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FULL PAPER

Comparison of the LIAISON®XL and ARCHITECT IgG, IgM, and IgG avidity assays for the diagnosis of *Toxoplasma gondii*, cytomegalovirus, and rubella virus infections

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SUMMARY

This study compared the performance of the LIAISON®XL system of immunoglobulin (Ig) G and IgM immunoassays for the diagnosis of *Toxoplasma gondii*, cytomegalovirus (CMV), and rubella virus infections with that of the ARCHITECT system. Patient serum samples, previously screened and clinically diagnosed with *T. gondii*, CMV or rubella, were used to compare LIAISON®XL and ARCHITECT IgG and IgM immunoassays. LIAISON®XL Toxo and CMV IgG avidity assays were also compared with equivalent ARCHITECT assays and reference methods. Overall agreement between the LIAISON®XL and ARCHITECT assays was 99% and 92% for the Toxo IgG and IgM assays, respectively, 98% and 96% for the CMV IgG and IgM assays, respectively, and 93% and 98% for the rubella virus IgG and IgM assays, respectively. LIAISON®XL IgG Toxo and CMV avidity assays showed high concordance with the VIDAS® Toxo IgG avidity assay and an in-house CMV avidity assay (reference methods), and faster IgG avidity maturation in a larger number of samples collected months after the primary infection compared with equivalent ARCHITECT assays. LIAISON®XL assays for detection of anti-*T. gondii*, CMV and rubella virus IgG and IgM are at least equal to the competitor assays on the ARCHITECT platform.

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INTRODUCTION

Maternal infections with TORCH pathogens (i.e., *Toxoplasma gondii*, rubella virus, cytomegalovirus [CMV] and herpes viruses) are a well-known cause of perinatal morbidity and mortality (Neu *et al*., 2015a; Wilson *et al*., 2015a). *T. gondii*, rubella virus and CMV infections in pregnant women increase the risk of spontaneous abortion or foetal death (Adams Waldorf *et al*. 2013a; Rasti *et al*., 2016). In addition, *T. gondii* is associated with foetal hydrocephalus, seizures, intracranial calcifications, eye disease and unilateral microphthalmia (Neu *et al*., 2015b; Adams Waldorf *et al*., 2013b), while rubella virus may cause malformations in multiple organ systems (clinical rubella syndrome), including eye, heart and neurological defects, and an increased risk of childhood type 1 diabetes mellitus (Neu *et al*., 2015c; Adams Waldorf *et al*., 2013c), and human CMV infections may cause foetal growth restriction, sensorineural hearing loss, brain damage and cerebral palsy (Adams Waldorf *et al*., 2013d). The prevalence of TORCH infections varies worldwide, with low- and middle-income countries having the highest burden of disease (Neu *et al*., 2015d). For instance, the seroprevalence of *T. gondii* among women in the USA, France and Brazil is approximately 11%, 44%, and 77%, respectively. Treatment or prevention strategies are available for most of these pathogens (Neu *et al*., 2015e). Prenatal screening allows for early diagnosis, which plays an important role in the effective management of patients with TORCH infections (Neu *et al*., 2015f; Kishore *et a*l., 2011). Along with detection of previous infection, estimation of the time of maternal infection is important in the management of foetal risk (Petersen *et al*., 2005a). Immunoglobulin (Ig) G avidity assays have therefore been developed. High IgG avidity indicates a past infection (≥4 months earlier for CMV and *T. gondii* infections); however, IgG avidity tests are less reliable for rubella infections due to their quick maturation (Wilson *et al*., 2015b). In this study, the performance of the LIAISON®XL (DiaSorin, Italy) IgG and IgM assays for detection of *T. gondii*-, CMV-, and rubella virus-specific antibodies, and of *T. gondii*- and CMV-specific IgG avidity assays, was compared with that of the ARCHITECT (Abbot, Germany) system using previously screened and clinically diagnosed patient serum samples.

Key words:
Prenatal screening, cytomegalovirus; rubella virus; *Toxoplasma* gondii, LIAISON® XL assays; ARCHITECT assays.

