Combination of line immunoassays Mikrogen *recom*Line CMV IgG and *recom*Line CMV IgG Avidity helps to date the onset of CMV primary infection

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ABSTRACT

CMV IgG avidity assays are widely used and can be helpful in pregnant women to date the onset of CMV primary infection; however, these tests are not standardized and sometimes give inconclusive results. We evaluated the performances of Mikrogen *recom*Line CMV IgG and IgG Avidity compared to the VIDAS CMV IgG Avidity. On a first sample set of 89 sequential sera collected from 40 women with precisely determined onset of CMV primary infection, the combination of Mikrogen *recom*Line CMV IgG and IgG Avidity showed an accurate interpretation in 83.1% (74/89), an incorrect result in 4.5% (4/89), and an inconclusive result in 12.4% (11/89) and showed a better sensitivity to diagnose infections <14 weeks compared to VIDAS (85.9% vs. 76.9%). On a second sample set of 89 sera with an intermediate VIDAS CMV IgG Avidity, the combination of line immunoassays provided additional information on the time of infection in 79% (70/89) of the samples. This combination of line assays is useful as additional confirmatory testing and can help to date more precisely the onset of CMV primary infection.

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1. Introduction

Human cytomegalovirus (CMV) is a herpesvirus which has a ubiquitous and worldwide distribution, and is the most frequent cause of congenital infection (Kenneson and Cannon, 2007). The prevalence is around 0.5–1% of all live births and is the leading cause of sensorineural hearing loss and mental retardation (Dollard et al., 2007; Stagno, 1986). CMV congenital infection can result from primary infection, reinfection, or reactivation among pregnant women. The risk of vertical transmission from mothers with primary infection during pregnancy ranges from 30% to 45% (Bodeus et al., 2010; Enders et al., 2011; Liesnard et al., 2000; Picone et al., 2013; Revello et al., 2011), and the risk is going down to 1.4% in case of nonprimary infection (Kenneson and Cannon, 2007). In order to counsel about the fetal risk, it is of major importance to differentiate CMV primary infections occurring before pregnancy from those occurring during pregnancy. The maternal infection is mostly asymptomatic, and when symptoms are present, they are mostly mild (Daiminger et al., 2005). Particularly before 12–14 weeks of gestation, the presence of IgG and IgM without knowing about the previous serologic status can be a source of anxiety for parents and of difficult management for professionals. Supplementary tests are then needed to define primary infection onset. IgG avidity assays are widely used in case of positive IgG and IgM in pregnant women. These tests determine the strength of antigen–antibody

bond, which increases over time after the primary infection. The IgG avidity is initially low during the early weeks and will mature to high avidity a few months after primary infection (Prince and Lapé-Nixon, 2014). In 12–25% of cases, the result of these conventional IgG avidity assays is inconclusive (intermediate avidity index); therefore, other markers are needed to help to date the onset of CMV primary infection (Enders et al., 2014; Leruez-Ville et al., 2013; Prince and Lapé-Nixon, 2014). Recently, other assays such as reactivity against specific CMV antigens and neutralizing antibodies were reported as additional tools for dating the onset of primary infection in pregnancy (Enders et al., 2013; Lilleri et al., 2016).

In this study, we aimed first to evaluate the ability of the line immunoassays Mikrogen *recom*Line CMV IgG and *recom*Line CMV IgG Avidity (Mikrogen GmbH, Neuried, Germany) to date the onset of CMV primary infection comparing to VIDAS CMV IgG Avidity (bioMérieux, Marcy l'Etoile, France) and, secondly, to evaluate the added value of the combination of *recom*Line CMV IgG and *recom*Line CMV IgG Avidity in cases of intermediate VIDAS avidity.

