CLINICAL EVALUATION OF AUTOMATED CYTOMEGALOVIRUS IgG, IgM AND IgG AVIDITY ASSAYS FOR THE LIAISON® XL ANALYSER PLATFORM

Claudia Pavia, Alessandra Moroni, Angela Chiereghin, Giulia Piccirilli, Gabriele Turello, Diego Squarzoni, Liliana Gabrielli, Tiziana Lazzarotto

Operative Unit of Microbiology, Laboratory of Virology, Policlinico S. Orsola Malpighi, University of Bologna, Bologna, Italy

INTRODUCTION

Foetal human cytomegalovirus (CMV) transmission occurs more frequently during primary infection than non-primary infection. This highlights the importance of the accurate determination of immune status during pregnancy. Maternal CMV infection is often asymptomatic, thus clinical diagnosis is unreliable and serological tests for CMV-specific immunoglobulin IgG and IgM are required. Moreover, the determination of CMV IgG avidity helps to establish the timing of infection as IgG avidity matures during the course of infection.

OBJECTIVES

Given the implications of CMV diagnosis, the assays must be thoroughly characterised with well-classified sera in order to ascertain their sensitivity and specificity and to ensure that they perform at least as well as, or better than, established routine tests on unselected samples.

The aim of this study was to evaluate the analytical performance and the clinical utility of a fully automated chemiluminescence immunoassay (CLIA) for the determination of human cytomegalovirus (CMV)-specific IgG, IgM and IgG avidity (LIAISON® XL, DiaSorin).

MATERIALS AND METHODS

Sixty-three serum samples from cord blood donors obtained at the end of pregnancy were reanalysed using the CLIA-IgG, IgM and IgG avidity tests in order to evaluate the analytical performance. The serum samples were previously examined using Abbott Architect CMV IgG and IgM assays (chemiluminescent microparticle immunoassay-CMIA) and CMV-specific IgM immunoblot (IB) (JCM 1998, 36:3337-41). The serological results were interpreted as suggested by the manufacturer (Table 1). Moreover, 214 sequential serum samples from 40 pregnant women with primary CMV infection were examined in order to evaluate the clinical utility of LIAISON® XL platform. The sequential sera had been divided into 6 groups based on the time of collection, from 30 to 210 days after the beginning of primary CMV infection.

	LIAISON® XL CLIA IgG	LIAISON® XL CLIA IgM	LIAISON® XL CLIA IgG Avidity	ARCHITECT CMIA IgG	ARCHITECT CMIA IgM
Units	IU/mL	AU/mL	%	AU/mL	Index
Negative range	x < 12	x < 18	x < 15	x < 6.0	x < 0.85
Equivocal range	12 ≤ x < 14	18 ≤ x < 22	15 ≤ x < 25	6.0 ≤ x < 15	0.85 ≤ x < 1.0
Positive range	x ≥ 14	x ≥ 22	x ≥ 25	x ≥ 15	x ≥ 1.0

Table 1: Interpretation of the results as suggested by the manufacturers.

RESULTS

Between June 2013 and December 2014, we screened 1210 serum samples from cord blood donors using the platform Architect CMV, CMIA-IgG and IgM assays. We found 63 samples (5.2%) with CMIA-IgM positive results (median value 1.06 index, range 0.85-4.53) and we were able to confirm IgM positivity with IB in only 8 samples. The 63 serum samples were reanalysed using the LIAISON® XL CLIA-IgG, IgM and IgG avidity tests, retrospectively. From 63 samples tested, 8 (12.7%) delivered positive results in all IgG and IgM assays (CMIA, CLIA and IB) (Table 2). Of 55 (87.3%) samples with discordant results (Table 3), 6 samples from uninfected CMV women were negative with all assays except CMIA-IgM test (median value 1.28 index, range 0.88-2.12). One sample was IgM-negative with IB assay and false IgM-positive with both tests, CLIA and CMIA (Table 3). The remaining 48 samples from women with not active CMV infection showed IgG positive results with CMIA and CLIA assays, however discordant results for CMV specific-IgM. In particular, the 48 samples were IgM-negative with IB and CLIA and borderline/positive with CMIA test (median value 1.28 index, range 0.88-2.12). The IgG avidity-CLIA level was high in all samples (Table 3). The diagnosis of primary CMV infection is based on the following criteria: seroconversion, decreasing levels of CMV specific-IgM antibody, and increasing levels of IgG antibody avidity. Two-hundred-fourteen sequential serum samples from women with primary CMV infection were examined with the CLIA-IgG, IgM and IgG avidity tests, retrospectively. In Figure 1, the correlation analysis between the avidity IgG-indices and the number of days after primary CMV infection gave a correlation coefficient of 0.67, indicating a good association between the two parameters. Considering the low and moderate values of IgG-CLIA avidity, we found 98% sensitivity in detecting a recent primary CMV infection within 90 days. The sensitivity decreased to 76.5% and 38% within 120 and 150 days after onset of primary infection, respectively (Figure 1). The 100% of sera collected after 180 days showed a high IgG-avidity index. The detection of CMV-specific IgM was 100% in all samples collected within 2 months after onset (Table 4). The sensitivity decreased to 73%, 45, and 33 within 90, 150 and 210 days after onset of primary CMV infection, respectively.