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MATERIALS AND METHODS

In this retrospective study, sera from patients (mainly pregnant women) who attended the outpatient clinic of the SC Clinical Microbiology and Virology of IRCCS

Polyclinic Foundation for TORCH screening were collected and analysed for routine diagnosis, and the residual sample was anonymised and stored frozen at -20° until used. The IgG sample was selected on the basis of the international unit (IU) value obtained in order to have low, intermediate and high titre according to tests employed. Some other sera came from routine analysis (150 for toxoplasmosis, 150 for CMV and 70 for rubella). Clinical data were also recorded. These anonymised sera samples, previously collected and screened for *T. gondii* IgG and IgM with the VIDAS® Toxo IgG and IgG Avidity assays (BioMérieux, France) and ISAGA IgM test (BioMérieux, France), were analysed with LIAISON®XL Toxo IgGII and IgM assays, and ARCHITECT Toxo IgG and IgM assays. Sera positive for *T. gondii* IgG and IgM or seroconversion were further analysed with the LIAISON®XL and ARCHITECT Toxo IgG, IgM and IgG Avidity tests. Sera previously screened for CMV IgG and IgM with ETI-CYTOK-M PLUS and ETI-CYTOK-G PLUS (DiaSorin, Italy) were evaluated with LIAISON®XL CMV IgG II and IgM II and ARCHITECT CMV IgG and IgM assays. Sera previously tested with an in-house CMV IgG avidity test were analysed with LIAISON®XL CMV IgG Avidity II and ARCHITECT CMV IgG Avidity assays; sera from CMV seroconversion were also tested with these methods. Sera previously analysed for rubella IgG and IgM with ETI-RUBEK-M PLUS and ETI-RUBEK-G PLUS (DiaSorin, Italy) were analysed with LIAISON® XL Rubella IgG and IgM and ARCHITECT Rubella IgG and IgM assays. Both the LIAISON® XL and ARCHITECT systems use two-step, indirect, chemiluminescent immunoassays (DiaSorin SpA LIAISON®XL Toxo IgG Avidity (ref 310795), 2015a; Abbott ARCHITECT system: Toxo IgG (ref 6C19), 2009a). In the first incubation step, pathogen-specific antibodies in samples/controls bind to the solid phase (pathogen-coated magnetic particles); in the second step, an antibody conjugate (containing a murine monoclonal antibody linked to an antibody-isoluminol conjugate for LIAISON®XL and acrydinium esters for ARCHITECT) reacts with the solid phase-bound pathogen-specific IgG and IgM antibodies. Unbound material is removed by washing after each incubation. Starter reagents are then added to induce the chemiluminescent reaction, which is measured as relative light units by a photomultiplier device.

The study was performed according to the guidelines of the Institutional Review Board on the use of biological specimens for scientific purposes in conformity to Italian law (art. 13 DI gs196/2003).

Statistical methods

Statistical analysis was conducted using Stata software, version 14.0 (Stata Corporation, USA). Descriptive statistics were used for all variables. Values are presented as mean and standard deviation (SD) for normally distributed variables, median and interquartile range (IQR) for non-normally distributed variables, and number with percentages for categorical variables. Linear regression models were used to assess correlation between test results as continuous values, and the Cohen κ coefficient was used to assess concordance between tests results as categorical values. Variables in regression models were log transformed to achieve normalization. Two-tailed tests were used throughout; p-values <0.05 were considered significant.

RESULTS

All the numeric results obtained and the cut off employed for all the tests under evaluation are reported in *Table 1* and *Table 2*.

Toxoplasma gondii-specific IgG and IgM assays

Two hundred sixty serum samples previously tested with VIDAS® Toxo IgG and ISAGA IgM (170 from routine analysis, 30 with 6-20 IU, 30 with 21-200 IU and 30 with >200 IU) assays were analysed with LIAISON®XL Toxo IgG II and IgM assays and ARCHITECT IgG and IgM assays. There was 99% and 92% agreement between the two systems for *T. gondii*-specific IgG and IgM, respectively (*Table 1*, *Figure 1A* and *1B*). For *Toxoplasma* IgM, we recorded fewer IgM-positive samples than with ARCHITECT but there were more IgM-equivocal samples that are considered as positive. Considering the equivocal samples as positive in the management of patients, concordance is 100% for the two assays.