2. Study design

2.1. Subjects and samples

For the first objective of this study, 89 sequential sera collected from 40 women with precisely determined onset of CMV primary infection were evaluated. We retrospectively selected sera drawn in the weeks
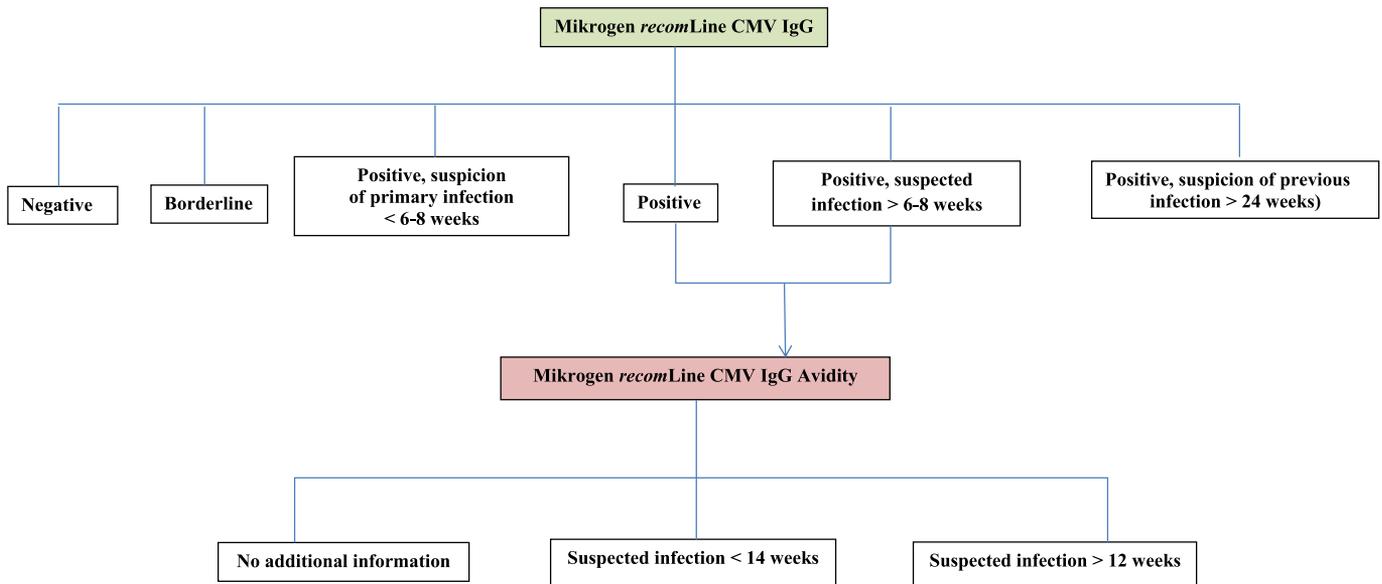

Fig. 1. Flow diagram of *recom*Line CMV IgG and CMV IgG Avidity interpretations.

and months following the diagnosis of primary infection. The number of sera per woman was between 1 and 8, depending on the availability of samples in the laboratory (1 serum for 16 women, 2 sera for 11 women, 3 sera for 7 women, 4 sera for 4 women, 6 sera for 1 woman, and 8 sera for another one).

The onset time of primary infection was determined by one of the following criteria:

- In presence of seroconversion of IgG and when both IgG and IgM are present at the first positive serology, the onset was arbitrary set at the mid time between the last IgG-negative and the first IgG-positive sample (n patients $= 16$, n sera $= 40$). For these 16 patients, the interval between last negative and first positive sample was ≤6 weeks, except for 3 patients (7, 7, and 8 weeks)
- In the presence of a seroconversion of IgG and when only IgM was positive at the first positive serology, the onset of primary infection was set at that point (n patients $= 11$, n sera $= 25$).
- In the presence of clinical symptoms and/or biological alterations compatible with CMV infection in the presence of CMV-specific IgM antibodies and a significant rise of IgG, the onset was established at the time of the symptoms (n patients $= 7$, n sera $= 16$)

The panel also included samples from women who experienced CMV primary infection in the past but presenting long-lasting CMV IgM (n patients $= 7$, n sera $= 8$).

