Detection of immunoglobulin G antibodies to Toxoplasma gondii: Evaluation of two commercial immunoassay systems

Nilgun Tekkesin a,*, Kenan Keskin b, Cumhur Kılınc a, Nuray Orgen a, Kaya Molo a

a Department of Biochemistry, Clinical Laboratory, Memorial Hospital, Istanbul, Turkey
b Department of Microbiology and Disease, Clinical Laboratory, Memorial Hospital, Istanbul, Turkey

Received 12 August 2009; received in revised form 20 October 2009; accepted 11 November 2009

KEYWORDS
Anti-T gondii IgG; Immunofluorescent assay; Toxo IgG

Background: Toxoplasmosis is a disease, which can cause severe congenital infection and is normally diagnosed by the detection of Toxoplasma gondii (T gondii)-specific antibodies in the serum of infected patients. Several different tests allow to distinguish recent from past infections and to quantify anti-T gondii-specific IgG, and the results can be used as markers for a chronic or recently seroconverted toxoplasma.

Methods: In the present study, the recent Cobas 6000 Toxo IgG assay (Roche Diagnostics, Indianapolis, IN, USA) for the serological diagnosis of toxoplasmosis was compared with the Axsym Toxo IgG assay (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA) employing a panel of negative, low- or high-reactive serum samples that were selected after routine screening in a laboratory of clinical analyses.

Results: The overall agreement between two methods was 99% (r = 0.99, p < 0.001). Of 91 analyzed samples, only one presented discrepant result, being positive in the Cobas 6000 Toxo IgG assay and negative in the Axsym Toxo IgG assay. By using an immunofluorescent assay as a confirmation test, this positive result was assayed to be negative.

Conclusions: Both assays performed in each analyzer were proven to be fast and fully automated procedures for reproducible measurement of IgG antibodies to T gondii. The new method, used for the determination of anti-T gondii IgG antibodies, should be evaluated with a further analysis with increased number of serum samples to get a broad performance of this newer test.

Copyright © 2011, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. All rights reserved.
Introduction

Toxoplasmosis, a ubiquitous protozoal disease, is caused by an intracellular parasite, Toxoplasma gondii (T gondii). This is mainly acquired by ingestion of food or water that is contaminated by mature oocysts shed by cats or by undercooked meat containing tissue cysts. Primarily, acute toxoplasmosis, which is mostly mild or asymptomatic, is generally benign in healthy people. However, it can be serious in the context of immunodeficiency, especially in the case of AIDS and bone marrow or heart transplant patients or in children infected in utero. However, reactivation of a latent Toxoplasma as a result of immunosuppression is frequently associated with meningoencephalitis. Primary maternal toxoplasmosis occurs during pregnancy may lead to severe damage of the fetus as the parasite can transmit across the placenta. Most infants with congenital infection do not present any clinical symptoms at birth but may develop severe sequelae later in life, such as mental and psychomotor retardation, chorioretinitis, and hear loss. The fetal rate increases with gestational age. However, the risk of severe clinical manifestations is higher in case of early maternal infection. Early drug therapy in acute toxoplasmosis during pregnancy can prevent congenital damage or ameliorate the severity of clinical manifestations.

A wide range of assays for the diagnosis of toxoplasma is available on the commercial market, and many are used in laboratories throughout Turkey. The assays vary in their format and in their ability to detect different types of antibody classes (some IgG specifically, others all antibody classes). This feature may lead to variation in the performance of the assays and makes the choice of assay for individual diagnostic laboratories complex. In addition, laboratories should use evidence-based medicine in their decision-making process.

In recent years, clinical laboratories have seen a drastic increase in demand for automated detection of antibodies to T gondii by assays based on immunoassay technology. The determination of IgG antibodies with a significant rise titers to T gondii is used to assess the serological status to toxoplasmosis and indicate a chronic or recently seroconverted toxoplasma. In the present study, the performance characteristics of the new Toxo IgG antibody assay on the Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA) was compared with those of Axsym Toxo IgG (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA) antibody assay on the Axsym automated analyzer systems with respect to reproducibility and correlation. We review the performance of these assays and offer the importance of the results in patient counseling for specialist and nonspecialist laboratories.

