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Comparative evaluation of eight commercial human cytomegalovirus IgG avidity assays

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ABSTRACT

Background: The interpretation of a positive IgM antibody result to human cytomegalovirus (HCMV) in a pregnant woman is of major importance for the correct management of the pregnancy. Determination of HCMV-specific IgG avidity is considered an useful approach for distinguishing IgM antibody due to primary HCMV infection from IgM antibody elicited during non-primary infection.
Objective: Comparative evaluation of eight commercial HCMV IgG avidity assays currently available in Europe.
Study design: A panel of 198 sequential samples collected from 65 pregnant women at 0–90, 91–180, and >180 days after the onset of primary HCMV infection was retrospectively tested by Abbott, BioMérieux, Bio-Rad, DiaSorin, Diesse, Euroimmun, Radim, and Technogenetics HCMV IgG avidity assays according to the manufacturer's instructions.
Results: None of the 198 samples tested yielded identical scores by the kits under evaluation. The Euroimmun and Radim assays showed the best correlation with expected results in terms of low (0–90 days), intermediate (90–180 days) and high (>180 days) avidity results, respectively. The best accuracy in diagnosing a recent (<90 days after the onset) or non-recent (>180 days after the onset) primary HCMV infection was shown by Radim followed by Euroimmun and Diesse. The best correlation with a well established in-house developed HCMV IgG avidity assay was shown by Radim.
Conclusions: HCMV IgG avidity kits need to be improved and standardized. In the meantime, highly specific IgM assays are preferable for screening purposes in pregnant women.

1. Background

The potential impact of human cytomegalovirus (HCMV) infection on pregnancy is well known.[1] Primary rather than recurrent maternal HCMV infection has been shown to be associated with an higher risk of congenital infection disease[2] and identification of pregnant women with primary infection is therefore critical for optimal pregnancy management. Seroconversion, i.e. appearance of HCMV-specific antibodies in a previously seronegative woman, represents the best serologic approach to diagnose primary HCMV infection. Unfortunately, such an approach is seldomly feasible as, more often, women are tested when they are already pregnant. In such a situation, in order to diagnose subclinical HCMV infections, IgG and IgM antibody are routinely determined.[3] It is well known, however, that the mere presence of IgM cannot be considered diagnostic of primary HCMV infection since a number of other conditions such as interfering factors, cross-reactivities, recurrencies and persistent IgM production may mediate a positive IgM result.[4] Moreover, HCMV-specific IgM production may last several months after primary infection.[5] Therefore, it is imperative that every positive IgM result in pregnancy be carefully interpreted since irrevocable decisions may be taken on the basis of laboratory results.

The most widely used approach for the interpretation of IgM-positive results relies on IgG avidity testing. Specifically, detection of low IgG avidity indicates an acute/recent primary infection and, consequently, an increased risk of intrauterine transmission, whereas high IgG avidity is more common in non-primary HCMV infection with little risk for the fetus.[6–8]

Abbreviations: HCMV, human cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; AUC, area under curve; AI, avidity index.

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Table 1
Characteristics of commercial HCMV IgG avidity assays included in the study.

Manufacturer Name of the assay	Technology	Dissociating agent	Range and interpretation of results (according to manufacturer's instructions)
Abbott Architect CMV IgG avidity	Chemiluminescent Microparticle immunoassay Automated	None	<50% low avidity 50–59.9% grey zone >59.9% high avidity
bioMérieux VIDAS CMV IgG avidity	Enzyme-linked fluorescent assay Semi-automated	Urea	<0.2 strong indication of a primary infection dating back <3 months 0.2–0.8 does not distinguish a recent infection from a former infection >0.8 strong indication of a primary infection dating back >3 months
Bio-Rad Platelia CMV IgG avidity	ELISA	Urea	<0.4 low avidity, more in favour of recent primary infection of <3 months 0.40–0.55 grey zone >0.55 high avidity, more in favour of past infection of >3 months
DiaSorin LIAISON CMV IgG avidity	Chemiluminescent immunoassay Automated	Urea	<0.2 low avidity, possible primary infection acquired <3 months 0.2–0.3 moderate avidity, does not rule out a recent infection >0.3 high avidity, may exclude a primary infection in the past 3 months
Diesse Cytomegalovirus IgG avidity	ELISA	Urea	<30% low avidity 30–40% borderline avidity >40% high avidity
Euroimmun CMV IgG avidity	ELISA	Urea	<40% low avidity 40–60% equivocal range >60% high avidity
Radim Cytomegalovirus IgG avidity	ELISA	Urea	<35% low avidity, strong indication of infection in the previous 3 months 35–45% mean avidity >45% high avidity
Technogenetics BEIA CMV IgG avidity	ELISA	Potassium thiocyanate	<25 low avidity, primary infection in the last 3 months 25–45 medium avidity, primary infection in the last 6 months >45 high avidity, exclude primary infection in the last 3 months