For the second objective of this study, 89 sera collected from 78 pregnant women presenting positive CMV IgG and IgM and an intermediate avidity with VIDAS CMV IgG Avidity were evaluated. For 11 patients, we tested 2 sequential sera with an interval of 1 to 7 weeks between samples.

2.2. Methods

The *recom*Line IgG and IgG Avidity is a line immunoassay allowing the identification of antibodies against 6 specific antigens of CMV: IE1, CM2, p150, p65, gB1, and gB2. The detection of phase-specific IgG and the avidity allow to differentiate between an acute and a previous infection. Assays were performed according to manufacturer's recommendations. For all samples, 2 test strips were incubated in parallel (1 treated with avidity reagent and the other not) in order to differentiate IgG antibodies with low avidity and IgG with high avidity. The test procedure lasts approximately 3 h.

After 2 h of drying, the evaluation of each strip was done using the test strip analysis software *recom*Scan (Mikrogen). The software measures the stain intensity of the occurring bands and determines the reactivity of each band. For each specific antigen band, a point value is given and the test result of the IgG is determined by adding the point values. Moreover, according to the pattern of IgG reactivity, the time of infection can be determined in a certain number of cases. The different possible interpretations of the IgG blot results are described in Fig. 1.

In cases of positive IgG result and positive IgG, suspected infection >6–8 weeks, the test strip incubated with avidity reagent was also interpreted.

By measuring the difference of intensity between both strips, the software is able to evaluate the avidity of IgG against IE1, CM2, p150, and gB2. A decrease of 40% or less indicates high avidity, a decrease of 50–100% indicates low avidity, and a decrease of 40–50% is regarded as intermediate avidity. According to the avidity of the respective IgG antibodies against the 4 different antigens, different possible interpretations are given by the software (Fig. 1). In our study, the final results were considered: either the combination of the 2 line assays or the result of the *recom*Line IgG only in cases of negative IgG, borderline IgG, positive IgG with suspicion of primary infection <6–8 weeks, and positive IgG with suspicion of previous infection >24 weeks. Positive IgG without additional information is considered as inconclusive result.

The VIDAS CMV IgG Avidity was performed and interpreted according to manufacturer's recommendations using a cutoff index <0.40 for low avidity and ≥0.65 for high avidity. Avidity indexes between 0.40 and 0.65 are intermediate and reported as inconclusive results. Avidity testing was not performed if concentration of VIDAS CMV IgG was below 6 AU/mL.

The tests routinely used for the diagnosis of CMV infection were the LIAISON® CMV IgG II and the LIAISON® CMV IgM II assays.

Table 1A
Results obtained with VIDAS on the sample set with precisely determined onset of CMV primary infection.

Onset of primary infection	VIDAS CMV IgG avidity		
	≤12 weeks	Inconclusive	>12 weeks
≤12 weeks $n = 52$	40	11	1
>12 weeks $n = 37$	0	13	24

Table 1B
Results obtained with *recom*Line on the sample set with precisely determined onset of CMV primary infection.

Onset of primary infection	MIKROGEN *recom*Line CMV IgG and CMV IgG Avidity							
	IgG negative	IgG borderline	Infection < 6–8 weeks	Infection >6–8 weeks and <14 weeks	Infection <14 weeks	Infection >12 weeks	Infection >24 weeks	IgG positive, no additional information
<14 weeks n = 57 (<6–8 weeks n = 32)	2[a]	0	9	1[b]	37	1[c]	0	7
>12 weeks n = 37 (>24 weeks n = 18)	0	0	0	1[d]	4[e]	21	5[f]	6

Five samples taken at 13 weeks after the onset of infection were counted twice: 2 of them were scored as infection <14 weeks, 1 as infection >12 weeks, and 2 as positive IgG with no additional information.
Supplementary information for some specific samples:
[a] Very recent infections: 0 week, 3 weeks.
[b] : Twelve weeks.
[c] : Thirteen weeks.
[d] : Fourteen weeks.
[e] : Thirteen, 13, 17, and 17 weeks
[f] : Eighteen, 22, 28, 31, and 62 weeks.