Among 64 samples positive for *T. gondii*-specific IgG and IgM plus 21 samples with seroconversion, the LIAISON®XL Toxo IgG Avidity assay showed faster maturation in older infections than did the ARCHITECT IgG Avidity assay (*Figure 1C*). In concordance assessments of the LIAISON®XL, ARCHITECT and VIDAS® Toxo IgG Avidity assays, κ values were 0.46 for LIAISON®XL versus ARCHITECT, 0.83 for LIAISON®XL versus VIDAS®, and 0.49 for ARCHITECT versus VIDAS®.

Among 21 samples from 8 patients (mean interval 3 weeks between 2 samples) with *T. gondii* seroconversion, the ARCHITECT Toxo IgM assay failed to detect *T. gondii*-specific IgM in two consecutive samples collected at the beginning of the infection (*Table 3*).

Table 1 - *Numeric results obtained in the evaluated tests.*

	IgG			*IgM*			*IgG avidity*		
	Positive	*Equivocal*	*Negative*	*Positive*	*Equivocal*	*Negative*	*Low*	*Intermediate*	*High*
Toxoplasma gondii (N=277)									
LIAISON®XL	160	6	111	61	12	204	33	24	27
ARCHITECT	155	10	112	65	8	204	41	10	12
CMV (N = 317)									
LIAISON®XL	86	2	229	221	1	95	20	25	20
ARCHITECT	83	0	234	208	8	101	46	3	16
Rubella (N=188)									
LIAISON®XL	136	13	39	9	1	178			
ARCHITECT	144	27	17	3	4	182			

Cytomegalovirus-specific IgG and IgM assays

In an analysis of 291 serum samples previously assessed for CMV IgG and IgM antibodies with ETI-CYTOK-M PLUS and ETI-CYTOK-G PLUS (22 with IU titres of 1220, 32 with 0-50 IU, 37 with IU titres >100 and 200 from the routine), there was 98% agreement between the LIAISON®XL CMV IgG II and ARCHITECT CMV IgG assays (*Table 1-2, Figure 2A*), and 96% agreement between the LIAISON®XL CMV IgM II and ARCHITECT CMV IgM assays (*Table 1; Figure 2B*).

Sixty samples previously tested with an in-house CMV IgG avidity assay were analysed with the LIAISON®XL CMV IgG Avidity II and the ARCHITECT CMV IgG Avidity assays (*Figure 2C*). In this analysis, CMV IgG avidity remained low on the ARCHITECT assay until 4 months after infection. In concordance assessments of CMV IgG

Table 2 - *Cut-offs for the tests included in the study.*

	Toxoplasma IgM		
	LIAISON®XL (U/mL)	*ARCHITECT (Index)*	*Biomerieux Isaga*
Negative	<6	<0.50	<7+
Equivocal	6-8	0.50-0.60	
Positive	>8	≥0.60	≥7+
	Toxoplasma IgG (IU/mL)		
	LIAISON®XL	*ARCHITECT*	*Biomeriuex Vidas*
Negative	<7.2	<1.6	<4
Equivocal	7.2–8.8	1.6–3.0	4-6
Positive	>8.8	≥3.0	≥6
	Toxoplasma Avidity		
	LIAISON®XL (AI)	*ARCHITECT*	*Biomeriuex Vidas (AI)*
Low	<0.20	<50%	<0.20
Intermediate	0.20-0.30	50-60%	0.20-0.30
High	≥0.30	≥60%	≥0.30
	Rubella IgG (IU/mL)		
	LIAISON®XL	*ARCHITECT*	
Negative	<9	<5	
Equivocal	9-11	5-10	
Positive	>11	≥10	
	Rubella IgM		
	LIAISON®XL (AU/mL)	*ARCHITECT (Index)*	
Negative	<20	<1.2	
Equivocal	20-25	1.2-1.6	
Positive	>25	≥1.6	
	Cytomegalovirus IgG		
	LIAISON®XL (U/mL)	*ARCHITECT (AU/mL)*	
Negative	<12.0	<6.0	
Equivocal	12.0–14.0		
Positive	≥14.0	≥6.0	
	Cytomegalovirus IgM		
	LIAISON®XL (U/mL)	*ARCHITECT (Index)*	
Negative	<18.0	<0.85	
Equivocal	18.0–22.0	0.85-1	
Positive	≥22.0	≥1	
	Cytomegalovirus Avidity		
	LIAISON®XL (AI)	*ARCHITECT*	
Low	<0.15	<50%	
Intermediate	0.15-0.25	50-60%	
High	≥0.25	≥60%	