Methods

The study related to 91 sera. They were obtained from the routine activity of the Central Clinical Laboratory at Memorial Hospital for the period March—April, 2007 and consisted of sera from pregnant women, newborns, immunocompetent, and immunocompromised patients. They included negative samples as well as samples with low, medium, and high levels of IgG antibodies to T gondii detected by Toxo IgG Axsym. Cobas 6000 Toxo IgG, marketed by Roche Diagnostics, is a relatively new product but increasingly being used in Europe. The sera were stored at −20 °C until testing. For each serum tested by Axsym Toxo IgG before, the biological interpretation (presence or absence of IgG) considered results were obtained with Cobas 6000 Toxo IgG.

Cobas 6000 Toxo IgG antibody immunoassay

Ten microliter sample, a biotinylated recombinant T gondii-specific antigen and T gondii-specific recombinant antigen labeled with a ruthenium complex form a sandwich complex. After addition of streptavidin-coated microparticles, the complex bind to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier. Results are expressed as international units per milliliter in the IgG assay. Interpretation of IgG results was based on the manufacturer’s criteria as follows: ≥3.0 IU/mL, positive for IgG antibodies; ≥1.0 to <3.0 IU/mL, equivocal result; and <1.0 IU/mL, negative for IgG antibodies.

Abbott Axsym Toxo IgG antibody immunoassay

The Abbott Axsym uses an automated system, which is based on microparticle enzyme immunoassay technology to detect antibodies to T gondii. After manual pipetting of sera into individual reaction cells, all subsequent steps are automated. The instrument delivers the sample and diluent buffers to the predilution well of the reaction cell. T gondii-coated microparticles and diluted sample are added to the incubation well, where the T gondii antibody binds to the T gondii-coated microparticles, forming an antigen-antibody complex. A diluted aliquot of this antigen-antibody complex is transferred to the glass fiber matrix, which irreversibly binds the microparticles. After washing the matrix to remove unbound materials, class-specific anti-IgG-alkaline phosphatase conjugate is dispensed onto the matrix, where it binds to the antigen-antibody complex. The matrix is again washed to remove unbound materials; this is followed by addition of the substrate, 4-methylumbelliferyl phosphate. The resulting fluorescent product is measured by the microparticle enzyme immunoassay optical assembly. The intensity of the fluorescence is proportional to the quantity of toxoplasma antibodies. Results are expressed as international units per milliliter. Interpretation of IgG results was based on the manufacturer’s criteria as follows: >3.0 IU/mL, positive for IgG antibodies; 2.0–3.0 IU/mL, equivocal result; and <2.0 IU/mL, negative for IgG antibodies.

System precisions as indicated by the coefficient of variation (CV%) were evaluated by intra- and interassay testing of negative and positive controls in each assay. Intra-assay precision was determined by running three consecutive runs of each negative and positive control for
IgG antibodies. Interassay precision was determined by testing one negative and one positive control, each in duplicate, for 10 days.

Results for sera with discrepant results for IgG antibodies were then verified by an immunofluorescent assay (Panbio, Inc., Columbia, MD, USA). System precisions as indicated by the CV% were evaluated by intra- and interassay testing of negative and positive controls in each assay.

Statistics

The CV% was used to determine intra- and interassay precision and reproducibility. CV% was used to determine the statistical agreement between the different tests using the McNemar test. Overall agreement was defined as the percentage of specimens that were positive or negative in the Axsym assay and gave the same positivity and negativity in the Cobas 6000 assay.

Results

The within-run and between-run reproducibility rates, calculated as the CV%, were between nonapplicable and 2.8% for serum samples tested by Axsym Toxo IgG and between 1.6% and 3.0% for those tested with the Cobas 6000 Toxo IgG assay. The results are presented in Table 1. The within-run reproducibility rates of negative and positive controls of each were below 0.1 for Axsym Toxo IgG and calculated CV% value was shown as nonapplicable. This indicated that Axsym Toxo IgG assay has a higher degree of assay and/or technical precision rather than Cobas 6000 Toxo IgG assay. The assays proved to be highly reproducible both within runs and between runs. After successfully completing reproducibility testing, evaluation of the clinical specimens began.

In the study comparing the Cobas 6000 and Axsym Toxo IgG assays, 17 sera of 91 routine laboratory sera were considered as positive; and for anti-\textit{T. gondii} IgG, 73 as negative and 1 as discordant. Figures 1 and 2 show a graphic representation of the reactivity variations for negative and positive samples.