2.3. Statistical analysis

We produced diagnostic accuracy measurements (sensitivity and specificity) for the VIDAS CMV IgG Avidity and for the *recom*Line IgG and IgG Avidity assays and computed 95% confidence interval (CI) according to the exact method. We excluded inconclusive test results from the computation of accuracy measurements, which are reported in the tables and in the text. As in the clinical practice, we have to consider the inconclusive avidity results, so we also calculated accuracy measurements including these results and counted them as incorrectly classified by the test.

We encountered a challenge in the computation of accuracy measurements since the line immunoassay can discriminate between infections of more than 12 weeks and less than 14 weeks, leaving out infections estimated at 13 weeks. Since infections occurring at 13 weeks are both >12 weeks and <14 weeks, we always considered them as classified correctly by the test.

3. Results

3.1. Samples with well-defined onset of infection

Fifty-two sera were drawn within 12 weeks of infection, and 37 were obtained more than 12 weeks after primary infection. The results
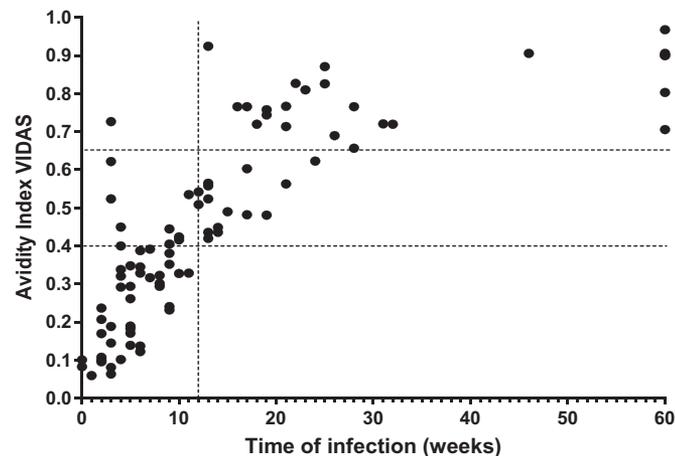

Fig. 2. VIDAS CMV IgG avidity index according to the time of infection. Samples taken more than 60 weeks after infection are plotted at 60 weeks.

obtained on the 89 samples with VIDAS CMV IgG Avidity and the combination of *recom*Line CMV IgG and *recom*Line CMV IgG avidity are summarized in Tables 1A and 1B.

According to the determined onset of primary infection, VIDAS CMV IgG Avidity showed an accurate interpretation in 71.9% (64/89) of cases, an incorrect result in 1.1% (1/89), and inconclusive results (intermediate avidity) in 27% (24/89). The falsely high avidity result obtained with VIDAS CMV IgG avidity came from a woman with a proven primary infection having taken place 3 weeks before sampling. The combination of *recom*Line CMV IgG and *recom*Line CMV IgG avidity scored this serum correctly as an infection <14 weeks.

Fig. 2 shows the VIDAS CMV IgG avidity index according to the time of infection.

According to the determined onset of primary infection, the combination of line immunoassays Mikrogen *recom*Line CMV IgG and *recom*Line CMV IgG Avidity showed an accurate interpretation in 83.1% (74/89) of samples tested, an incorrect result in 4.5% (4/89), and an inconclusive result in 12.4% (11/89) (positive IgG). The 4 samples not correctly scored by the combination of immunoblot were as follows: 2 infections scored <14 weeks at 17 weeks and 2 infections scored >24 weeks at 18 and 22 weeks of infection. For one of the samples not

Table 2
Accuracy of VIDAS CMV IgG Avidity to diagnose an infection ≤12 weeks and of MIKROGEN *recom*Line CMV IgG and CMV IgG Avidity to diagnose an infection <14 weeks.