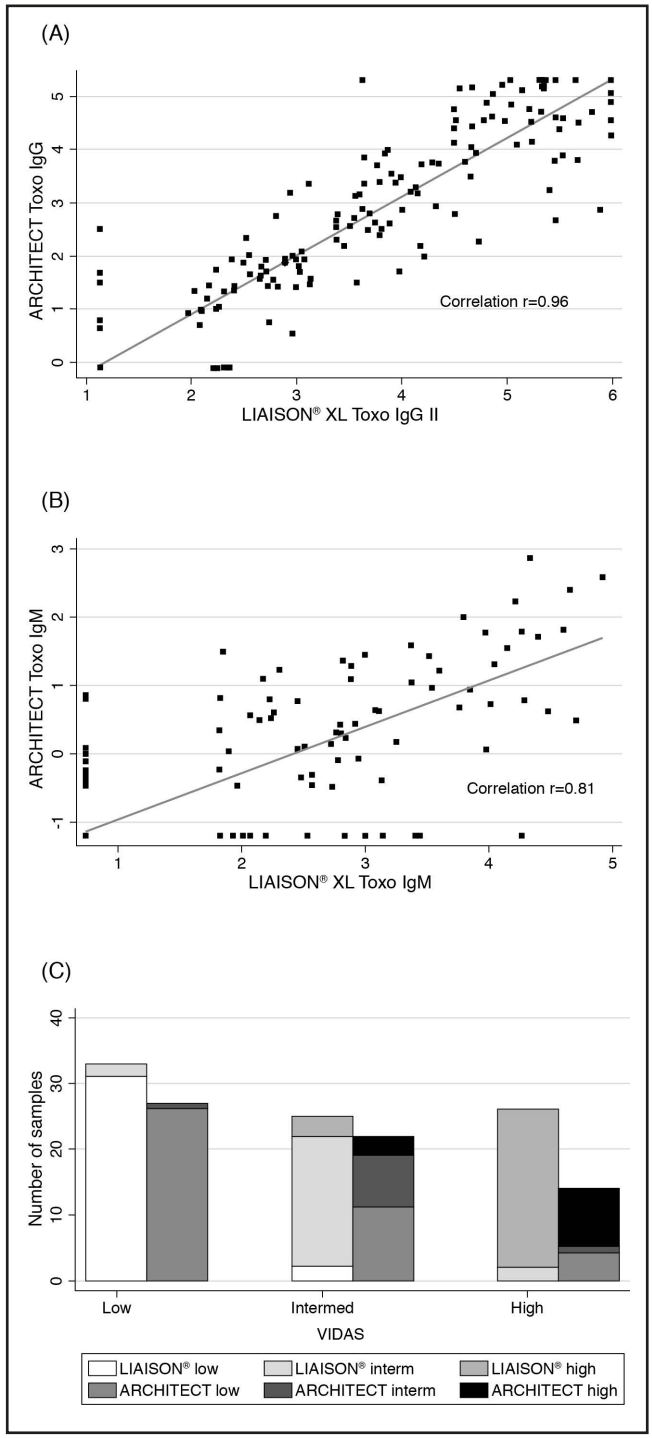

Figure 1 - *Comparison of LIAISON®XL with ARCHITECT assays for detection of T. gondii-specific (A) IgG and (B) IgM antibodies, and (C) the IgG avidity assays, using the VIDAS® IgG Avidity assay as a control.*

avidity assays, κ values were 0.29 for LIAISON®XL versus ARCHITECT, 0.98 for LIAISON®XL versus the in-house assay, and 0.31 for ARCHITECT versus the in-house assay. Among 19 samples from 5 patients (mean interval 3 weeks between samples) with CMV seroconversion, the ARCHITECT CMV IgM assay failed to detect CMV-specific IgM antibodies in one sample at the beginning of infection, whereas LIAISON®XL IgM showed positive results (*Table 3*).

