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Comparison of Three Diagnostic Methods for the Detection of Cytomegalovirus and *Toxoplasma gondii* IgG Antibodies at Prenatal Screening

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Abstract: *Toxoplasma gondii (T. gondii)* and cytomegalovirus (CMV) infections are typically asymptomatic infections, but they can have serious consequences mainly in newborns and immunocompromised patients. In many parts of the world, these infections are routinely screened during pregnancy (toxoplasmosis) and, in others, high-risk individuals are tested using fully automated screening assays. In this study, we investigated the performance of the three fully automated immunoassays, LIAISON® XL DiaSorin, Abbott Architect and Roche Cobas®, for the determination of specific IgG antibodies to Cytomegalovirus and *Toxoplasma gondii* in human serum or plasma samples in terms of prevalence of CMV and Toxo IgG detected, and both sensitivity and specificity. Performance of the LIAISON® assays was investigated compared to two other assays, ARCHITECT (CMV IgG and Toxo IgG assays) and Cobas® (CMV IgG and Toxo IgG assays). Discrepant anti CMV IgG and anti Toxoplasma IgG samples were tested for IgM to CMV and Toxoplasma to exclude early acute infection where IgG could be detected differently by the methods. Overall, for both CMV IgG and Toxo IgG, the LIAISON® assay was better than both the Cobas® and ARCHITECT assays in terms of CMV and Toxo IgG detected, and both diagnostic sensitivity and specificity performance although the difference is statistically significant only compared to Cobas®.

Keywords: *Toxoplasma gondii*, Cytomegalovirus, Prenatal Screening, Anti-cytomegalovirus IgG Antibodies, Anti-toxoplasma IgG Antibodies

1. Introduction

Toxoplasma gondii and cytomegalovirus (CMV) infections are significant causes of severe neonatal and childhood illnesses worldwide [1, 2]. These infections can also be a significant issue in immunocompromised individuals [3, 4]. *Toxoplasma gondii* is a ubiquitous intracellular parasite [5]. Toxoplasmosis, the disease that results from infection with *Toxoplasma gondii,* is typically asymptomatic, but primary infection in pregnancy can have severe consequences for the developing foetus [6], and can be severe in immunocompromised patients [7]. Ocular toxoplasmosis is

the most frequent cause of infectious posterior uveitis and it is often a consequence of congenital infection [14], CMV, also frequently asymptomatic, is a common cause of congenital infection, and a leading cause of sensorineural hearing loss, brain damage and cerebral palsy in the US [9].

Diagnosis of these infections is often challenging, largely due to their asymptomatic nature, and when signs and symptoms are present, they are often non-specific [10, 7]. Diagnosis is therefore usually based on serological testing [11], and these infections are universally screened for during pregnancy, or only high-risk individuals are screened in many parts of the world [12] in order to find non-immune

patients to prevent, by hygienic and dietary prophylaxis, maternal and subsequently congenital infection. To this end, there is a need for an IgG test with high specificity in order to correctly classify non-immune patients to be counselled for hygienic and dietary prophylaxis and serological follow-up.

Novel, fully automated screening assays have been developed to cope with increasing test volumes and to overcome limitations of conventional screening methods and for the full traceability of results. The aim of this study was to investigate and compare diagnostic performance of the three systems LIAISON® XL, ARCHITECT and Cobas® for detection Toxoplasma IgG and CMV IgG.

2. Methods

This was a comparison study, conducted at CerbaXpert part of Cerba Research Saint-Ouen l'Aumône, France, in pregnant women and in patients undergoing routine TORCH screening (n=501), with routine samples. Fresh serum samples were prospectively collected without any selection and stored at 2–8°C for a maximum of 72 hours in the primary collection tubes before testing.

CerbaXpert part of Cerba Research collected all samples and relevant required data and ran all testing as well. In compliance with national and international regulation, all samples were obtained without any link to the patient's identity, and no further procedures were required, performed or modified for reasons depending on the study.

2.1. Assay Systems

At the clinical site, two different test runs were conducted in parallel for both *Toxoplasma gondii* and CMV IgG on each of the three assay systems ARCHITECT, Cobas® and LIAISON® XL. The LIAISON® Toxo IgG II (DiaSorin - Saluggia, Italy) uses the chemiluminescent immunoassay (CLIA) technology, based on isoluminol derivative, for the quantitative determination of specific IgG antibodies to *Toxoplasma gondii* in human serum or plasma samples. The LIAISON® Toxo IgG II is an indirect chemiluminescence immunoassay. The inactivated *Toxoplasma gondii* (RH strain) obtained from sonicated and detergent-extracted trophozoites is coated with magnetic particles.