	VIDAS CMV Ig Avidity Diagnosis of an infection ≤12 weeks	Mikrogen *recom*Line[a] Diagnosis of an infection <14 weeks
Number of inconclusive results excluded	24/89 (27%)	11/89 (12.4%)
Number of samples evaluated	65	83[a]
Sensitivity	97.6% (40/41) 95% CI 87.1–99.9%	98% (49/50) 95% CI 89.35–99.95%
Specificity	100% (24/24) 95% CI 91.2–100%	78.8% (26/33) 95% CI 61.09–91.02%
Number of inconclusive results included	24/89 (27%)	11/89 (12.4%)
Number of samples evaluated	89	89
Sensitivity	76.9% (40/52) 95% CI 67.20–84.43%	85.9% (49/57) 95% CI 76.76–91.91%
Specificity	64.9% (24/37) 95% CI 52.18–75.75%	70.3% (26/37) 95% CI 57.31–80.63%

[a] The test has a double threshold of >12 weeks/<14 weeks. Samples whose true status was 13 weeks (n = 3) were always considered correct.

Table 3

Accuracy of MIKROGEN *recom*Line CMV IgG and CMV IgG Avidity to diagnose an infection >24 weeks and an infection <6–8 weeks.

	>24 weeks	<6–8 weeks[a]
	Total number of samples evaluated: 78 Number of samples excluded due to an inconclusive result: 11 (12.4%)	Total number of samples evaluated: 79 Number of samples excluded due an inconclusive result: 11 (12.4%)
Sensitivity	16.7% (3/18) 95% CI 3.58–41.42%	34.4% (11/32) 95% CI 18.57–53.19%
Specificity	96.7% (58/60) 95% CI 88.47–99.59%	100% (47/47) 95% CI 71.51–100%

[a] The test has a double threshold of ><=6 weeks/>8 weeks. Samples whose true status was 7 weeks (*n* = 1) were always considered correct.

correctly scored at 17 weeks, the time of infection was set at the mid time between the last negative serology and the first positive serology, but the interval in between the two samples was 8 weeks, inducing a possible misclassification of this sample. The 2 samples incorrectly scored at 18 and 22 weeks were more precisely timed, based on the first positive serology with IgM positive only, and came from the same patient.

Tables 2 and 3 show the sensitivity and specificity of both tests to date correctly a CMV infection. Table 2 presents the computation either by excluding the inconclusive results or by including them in order to be more representative of the reality of the clinical setting.

3.2. Samples with unknown onset of infection and intermediate VIDAS CMV IgG Avidity

The combination of line immunoassays yielded 5 infections <6–8 weeks, 8 infections >6–8 weeks without additional statement, 11 infections between 6–8 and 14 weeks, 13 infections >12 weeks, 1 infection >24 weeks, 29 infections <14 weeks, 2 negative IgG, 1 intermediate IgG, and 19 cases of noncontributory positive IgG (Table 4).

Taking into account all the results giving useful information (including the negative and borderline IgG), the combination of line immunoassays provided additional information on the time of infection in 79% (70/89) of the samples.

4. Discussion

In this study, we evaluated the performance of the *recom*Line IgG and IgG Avidity with a total of 178 samples. Half of them came from women with precisely determined onset of CMV primary infection, and half of them were sera with an intermediate VIDAS IgG avidity.

CMV IgG avidity tests are widely used as a useful tool to date CMV infection, especially in pregnant women presenting with positive CMV IgG and IgM, but they are not standardized (Revello et al., 2010). Several comparative studies have shown discrepancies between commercial assays, in particular false high positive results (Revello et al., 2010; Sellier et al., 2015; Vauloup-Fellous et al., 2013). We also observed a false high positive result in this study, previously described as a case report (Delefortrie et al., 2016). Other limitations are, on the one hand, the restricted usefulness of these tests after the first trimester of pregnancy and, on the other hand, the reported unusually long persistence of

intermediate or low CMV IgG avidity in some cases (Lumley et al., 2014; Revello et al., 2010; Sellier et al., 2015; Vauloup-Fellous et al., 2013). Therefore, we have to be cautious with their interpretation, and other tests such as immunoblot assays may be useful in this setting.