2. Objectives

Presently, a number of assays for IgG avidity determination are commercially available. The objective of this study was to evaluate and compare the performances of eight CE-labelled HCMV IgG avidity assays.

3. Study design

3.1. Sera and diagnosis of primary HCMV infection

The panel of sera used for the analysis consisted of 198 sequential sera collected from 65 pregnant women with primary HCMV infection. Primary HCMV infection was diagnosed by IgG seroconversion in 56 women (36 women had concomitant signs and/or symptoms) and by kinetics of IgM antibody, IgG avidity, and DNAemia in the remaining nine women.[9] Precise dating of the onset was established either based on the presence of clinical symptoms and/or abnormal laboratory findings[3] or, when absent, it was arbitrarily set between the last IgG-negative and the first IgG-positive serum sample. Sera were collected 7–275 (median 75) days after the onset. In detail, 116, 60 and 22 sera were collected 7–90 (median 50), 91–178 (median 121), and 183–275 (median 214) days after the onset of primary HCMV infection, respectively.

3.2. In-house IgG avidity assay

All samples had been previously tested for diagnostic purposes by an in-house developed IgG avidity assay.[9] Sera used in the study were collected and handled aseptically. They were stored frozen (−20 °C) in aliquots for 6 months to 8 years and underwent 1–2 freeze–thaw cycles (including the one necessary for the present study). During the study period (2 months) aliquots of tested sera were stored at +4 °C. Briefly, test serum is added to wells coated with HCMV antigen. Wells are then washed with 6 M urea solution and residual antigen-bound IgG detected by addition of peroxidase-conjugated anti-human IgG and a chromogen substrate solution. The ratio of absorbance in wells treated with urea to the absorbance in untreated wells is multiplied by 100 and the avidity index (AI) calculated. AIs <35% are mostly associated with primary infection acquired within <3 months, AIs ≥51% indicate infections acquired at least 6 months before.

3.3. Commercial HCMV IgG avidity assays

The eight kits tested (5 ELISA, 2 chemiluminescent and 1 enzyme-linked fluorescent assay) are listed in Table 1 together with range and interpretation of results. Seven kits are based on the same test principle, i.e. the dissociation of previously formed IgG-HCMV immunocomplexes by a dissociating agent followed by calculation of the ratio of reactivity (expressed as optical density, relative light units, or relative fluorescence units) in wells treated with dissociating buffer to that of untreated wells. On the other hand, the Abbott Architect assay includes a pre-adsorption step with diluted HCMV antigen during which preferential blocking of IgG of high avidity is likely to occur. Treated samples are then tested for residual HCMV-specific IgG reactivity and results compared to untreated samples. Serum samples were tested blindly and results interpreted according to the manufacturer's instructions. A single lot of each kit was used.

3.4. Statistical analysis

Expected IgG avidity results according to the three selected time intervals, i.e. low avidity for the 0–90-day interval, moderate avidity for the 91–180-day interval, and high avidity for the >180-day interval after the onset, were the gold standard for assessing assay performance. Agreement between commercial kits (categorized measures), and between commercial kits and the Pavia assay was evaluated with kappa statistics. Accuracy in diagnosing a recent or a non-recent primary infection was assessed by computing the area under the receiver operating characteristic curve (AUC) and its 95%