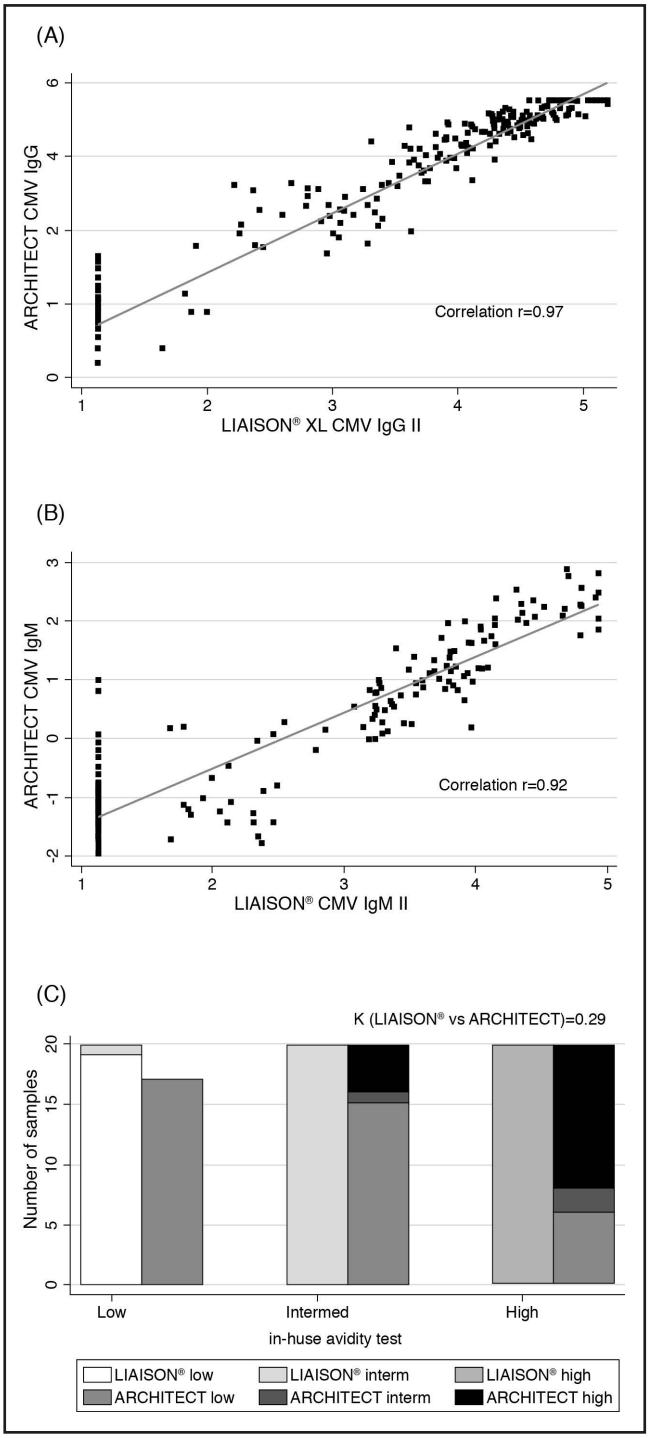

Figure 2 - *Comparison of LIAISON®XL with ARCHITECT assays for detection of CMV-specific (A) IgG and (B) IgM antibodies, and (C) the IgG avidity assays using an in-house method as a control.*

Rubella virus-specific IgG and IgM assays

Among 188 samples previously assessed for rubella virus IgG and IgM antibodies with ETI-RUBEK-M PLUS and ETI-RUBEK-G PLUS (42 with IU titres 5-10, 21 with 11-20 IU, 50 with >100 IU and 65 from routine), the LIAISON®XL and ARCHITECT systems showed 93% and 98% agreement for detection of rubella virus-specific IgG and IgM, respectively (*Figure 3A* and *3B*). The discrepancies for Rubella IgG antibodies could be due to the LIAISON®XL cut-off employed in this study (equivocal between 9-11 IU). If the other cut-off (equivocal between 5-10 IU) were used, the number of positive samples could increase and concordance would be 97%. All concordance associations were statistically significant.