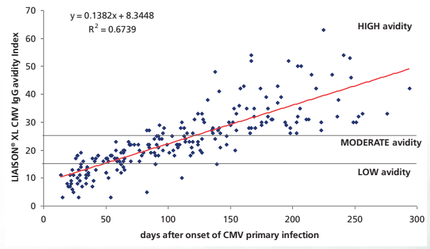

Figure 1: Kinetic of LIAISON® XL CMV IgG - CLIA avidity

CONCLUSION

The CMV LIAISON® XL CLIA-IgM assay showed a very good specificity in samples from active and no active CMV infection compared to that one found with Architect CMIA-IgM assay (98.4% versus 12.7%). We observed a very good agreement between the results obtained from CLIA-IgM and IB-IgM tests. Among the 63 serum samples from cord blood donors, 62 were positive or negative with both assays whereas the CMIA-IgM test was in agreement with IB-IgM test in only 8 of the 63 samples. In pregnancy, the most important requirement for a CMV IgG avidity assay is to help in identifying or excluding a primary infection occurring in the previous 3 months in women presenting with a positive CMV IgM result. The 98% of samples taken <90 days after infection onset were reported as containing low-moderate avidity CMV IgG and 100% of samples taken >180 days after infection onset were reported as high-avidity CMV IgG by the LIAISON® XL CMV IgG Avidity assay. Between 90 and 120 days after infection onset the percentage of samples with low-moderate IgG avidity was still very high (76.5%). A mixture of low-moderate avidity and high-avidity were observed in samples taken 120–180 days after infection onset. The latter observation is consistent with the fact that the kinetics of the IgG avidity maturation process is likely to vary between individuals. Therefore, the LIAISON® XL system appears useful for accurately diagnosing of CMV infection in pregnant women.

Number of samples	Architect CMIA IgG AU/mL	LIAISON® XL CLIA IgG IU/mL	Architect CMIA IgM Index	LIAISON® XL CLIA IgM AU/mL	LIAISON® XL CLIA Avidity IgG Index %	IB IgM
6	positive	positive	positive	positive	high	POS
median value range	180.7 60 – 250	110 31 – 173	1.61 1.18 – 4.44	27.3 22 – 41.4	63 48 - 83	
2	positive	positive	positive	positive	low	POS
median value range	96.45 0.1 – 5.1	69.85 5 – 11.5	4.45 4.4 – 4.5	46.55 31.8 – 61.3	12.5 12 - 13	

Table 2: Comparison of the results of tests performed on 8 serum samples in agreement for the diagnosis of CMV infection.

Number of samples	Architect CMIA IgG AU/mL	LIAISON® XL CLIA IgG IU/mL	Architect CMIA IgM Index	LIAISON® XL CLIA IgM AU/mL	LIAISON® XL CLIA Avidity IgG Index %	IB IgM
48	positive	positive	BL/positive	negative	high	NEG
median value range	222.2 39 – 250	117.5 30 – 180	1.01 0.85 - 2.91	6.85 5 - 17	60 39 - 83	
6	negative	negative	BL/positive	negative	NOT DOSABLE	NEG
median value range	0.25 0.1 – 5.1	5 5 – 11.5	1.28 0.88 - 2.12	5 5 - 17		
1	positive 224.9	positive 106	BL 1	BL 23	high 63	NEG

Table 3: Comparison of the results of tests performed on 55 serum samples with discordant results for the diagnosis of CMV infection.

DAYS AFTER ONSET OF PRIMARY CMV INFECTION						
LIAISON® XL CMV IgM	0-30	31-60	61-90	91-150	151-180	181-210
% positive results	100	100	73	45.5	38.8	33.3
% borderline results	0	0	8.1	22.7	16.8	8.3
% negative results	0	0	18.9	31.8	44.4	58.4
Median Value AU/mL	50.0	35.0	30.0	21.5	19.0	16.3
Min (AU/mL) Max (AU/mL)	25 97	22 65.9	6 60.3	5 50	5 50	5 47

Table 4: Spread of results with LIAISON® XL IgM-CLIA test