The first part of the study evaluated the accuracy of *recom*Line IgG and IgG Avidity to date CMV infection: In our study, 83.1% of samples tested matched onset dates compared to 71.9% with VIDAS avidity. Moreover, the number of inconclusive results (intermediate avidity for VIDAS, positive IgG without additional statement for *recom*Line) is smaller with the combination of line immunoassays (27% vs. 12.4%).

Considering the performances of the combination of *recom*Line assays for timing primary infection according to the interpretations proposed by the manufacturer, we showed that their sensitivity to diagnose an infection <14 weeks is good (98%) even after including the inconclusive results (86%). An advantage of the *recom*Line assay should be its capacity to diagnose correctly infections >24 weeks, in order to rule out a primary infection during pregnancy when women attend prenatal care late after the first trimester. Unfortunately, our results showed a sensitivity of 16.67% (3/18) to exclude an infection <24 weeks. For the 15 other samples, the test scored correctly as infections >12 weeks, but this result does not help in the management of these women presenting late in the pregnancy. This poor sensitivity has also been observed by Enders et al. They did not obtain the characteristic blot pattern for late infection >24 weeks among a sample set of 30 infections >20–40 weeks. However, we have to mention that the manufacturer improved the quality of the antigen used for the *recom*Line assay compared to *recom*Blot tested by Enders, so the results may not be fully comparable. As mentioned by Enders et al., these results highlight the importance of performing CMV antibody testing during the first trimester or beginning of the second trimester in order to date correctly a primary infection (Enders et al., 2013). We observed also a low sensitivity of the *recom*Line assay to diagnose precisely an infection <6–8 weeks (34.38%, 11/32), but in this sample set, the test scored correctly the majority of the serum as infections <14 weeks (21/32).

Conventional CMV IgG avidity assays have proved to be a helpful tool to date infection in pregnant women with positive CMV IgG and IgM; however, they give an intermediate or inconclusive result in 12–25% of cases, depending on the population studied and the method used (Enders et al., 2014; Leruez-Ville et al., 2013; Prince and Lapé-Nixon, 2014, personal data). In the second part of our study, we showed that among the sample set of inconclusive results with VIDAS CMV IgG Avidity, the combination of line immunoassays could help to determine the timing of infection in 79% of samples. We took into account in these useful interpretations 2 negative and 1 borderline IgG. This is perhaps questionable, but as these samples were clearly positive with 2 different IgG immunoassays (and IgM positive), we considered that this information was useful, helping to suspect a very recent infection. Obviously, this has to be confirmed on a second sample. Considering the results of the second part of our study, the sequential use of conventional avidity testing followed by the line assays in case of inconclusive conventional avidity results may therefore help in the management of pregnant women presenting with a positive CMV IgG and IgM serology, especially during the first trimester of pregnancy.

In conclusion, we assessed the performances of the Mikrogen *recom*Line CMV IgG and CMV IgG Avidity, and we demonstrated that this combination of line assays is useful as additional confirmatory testing and can help to date more precisely the onset of CMV primary infection.

Table 4

Results obtained on the sample set with borderline VIDAS CMV IgG Avidity (unknown onset of infection).

	MIKROGEN *recom*Line CMV IgG and CMV IgG Avidity							
	IgG negative	IgG borderline	Infection <6–8 weeks	Infection >6–8 weeks	Infection > 6–8 weeks and <14 weeks	Infection <14 weeks	Infection >12 weeks	Infection >24 weeks	Positive, no additional information
n = 89	2	1	5	8	11	29	13	1	19

Conflict of interests

Contributors

M.L.D. designed the study and wrote the manuscript; J.E. performed the experiments; C.D., M.L.D., D.S., and I.M. recruited patients; E.C. performed statistical analysis. All contributors critically revised the paper.