Table 1 shows the results of 17 serum samples that were positive in both automated assays. The reactivity indices showed that the reactivity was significantly higher in the Cobas 6000 when compared with the Axsym. The average reactivity of the Cobas 6000 assay was 302.4 IU/mL, whereas the Axsym assay showed an average reactivity of 54.5 IU/mL.

The reproducibility studies were also the same as the mean value of Cobas 6000. Toxo IgG was higher than Axsym Toxo IgG (48.6 IU/mL vs. 19.3 IU/mL for positive controls and 0.767 IU/mL vs. 0.1 IU/mL for negative controls).

Only one serum sample has a discrepant result. It was Cobas 6000 Toxo IgG assay positive (titer, 41 IU/mL) but Axsym Toxo IgG assay negative (titer, 0 IU/mL). This sample was retested in each analyzer and the same results were obtained. The same serum was negative by indirect fluorescent antibody (IFA) test. The overall agreement between two methods was 99% ($r = 0.99$, $p < 0.001$). These results indicate a close quantitative correlation between Axsym Toxo IgG assay and the Cobas 6000 Toxo IgG assay. Table 2 illustrates the comparative performances of the Cobas 6000 and Axsym assays.

Discussion

The detection of anti-\textit{T. gondii} IgG is considered to be a marker for a chronic or recently seroconverted toxoplasmosis, and is very important as a reference for clinical and epidemiological patient management and counseling. Today, in the case of toxoplasmosis, new diagnostic parameters, such as IgG avidity, are assessed to identify the most likely time of and to allow the physician to better evaluate the clinical management of each patient. In many health care services, the detection of anti-\textit{T. gondii}-specific IgG implicates that the patient is considered protected against the disease in case of a new contact with the parasite. Consequently, the performance of assays that detect specific IgG is of vital importance, and several investigations on this diagnostic approach are underway.

A number of techniques have been applied to detect IgG antibodies in patients with toxoplasmosis, including the dye test, direct agglutination assay, IFA, and ELISA. Among these tools, the automated and standardized assay for anti-\textit{T. gondii}-specific IgG detection newly developed by Roche Diagnostics occupies an important position.

Evaluation of clinical specimens for IgG-specific antibodies to \textit{T. gondii} was performed by using the Cobas 6000 and Axsym assays. The Cobas 6000 is a new product but is increasingly being used in Turkey. The Axsym Toxo IgG assay

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of precision data for Axsym and Cobas 6000 Toxo IgG assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum sample</td>
<td>Axsym Toxo IgG</td>
</tr>
<tr>
<td></td>
<td>Mean (IU/mL)</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.1</td>
</tr>
<tr>
<td>Positive control</td>
<td>19.3</td>
</tr>
</tbody>
</table>

a Three replicates per run.
b Two samples per run for 10 days.
c $p < 0.0001$.
CV = coefficient of variation; NA = nonapplicable.
has been used throughout Turkey for many years. The Cobas 6000 assay had not previously been evaluated with the Axsym assay. We review the performance of these assays and offer the best advice for specialist and nonspecialist laboratories in Turkey. Detection and measurement of IgG-specific antibodies is rarely problematic, and good sensitivity and specificity have been achieved by a variety of methods.\textsuperscript{18–22} A clinical laboratory must not rely on the manufacturer to ensure a continuing supply of reagents but must institute its own verification procedures for each kit as well as performing an initial evaluation.\textsuperscript{23} Kit inserts have demonstrated a sensitivity of 99.7% and specificity of 99.1% for Abbott Axsym and 99.5% and 98.8%, respectively for Cobas 6000. As we could not have any chance to verify the other samples qualitatively by IFA, it would not be true to calculate the sensitivities and specialties of both assays. Further analysis should be organized for this purpose. Overall, the agreement between two assays was found very high ($r = 0.99$, $p < 0.001$).

Precision rates are generally presented as the assay precision rate but actually reflect the combination of the technologist’s precision and that of the assay. With both assays, the addition of a measured amount of undiluted patient serum to the test cassette is the only manual procedure required. One would therefore expect improved precision with the automated assays as opposed to the manual assays. The Cobas 6000 Toxo IgG is proved to be highly reproducible both within runs and between runs, with a higher detection titer than Axsym Toxo IgG in positive and negative reactive samples. Low titers of anti-\textit{T. gondii} IgG antibodies often maintain ambiguity on the protective immunity notion. In this regard, the high reactivity performance of Cobas 6000 Toxo IgG assay might have an advantage.