In 2018, DiaSorin developed a new diagnostic kit for rubella in order to improve sensitivity and specificity. Several peer-reviewed articles have highlighted the lack of standardization between rubella virus IgG assays reporting results in international units per millilitre. The investigation into the reasons for the lack of standardization found that the current WHO international standard (RUB-1-94) fails by three key metrological principles. The standard is not a pure analyte but is composed of pooled human immunoglobulin. It was not calibrated by certified reference methods; rather, superseded tests were used. Finally, no measurement uncertainty estimations have been provided. There is an analytical and clinical consequence to the lack of standardization of rubella virus IgG assays, which leads to misinterpretation of results **(Clin Microbiol Rev 2016;29:163-74).**

DISCUSSION

Overall, the LIAISON®XL system of assays showed good agreement with the ARCHITECT system in the detection and quantification of *T. gondii*-, CMV-, and rubella virus-specific IgG and IgM antibodies. In the LIAISON®XL Toxo IgG Avidity assay, IgG avidity was higher in older infections than when using the ARCHITECT assay, and was superimposable on that observed with the VIDAS® Toxo IgG Avidity assay. With CMV IgG avidity assays, several samples with high avidity in old infections on the LIAISON®XL assay still showed low avidity when tested with the ARCHITECT assay. Furthermore, the LIAISON®XL assay showed good concordance with the in-house CMV IgG avidity test.

Previous studies comparing LIAISON®XL assays with other automated systems have shown generally similar levels of agreement with the current study. In a comparison of the VIDAS®, LIAISON®XL, and ARCHITECT systems, the *T. gondii* IgG and IgM assays showed 95% and 98% agreement, respectively, between VIDAS® and ARCHITECT, and 97% and 95% agreement, respectively, between VIDAS® and LIAISON®XL (Murat *et al.*, 2013). In a subsequent evaluation of nine immunoassays for *T gondii*-specific IgG and IgM detection, including the LIAISON®XL, ARCHITECT, and VIDAS® assays, IgG and IgM sensitivities ranged from 97.1% to 100% and 65% to 97.9%, respectively, and marked differences between assays in IgG titres were observed (Villard *et al.*, 2016a).

In a previous evaluation of the LIAISON®XL CMV II IgG and IgM assays, overall agreement for CMV IgG assays was 98.8% between the LIAISON®XL and VIDAS® assays, and 95% between the LIAISON®XL and ETI-CYTOK-M reverse plus assays (Delforge *et al.*, 2015a).

Table 3 - *Results of the evaluated tests in discordant consecutive samples.*

	Sample date	*Antibody*	*Vidas*	*ISAGA*	*LIAISON®XL*	*ARCHITECT*	*Vidas IgG Avidity*	*LIAISON®XL IgG Avidity*	*ARCHITECT IgG Avidity*
T.GONDII/1	02/02/14	IgG	<4		<3	<0.5	ND	ND	ND
		IgM		9+	7.42	<0.5			
	20/03/14	IgG	27		43	40.5	0.047	0.0415	ND
		IgM		9+	6.14	<0.5			
	21/05/14	IgG	40		90	81	0.049	0.0705	24.46
		IgM		9+	<6	<0.5			
T.GONDII/2	24/2/15	IgG	<4		<3	<0.5	ND	ND	ND
		IgM		12+	6.5	<0.5			
	09/04/15	IgG	18		29	9.8	ND	ND	ND
		IgM		12+	15.9	<0.5			
	30/04/15	IgG	17		42.5	13.6	0.055	0.618	19.9
		IgM		12+	15.9	4.7			
CMV/1	25/11/13	IgG			<5	<6			
		IgM			25.5	0.88			
	03/12/13	IgG			6.44	0.7			
		IgM			44.6	2.52			
	08/01/14	IgG			54.3	39.8		0.096	16.28
		IgM			43.5	2.21			

In an analysis of 14 rubella IgG immunoassays, the LIAISON®XL Rubella IgG assay showed 100% sensitivity and specificity; however, concordance across all of the evaluated immunoassays was low (Huzly *et al.*, 2016).