The LIAISON® CMV IgG II (DiaSorin – Saluggia, Italy) uses chemiluminescent immunoassay (CLIA) technology, based on isoluminol derivative, for the quantitative determination of specific IgG antibodies to hCMV in human serum or plasma samples. The LIAISON® CMV IgG II is an indirect chemiluminescence immunoassay. The magnetic particles are coated with inactivated hCMV antigen (AD 169 strain).

The ARCHITECT Toxo IgG (Abbott – Wiesbaden, Germany) is a two-step immunoassay for the quantitative determination of IgG antibodies to *Toxoplasma gondii* in human serum and plasma using the chemiluminescent microparticle immunoassay (CMIA) technology based on acridinium derivative. Recombinant *Toxoplasma gondii* antigen (containing recombinant antigens P30 (SAG1) and P35 (GRA8)) is coated with paramagnetic microparticles.

The ARCHITECT CMV IgG (Abbott – Wiesbaden, Germany) is a two-step immunoassay for the qualitative detection and semi-quantitative determination of IgG antibodies to Cytomegalovirus in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology based on acridinium derivative. The paramagnetic microparticles are coated with CMV virus lysate (AD 169 strain).

The Cobas® Toxo IgG (Roche – Mannheim, Germany) is a sandwich immunoassay for the in vitro quantitative determination of IgG antibodies to *Toxoplasma gondii* in human serum and plasma using electrochemiluminescence technology (ECLIA). The assay uses a biotinylated recombinant *T. gondii*-specific antigen, and a *T. gondii*-specific recombinant antigen labeled with a ruthenium complex.

The Cobas® CMV IgG (Roche – Mannheim, Germany) is a sandwich immunoassay for the in vitro quantitative determination of IgG antibodies to CMV in human serum and plasma using electrochemiluminescence technology (ECLIA). The assay uses biotinylated recombinant CMV-specific antigens, and CMV-specific recombinant antigens labeled with a ruthenium complex.

For all three assays, and both pathogens, the presence of IgG antibodies was classified as positive, negative or equivocal, according to the values specified for each assay (Table 1). Samples close to the cut-off values for each system (Table 1) were transferred to a secondary tube and centrifuged, and the runs were then repeated twice.

Table 1. Comparison of IgG cut-off values with the three assays used.

CMV IgG	LIAISON®XL (U/mL)	Cobas® (U/mL)	ARCHITECT (AU/mL)
Negative samples	<12.0	<0.5	<6.0
Equivocal samples	12.0–14.0	0.5–1.0	6.0–15.0[a]
Positive samples	≥14.0	≥1.0	≥15.0
Toxo IgG (IU/mL)	LIAISON®XL	Cobas®	ARCHITECT
Negative samples	<7.2	<1.0	<1.6
Equivocal samples	7.2–8.8	1–3[b]	1.6–3.0
Positive samples	>8.8	≥3.0	≥3.0

Equivocal samples must be retested to confirm the initial result. A second sample should be collected and tested no less than one week later when the result is repeatedly equivocal.

[a]Specimens with concentration values ≥ 6.0 AU/mL are considered reactive for IgG antibodies to CMV. However, it is recommended that samples between 6.0 AU/mL and 15.0 AU/mL be re-tested or a second sample taken, if possible, within a reasonable period of time and used to repeat ARCHITECT CMV IgG testing.
[b]When IgM antibodies are run in parallel to IgG or their result is negative, the 'grey zone' for this assay broadens to between 1 and 30 IU/mL.

Once the testing was completed, data were analysed, and the combination of the results was used to define the expected results., The classification of samples was assigned based on the consensus of the 3 results, i.e., a sample with concordant result by all the three methods was defined positive or negative accordingly. If the results obtained by the three methods were either discordant among themselves or concordant but equivocal, the sample was classified as equivocal/doubtful and further analysed with additional testing Discrepant CMV samples were tested for IgM to CMV to exclude early acute infection where IgG could be detected differently by the methods and run by bioMérieux Vidas CMV IgG and in-house EIA IgG methods together with Mikrogen (Neuried Germany) RecomLine CMV IgG to confirm the initial test.

Discrepant IgG anti-Toxoplasma results were evaluated by testing the samples for IgM to Toxoplasma with LIAISON® Toxo IgM to rule out potential onset of infection when IgG could be at low levels near the threshold and tested by Toxo II IgG VIDAS bioMérieux (Marcy-l'Etoile France) Vidas Toxo IgG II together with LDBio (Lyon France) Toxo II Immunoglobulin G Western Blot to confirm the initial data.