In this study, the material analyzed consisted of both negative and positive IgG serum samples and included a large range of positive IgG titers. The different IgG titers obtained from the same serum samples with different tests

\begin{figure}
\centering
\includegraphics[width=\textwidth]{sera_positivity.png}
\caption{Positive reactivity of serum samples in each system.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{sera_negativity.png}
\caption{Negative reactivity of serum samples in each system.}
\end{figure}
emphasize the variability in the quantitative results according to the serological methods. It is well established that identical titers cannot be obtained with commercial kits using different antigenic extracts from *T. gondii*. In studies that compare the performances of various methods to detect IgG antibodies to *T. gondii*, it has been shown that no method is perfect. Such studies have shown that certain sera yield discordant results. In our study, the results indicate close quantitative agreement between Axsym Toxo IgG assay and the Cobas 6000 Toxo IgG assay. Compared with Axsym Toxo IgG assay, Cobas 6000 showed only one discrepant result, confirmed by IgG-specific IFA test. The same sample was analyzed for the second time by another Cobas 6000 Toxo IgG kit perhaps resulting variability among kit lots. But, again it was positive, probably because of a cross-reactivity, an undefined interference or may be explained by differences in the sensitivity and in the kinetics of these two methods. This suggests that the result obtained for this discrepant serum by the Cobas 6000 Toxo IgG assay is false-positive.

For this study, an immunofluorescent assay for *T. gondii* IgG antibody was designated as the reference assay for the determination of discrepancy reason because of the depth of experience and data accumulated with this assay. Sera collected during various stages of and convalescence often could give different results because methods have different sensitivities, measure different immunoglobulin classes of antibodies, or measure antibodies to different viral components. One of the reasons for these test differences may be the different antigens coated on the solid phase of each assay and the resulting ability of each assay to detect these immunoglobulin classes and viral components. Specimens that yielded discordant test results following evaluation by Cobas 6000 may be explained by such differences in solid-phase coating. The remaining discrepant *Toxoplasma* IgG specimen had insufficient volume for further testing. Otherwise, it was evaluated further by the Sabin-Feldman dye test that would be the second step confirmatory analysis. The authors determined that the dye test should be retained as a reference test and as an additional confirmatory test for the validation of commercial kits. As the results were obtained using retrospective samples, we have no chance to take a subsequent sample afterward. In a practical way, when positive results are obtained with the two methods, a subsequent serum sample taken 3 weeks later is tested, so as to verify the presence of a positive and stable titer of anti-*T. gondii* IgG antibodies. In fact, the host defense mechanisms against *Toxoplasma* depend mainly on cell-mediated immunity. On the other hand, in case of discordant results, it is advisable to consider the patient as nonimmune, thereby necessitating a serological follow-up during the pregnancy.

As we could not have any chance to verify the other samples qualitatively by IFA, it would not be true to calculate the sensitivities and specialties of both assays. Besides, Cimon et al. found a lower sensitivity of IFA compared with Toxo IgG Axsym and may be related to the lack of antigen affinity purification and the sometimes-delicate microscope reading at the threshold dilution. Also, serological methods are often not sufficient in the follow-up of immunocompromised patients. For Toxo IgG Axsym and IFA, Roux-Buisson et al. found false-negative and false-positive results in immunocompromised patients. In practice, IFA should be used in a laboratory in combination with another method with different antibody kinetics in necessity.

The knowledge of the specificity and sensitivity of test performance is fundamental for a physician so that he or she can make correct decisions and patient counseling, especially during pregnancy. A further analysis with a much larger sample of specimens should be done to evaluate a broad performance of this newer test. However, the results of this study should be taken into consideration when interpreting a positive result obtained with Cobas 6000 Toxo IgG kit.

**References**

8. Schaefler LE, Dyke JW, Meglio FD, Murray PR, Crafts W, Nile AC. Evaluation of microparticle enzyme immunoassays for

**Table 2** Comparison of performances of Axsym and Cobas 6000 Toxo IgG assays

<table>
<thead>
<tr>
<th>No. of serum samples (n = 91)</th>
<th>No. of serum samples with indicated results</th>
<th>% Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axsym Toxo IgG negative</td>
<td>Axsym Toxo IgG positive</td>
</tr>
<tr>
<td>Cobas 6000 negative</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>Cobas 6000 positive</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

\* \( r = 0.99 \) between two assays \((p < 0.001)\).