Acknowledgments

References

Bodeus M, Kabamba-Mukadi B, Zech F, Hubinont C, Bernard P, Goubau P. Human cytomegalovirus *in utero* transmission: follow up of 524 maternal seroconversions. J Clin Virol 2010;47:201–2.

Daiminger A, Bader U, Enders G. Pre- and periconceptional primary cytomegalovirus infection: risk of vertical transmission and congenital disease. BJOG 2005;112:166–72.

Delefortrie Q, Delforge ML, Cirriez JM, Schatt P, Auger L, Vankerkhoven P, et al. CMV seroconversion associated with a high avidity in a previously seronegative pregnant woman during her first trimester of pregnancy. Curr Trends Biomed Eng Biosci 2016;1(1). [CTBEB.MS.ID.555552].

Dollard S, Grosse S, Ross D. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007;17:355–63.

Enders G, Daiminger A, Bäder U, Exler S, Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation with gestational age. J Clin Virol 2011;52:244–6.

Enders G, Daiminger A, Bäder U, Exler S, Schimpf Y, Enders M. The value of CMV IgG avidity and immunoblot for timing the onset of primary CMV infection in pregnancy. J Clin Virol 2013;56:102–7.

Enders M, Daiminger A, Exler S, Enders G. Author's reply to letter to the editor regarding "Comments on an alternative calculation of cytomegalovirus IgG avidity indexes on VIDAS" [J Clin Virol 60 (2014) 76–77]. J Clin Virol 2014;61(2):309–10.

Kenneson A, Cannon M. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007;17:253–76.

Leruez-Ville M, Sellier Y, Salomon L, Stirnemann J, Jacquemard F, Ville Y. Prediction of fetal infection in cases with cytomegalovirus immunoglobulin M in the first trimester of pregnancy; a retrospective cohort. Clin Infect Dis 2013;56:1428–35.

Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet Gynecol 2000;95:881–8.

Lilleri D, Gerna G, Furione M, Zavattoni, Spinillo A. Neutralizing and ELISA IgG antibodies to human cytomegalovirus glycoprotein complexes may help date the onset of primary infection in pregnancy. J Clin Virol 2016;81:16–24.

Lumley S, Patel M, Griffiths PD. The combination of specific IgM antibodies and IgG antibodies of low avidity does not always indicate primary infection with cytomegalovirus. J Med Virol 2014;86:834–7.

Picone O, Vauloup-Fellous C, Cordier AG, Guitton S, Senat MV, Fuchs F, et al. A series of 238 cytomegalovirus primary infections during pregnancy: description and outcome. Prenat Diagn 2013;33:1–8.

Prince H, Lapé-Nixon M. Role of cytomegalovirus (CMV) IgG avidity testing in diagnosing primary CMV infection during pregnancy. Clin Vaccine Immunol 2014;21(10):1377–84.

Revello MG, Genini E, Gorini G, Klersy C, Piralla A, Gerna G. Comparative evaluation of eight commercial human cytomegalovirus IgG avidity assays. J Clin Virol 2010;48:255–9.

Revello MG, Fabbri E, Furione M, Zavattoni M, Lilleri D, Tassis B, et al. Role of prenatal diagnosis and counseling in the management of 735 pregnancies complicated by primary human cytomegalovirus infection: a 20-year experience. J Clin Virol 2011;50:303–7.

Sellier Y, Guilleminot T, Ville Y, Leruez-Ville M. 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 2015;72:46–8.

Stagno S. Cytomegalovirus infection: a pediatrician's perspective. Curr Probl Pediatr 1986;16:629–67.

Vauloup-Fellous C, Berth M, Heskia F, Dugua JM, Grangeot-Keros L. Re-evaluation of the VIDAS CMV IgG avidity CMV-IgG assay: determination of new cut-off values based on the study of kinetics of CMV-IgG maturation. J Clin Virol 2013;56:118–23.