The following variables were measured: prevalence and frequency distribution, sensitivity and specificity after resolution of discordant sample..

2.2. Statistical Analysis

The seroprevalence of IgG positive subjects was calculated as percentage rate for all methods by markers with the relevant 95% confidence limits by applying Clopper-Pearson "exact" method.

Diagnostic sensitivity and specificity with relevant 95% confidence interval were calculated by methods and markers.

3. Results

Of the 501 patients, 82% were female, with average age of 35.3 years, while males were older, with average age of 54.8 years.

3.1. Anti-cytomegalovirus Immunoglobulin G

Overall concordance for the 3 tests was high (Cohen's kappa= 0.81 95%CI 0.77-0.83).

The 'true' result for each sample was determined based on the results of the three assays run in parallel: 162 were negative and 275 positive for all three tests. Of the 64 discordant samples, after resolution with Mikrogen recomline CMV IgG blot, 5 were confirmed positive, 4 doubtful and 55 samples were confirmed CMV IgG negative.

Thus, a total of 280 were true positive (TP), 217 true negative (TN) and 4 remained equivocal. The equivocal results were mostly from samples found to be negative with LIAISON® XL, positive with Cobas® and equivocal with ARCHITECT. While sensitivity was high and similar between the three methods: 100% of Cobas (95%CI 98.7-100%), 98.2% (95%CI 95.9-99.4%) for LIAISON®XLand 98.6% (95% CI 96.4 -99.6%) for Architect, there were notable differences in specificity between the three assays. Specificity for Cobas® was significantly lower than the other (p<0.001 in both comparisons), at 75.1% (95%CI 68.8-80.8%), compared with 94.9% (95%CI 91.1-97.4%) for ARCHITECT and 98.6% (95%CI 96.0-99.7%) for LIAISON® XL as Cobas® identified all the 57 confirmed negative samples as erroneously positive and LIAISON® XL and ARCHITECT correctly identified 52 and 44 samples, respectively, as negative (Table 2).

Table 2. *Cytomegalovirus IgG sensitivity and specificity calculated for each commercial kit after resolution of the discordant samples.*

CMV IgG	LIAISON XL®	Cobas®	ARCHITECT
Sensitivity	98.2%	100%	98.6%
95% C. I.	95.9-99.4%	98.7-100%	96.4-99.6%
Specificity	98.6%	75.1%*	94.9%
95% C. I.	96.0-99.7%	68.8-80.8%	91.1-97.4%

*Statistically significant difference.

The seroprevalence of CMV IgG among the samples tested ranged from 54.9% (95%CI 50.4-59.3%) and 55.1% (95%CI 50.6-59.5%) for LIAISON® and Architect respectively to 67.5% (95%CI 63.2-71.6%) with Cobas®. The percentage of samples classified as equivocal was low for both LIAISON® and Cobas® (0.8% and 0%, respectively), while 3% of samples tested as equivocal on ARCHITECT (Table 3).

Table 3. *Cytomegalovirus IgG% positive, negative and equivocal samples calculated for each commercial kit.*

CMV IgG	LIAISON® XL	Cobas®	ARCHITECT	Confirmatory test
Negative samples	222 (44.3%)	163 (32.5%)	210 (41.9%)	217 (43.3%)
Equivocal samples	4 (0.8%)	0 (0%)	15 (3%)	4 (0.8%)
Positive samples	275 (54.9%)	338 (67.5%)	276 (55.1%)	280 (55.9%)
Total samples	501	501	501	501

3.2. Toxoplasma Gondii IgG

The seroprevalence of anti*Toxoplasma gondii* IgG among the samples tested in this study ranged from 35.7% (95%CI 31.5-40.1%) for ARCHITECT and 37.5% (95%CI 33.3-41.9%) for LIAISON® to 46.3% (95%CI 41.9-50.8%) with Cobas®. Both LIAISON® XL and Cobas® had a low rate of equivocal samples (0.4% and 0.2%, respectively), with a rate of 2.6% for ARCHITECT (Table 4).

Table 4. *Toxoplasma gondii IgG% positive, negative and equivocal samples calculated for each commercial kit.*

Toxo IgG	LIAISON XL®	Cobas®	ARCHITECT	Confirmatory test
Negative samples	311 (62.1%)	268 (53.5%)	309 (61.7%)	314 (62.7%)
Equivocal samples	2 (0.4%)	1 (0.2%)	13 (2.6%)	1 (0.2%)
Positive samples	188 (37.5%)	232 (46.3%)	179 (35.7%)	186 (37.1%)
Total samples	501	501	501	501

Overall concordance for the 3 tests was high (Cohen's kappa= 0.83, 95%CI 0.80-0.87).