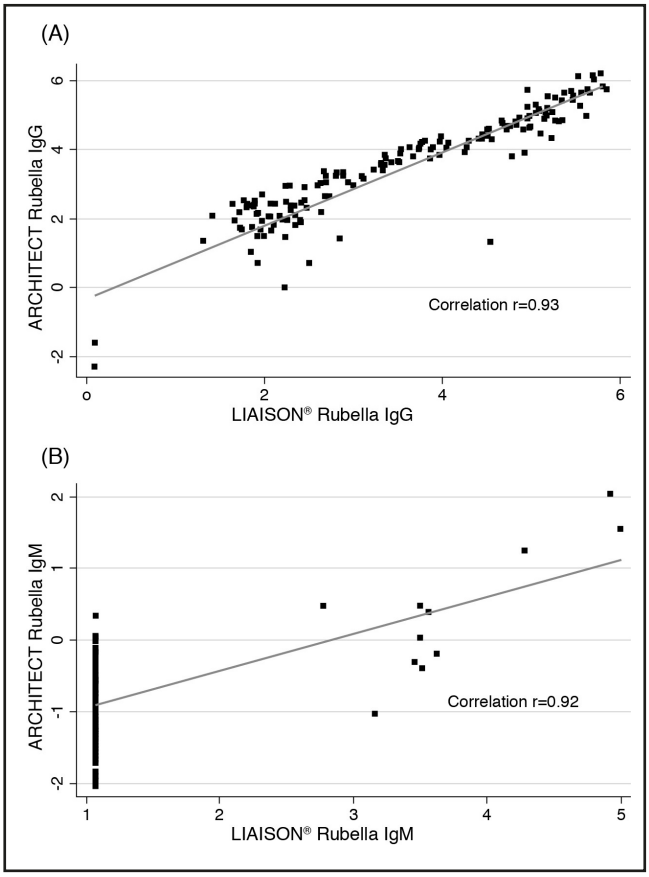

Figure 3 - *Comparison of LIAISON®XL with ARCHITECT assays for the detection of rubella virus-specific (A) IgG and (B) IgM antibodies.*

The LIAISON®XL system of assays was the first to become available for measurement of the IgG avidity index in samples with low levels of *T. gondii*-specific IgG antibodies (Petersen *et al.*, 2005e). The differences between LIAISON®XL and ARCHITECT avidity assays observed in this study are consistent with those previously observed when comparing VIDAS® and ARCHITECT *T. gondii* IgG avidity assays, i.e., the VIDAS® avidity assay showed higher accuracy than ARCHITECT (93% vs 87%) when compared to the estimated date of infection (Smets *et al.*, 2016a).

This variation in avidity assay results may be due to the recombinant antigens used in the ARCHITECT assay (Smets *et al.*, 2016a). In a comparison of the LIAISON®XL and VIDAS® CMV IgG avidity assays, the LIAISON®XL assay showed high avidity status sooner than the VIDAS® assay, although avidity was falsely high with the LIAISON®XL assay in 1.4% of samples (Sellier *et al.*, 2015). In another comparison of the LIAISON®XL and VIDAS® CMV IgG avidity assays, there was 81% agreement between the two assays (using a VIDAS® cut-off of 0.65) (Delforge *et al.*, 2015a).

The current study provides a comprehensive comparison of the LIAISON®XL and ARCHITECT assay systems for detection of *T. gondii*-, CMV-, and rubella virus-specific IgG and IgM antibodies, as well as of the IgG and IgM avidity assays for *T. gondii* and CMV. Furthermore, this study confirms equivalent performance of the LIAISON®XL and VIDAS® assays for detection of *T. gondii*-specific IgG and IgM and assessment of IgG avidity. Potential limitations of this study include the relatively small sample size compared with an earlier study (Murat *et al.*, 2013b) and the post-hoc nature of the analysis.

