in follow-up serology. Decreasing or stable IgG (depending on gestational age and test interval) denotes chronic infection in immunocompetent pregnant women, while a significant IgG-increase within two samples proves acute infection (9). Additional tests (e.g. IgG avidity, differential agglutination (AC/ HS)) and serial serum samples collected at an interval of 2 – 4 weeks are mandatory in the detection of acute infection with T. gondii. It is recommended that sera with positive IgM test results obtained at non-reference laboratories should be sent to a Toxoplasma reference laboratory. The serologic diagnosis must be verified by confirmatory testing before treatment is considered.

VITROS ECiQ is a practicable, easy-to-use test system with screening results in less than 40 minutes (with automated samples dilution < 2 hours). Nonetheless, our results revealed 34 (6.2%) false-negative results in the VITROS system even though Sabin-Feldman and ISAGA IgM demonstrated a chronic infection status of these study patients. In case of negative serological findings by the VITROS system a re-testing in a subsequent pregnancy is recommended to rule out an acute Toxoplasma infection.

ACKNOWLEDGEMENTS
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REFERENCES


Table 1. Comparison of the ISAGA IgM to the VITROS Toxoplasma-specific IgM assay.

<table>
<thead>
<tr>
<th>VITROS IgM test results (%)</th>
<th>Negative</th>
<th>Borderline</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ISAGA IgM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>391 (54.4%)</td>
<td>12 (1.7%)</td>
<td>4 (0.6%)</td>
<td>407 (56.6%)</td>
</tr>
<tr>
<td>Borderline</td>
<td>27 (3.8%)</td>
<td>13 (1.8%)</td>
<td>13 (1.8%)</td>
<td>53 (7.4%)</td>
</tr>
<tr>
<td>Positive</td>
<td>51 (7.1%)</td>
<td>36 (5.0%)</td>
<td>172 (23.9%)</td>
<td>259 (36.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>469 (65.2%)</td>
<td>61 (8.5%)</td>
<td>189 (26.3%)</td>
<td>719 (100.0%)</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the Sabin-Feldman dye test to the combination of both VITROS anti-Toxoplasma-specific IgG and IgM assays.

<table>
<thead>
<tr>
<th>VITROS IgG and IgM assay results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Sabin-Feldman dye test</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

\(^{a}\)All 34 samples that were VITROS Toxoplasma IgG and/or IgM negative but Sabin-Feldman dye test positive were chronic infections.
Table 3. Criterion values and characteristics of the ROC curve analysis.

<table>
<thead>
<tr>
<th>Criterion value</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
<th>+LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1.2(^a)</td>
<td>92.2</td>
<td>84.6 - 96.8</td>
<td>77.0</td>
<td>73.1 - 81.0</td>
<td>4.05</td>
<td>0.10</td>
</tr>
<tr>
<td>≥ 1.47(^b)</td>
<td>92.2</td>
<td>84.6 - 96.8</td>
<td>81.6</td>
<td>79.1 - 86.2</td>
<td>5.38</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^a\)Cut-off value according to the manufacturer’s recommendation
\(^b\)Optimal cut-off value calculated in this study; +LR: positive Likelihood Ratio; -LR: negative Likelihood Ratio
Figure 1. Receiver operating characteristic curve analysis of the VITROS IgM values for the discrimination of acute (n = 90) and chronic (n = 461) Toxoplasma infection.