The 'true' result for each sample was determined based on the results of the three assays run in parallel: 264 were negative and 177 positive for all three tests. Of the 60 discordant samples, after resolution with Toxo IgG II VIDAS (Biomerieux – France) and Toxo II IgG Western blot (LdBio – France), 9 were confirmed positive, 1 doubtful and 50 samples were confirmed Toxo IgG negative.

Thus, a total of 186 were true positive (TP), 314 true negative (TN) and 1 remained equivocal.

As seen for the CMV IgG assay, specificity was significantly ($p<0.001$ in both comparisons) lower for Cobas® 85.3%(95%CI 80.9-89.1%), with results again suggesting that the broader grey zone with a cut-off of 30 IU/mL would be more appropriate for this assay, while ARCHITECT showed 96.5% (95%CI 93.8-98.2%) and LIAISON® XL 96.8% (95%CI 94.2-98.5%). Sensitivity was lower though not significantly different for ARCHITECT at 95.7% (95%CI91.7-98.1%), and LIAISON® XL at 96.2% (95%CI 92.4-98.5%), whereas for Cobas® sensitivity was good, 100% (95%CI 98.0-100%) (Table 5).

Table 5. *Toxoplasma gondii IgG sensitivity and specificity calculated for each commercial kit after resolution of the discordant samples.*

Toxo IgG	LIAISON XL®	Cobas®	*ARCHITECT*
Sensitivity	95.7%	100%	96.2%
95% C. I.	91.7-98.1%	98.0-100%	92.4-98.5%
Specificity	96.8%	85.3%*	96.5%
95% C. I.	94.2-98.5%	80.9-89.1%	93.8-98.2%

*Statistically significant difference.

The frequency distribution analysis shows the negative and positive population overlapping.

No positive anti-CMV or anti-Toxoplama IgM antibodies were detected in the study samples.

4. Discussion

The strengths of this study include the fact that two alternative assays were used to compare the prevalence, distribution, sensitivity and specificity of the LIAISON® assay, as well as the additional use of the VIDAS® assay and Western blot to confirm the results.

We can conclude that no differences emerged among the 3 tests for the confirmed CMV IgG and Toxo IgG positive results, and we can confirm that the sensitivity of the three assays is comparable. We cannot say the same for specificity.

LIAISON® XL CMV IgG and ARCHITECT CMV IgG showed a better specificity than Cobas® ($p<0.001$ in both comparisons), as Cobas® identified all the 57 confirmed negative samples as erroneously positive and LIAISON® XL

and ARCHITECT correctly identified 52 and 44 samples, respectively, as negative.

For the confirmed Toxo IgG negative, LIAISON® XL and ARCHITECT showed a better specificity than Cobas® ($p<0.001$ in both comparisons), as Cobas® identified 45 out of the 50 confirmed negative samples as erroneously positive or doubt, while LIAISON® XL and ARCHITECT correctly identified 40 and 39 samples as negative.

Furthermore, we must report that, with the ARCHITECT assays, the number of undetermined results is higher and the interpretation of results are more difficult than LIAISON® XL and Cobas®. The fully automated assays are still the preferential tools to assess the serological status of pregnant women. Nevertheless, the specificity could be related to the method and need to be carefully assessed by users in order to avoid false positive results and allow a proper clinical management. False positive results are of great concern, in consideration that seronegative women can actually lower the risk of acquiring a primary infection when properly counselled about hygienic prophylactic measures. The primary prevention strategy of maternal infection and, ultimately, congenital infection is based on the identification and provision of adequate information to susceptible pregnant women at risk for infection. On the other hand, a woman considered as immune will not follow the hygienic prophylactic measures and the correct follow-up. In these cases, seroconversion could be undiagnosed or detected later, the correct therapy (for toxoplasmosis) not given promptly and the consequence for the foetus could be more serious. In transplanted patients, the possibility of a false positive result could prevent the patients' follow-up and the preventive measures adopted in all the cases of mismatch (13).

5. Conclusion

Overall, we can conclude that for both CMV IgG and Toxo IgG, the LIAISON® assay was better than both the Cobas® and ARCHITECT assays in terms of CMV and Toxo IgG detected, and both diagnostic sensitivity and specificity performance although the difference is statistically significant only compared to Cobas®.

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