LIAISON®XL assays for detection of anti-*T. gondii*, CMV and rubella virus IgG and IgM are at least equal to the competitor assays on the ARCHITECT platform. LIAISON®XL IgG Avidity assays for *T. gondii*- and CMV-specific IgG show higher avidity in a larger number of samples collected at ≥4 months after the primary infection compared with equivalent ARCHITECT assays. In

this study, we found slower maturation of *Toxoplasma* IgG avidity in the ARCHITECT assay and, consequently, better definition of the infection with the LIAISON®XL Toxo IgG Avidity assay.

Conflicts of interests

The authors have no conflicts of interest to declare. The study received no external funding.

Abbreviations: CMV, cytomegalovirus; Ig, immunoglobulin; TORCH, *Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes viruses.

References

Abbott. ARCHITECT system: Toxo IgG (ref 6C19), 2009. http://www.ilex-medical.com/files/PDF/ToxoIgG_ARC.pdf.

Adams Waldorf K.M., McAdams R.M. (2013). Influence of infection during pregnancy on fetal development. *Reproduction*. **146**, R151-162.

Delforge M.L., Desomberg L., Montesinos I. (2015). Evaluation of the new LIAISON® CMV IgG, IgM and IgG Avidity II assays. *J Clin Virol*. **72**, 42-45.

DiaSorin SpA. LIAISON® XL Toxo IgG Avidity (ref 310795), 2015. http://www.diasorin.com/en/liaisonr-xl-toxo-igg-avidity.

Dimech W., Grangeot-Keros L., Vauloup-Fellous C. (2016). Standardization of Assays That Detect Anti-Rubella Virus IgG Antibodies. *Clin Microbiol Rev.* **29**, 163-174.

Huzly D., Hanselmann I., Neumann-Haefelin D., Panning M. (2016). Performance of 14 rubella IgG immunoassays on samples with low positive or negative haemagglutination inhibition results. *J Clin Virol.* **74**, 13-18.

Kishore J., Misra R., Paisal A., Pradeep Y. (2011). Adverse reproductive outcome induced by Parvovirus B19 and TORCH infections in women with high-risk pregnancy. *J Infect Dev Ctries.* **5**, 868-873.

Murat JB, Dard C, Fricker Hidalgo H, Darde ML, Brenier-Pinchart MP, Pelloux H. (2013). Comparison of the Vidas system and two recent fully automated assays for diagnosis and follow-up of toxoplasmosis in pregnant women and newborns. *Clin Vaccine Immunol.* **20**, 1203-1212.

Neu N., Duchon J., Zachariah P. (2015). TORCH infections. *Clin Perinatol.* **42**, 77-103, viii.

Rasti S., Ghasemi F..S, Abdoli A., Piroozmand A., Mousavi S.G., Fakhrie-Kashan Z. (2016). ToRCH "co-infections" are associated with increased risk of abortion in pregnant women. *Congenit Anom* (Kyoto), **56**, 73-78.

Sellier Y., Guilleminot T., Ville Y., Leruez-Ville M. (2015). Comparison of the LIAISON® CMV IgG Avidity II and the VIDAS® CMV IgG Avidity II assays for the diagnosis of primary infection in pregnant women. *J Clin Virol*. **72**, 46-48.

Smets A., Fauchier T., Michel G., Marty P., Pomares C. (2016). Comparison of *Toxoplasma* gondii IgG avidity ARCHITECT and Vidas assays with the estimated date of infection in pregnant women. *Parasite.* **23**, 45.

Villard O., Cimon B., L'Ollivier C., Fricker-Hidalgo H., Godineau N., Houze S., et al. (2016). Help in the choice of automated or semiautomated immunoassays for serological diagnosis of toxoplasmosis: evaluation of nine immunoassays by the French National Reference Center for Toxoplasmosis. *J Clin Microbiol.* **54**, 3034-3042.

Wilson C., Nizet V., Maldonado Y., Remington J., Klein J., Infectious diseases of the fetus and newborn infant, 8th ed., Elsevier - Health Sciences Division, Philadelphia, USA, 2015.