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NOTES

Performance of the Epstein-Barr Virus and Herpes Simplex Virus Immunoglobulin M Assays on the Liaison Platform with Sera from Patients Displaying Acute Parvovirus B19 Infection[▽]

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Acute parvovirus B19 infection has been reported to cause false-positive results frequently in the Epstein-Barr (EBV) and herpes simplex virus (HSV) immunoglobulin M (IgM) assays from DiaSorin performed on the Liaison platform. We tested 65 sera from patients with a presumptive or conclusive diagnosis of acute parvovirus B19 infection in both assays and obtained no false-positive results in the EBV IgM test and 10.4% nonspecific reactivities in the HSV IgM assay. Our data support the specificity of both assays in this clinical setting.

Biological diagnosis of febrile diseases of viral origin commonly relies on serological tests, demonstration of seroconversion being the "gold standard." Convalescent-phase sera are often unavailable, so that the presence of virus-specific immunoglobulins M (IgMs) in acute-phase sera is the only marker supporting the diagnosis, unless sufficient levels of virus-specific IgGs are detected, and thus avidity index (AI) values can be determined. The interpretation of an isolated positive IgM test is frequently complicated by the concurrent appearance in sera of IgMs against heterologous viral agents. The presence of IgMs against Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6, measles virus, and rubella virus has been reported to occur in sera from patients with acute human parvovirus B19 infection (3, 5–7). IgM antibodies against unrelated viruses appearing in the setting of acute parvovirus B19 infection may be either cross-reactive antibodies or truly virus-specific antibodies secreted as a result of heterologous virus reactivation driven either directly or indirectly by parvovirus B19 infection. Alternatively, false-positive results in viral IgM assays may occur as a result of spurious binding of nonspecific serum antibodies to the solid phase. In this context, it has been recently reported (1) that an exceedingly high percentage of sera drawn from patients with either a conclusive or a presumptive diagnosis of acute parvovirus B19 infection gave a false-positive result in several IgM immunoassays (EBV, CMV, herpes simplex virus, and *Borrelia burgdorferi* sensu lato) from DiaSorin (Saluggia, Italy) performed on the Liaison platform. The false-positive reactivities were apparently due to nonspecific binding of IgMs to the antigen-coated beads used in the assay (2) and were found to be partially eliminated (although sera remained positive) by adding polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) to the dilution buffer. The diagnostic relevance of the above findings prompted us to evaluate the performance of the DiaSorin EBV and HSV IgM assays with sera from acutely parvovirus B19-infected patients in our setting. Sixty-five sera from 65 patients (45 females and 20 males, aged 4 to 70 years, median age of 15 years) with a presumptive clinical and/or biological diagnosis of acute parvovirus B19 infection sent to our laboratory from January 2005 to January 2009 were retrieved for analysis. These sera had been tested in the parvovirus B19 enzyme immunoassay (EIA) from Biotrin International (Dublin, Ireland) and found to be IgG and IgM positive ($n = 53$) or IgG negative and IgM positive ($n = 12$). In the Biotrin assay, antibodies against a baculovirus-expressed VP2 conformational protein are detected. The B19-specific IgM assay is a mu capture EIA, while the IgG assay is an antigen capture EIA. This immunoassay has 89.1% sensitivity and 99.4% specificity for IgM detection (4). Clinical data were available for 41 patients. These patients displayed fever and one or more of the following clinical or biological signs compatible with acute parvovirus B19 infection: exanthema, arthralgia, and mono- or pancytopenia. To confirm the acute nature of parvovirus B19 infection, IgG avidity tests were performed. In brief, parvovirus B19 IgG avidity was determined with the Biotrin assay. The first step of the assay was modified to include two washes (5 min each) with a washing buffer containing urea (4 M). The AI value (as a percentage) was calculated as follows: (absorbance of parvovirus B19 IgGs in the presence of urea/absorbance of parvovirus B19 IgG in the absence of urea) × 100. AI values of <25% are seen early after infection, and AI values of >80% are observed in past infections (8). In our experience, AI values of <40% should be considered indicative of a recent infection when using urea at 4 M in the washing buffer (unpublished observation). IgG AI values were determined for 20 of

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TABLE 1. Performance of the DiaSorin EBV, HSV, and *B. burgdorferi* sensu lato IgM assays with sera from patients with acute parvovirus B19 infection

Parvovirus B 19 serological profile (no. of sera)	Results of DiaSorin IgM assays (no. of sera positive/Ind[a]/negative)		
	EBV[b]	HSV[c]	*B. burgdorferi*[d]
IgG^-/IgM^+ (12)	0/1/11	3/0/3	0/0/6
IgG^+/IgM^+ (53)	0/4/49	2/2/38	0/0/6

[a] Ind, indeterminate result.
[b] Sixty-five sera were tested in the EBV IgM assay.
[c] Forty-eight sera (displaying a negative result in the Vircell HSV IgM test) were tested in the HSV IgM assay from DiaSorin.
[d] Twelve sera were tested in the *B. burgdorferi* sensu lato IgM assay.

the 53 parvovirus B19 IgG-positive sera of which a sufficient sample volume was available for analysis. All 20 sera gave AI values of <40% (median, 32%; range, 6.6 to 39.42%), thus confirming the acute nature of the parvovirus B19 infection in these patients. In 8 of the 12 patients with an isolated IgM reactivity profile in the acute-phase serum specimen, acute parvovirus B19 infection was confirmed by demonstration of seroconversion in convalescent-phase sera. No follow-up samples were available for the remaining four patients. Thirty-four of these sera had been tested for viral capsid antigen IgM antibodies (Captia VCA IgM; Trinity-Biotech, Bray, Ireland) as requested and found to be negative. Fifty-five sera were tested for HSV IgMs (Herpes simplex 1 + 2 IgM test; Vircell, Granada, Spain). Forty-four sera had a negative result, seven displayed a positive result, and four had an indeterminate result. The sera were tested in the EBV IgM ($n = 65$) and HSV IgM ($n = 55$) assays from DiaSorin on the Liaison platform. Interpretation of results was done according to the instructions of the manufacturer. Data are shown in Table 1. None of the sera tested positive in the EBV IgM assay. Five of the 44 HSV IgM-negative sera (in the Vircell assay) tested positive in the DiaSorin HSV IgM assay. These sera, however, had a negative result when PVP (0.1%) and PVA (0.005%) (both purchased form Sigma-Aldrich) were added to the dilution buffer. Of the seven sera testing positive in the HSV IgM assay from Vircell, five did so in the DiaSorin HSV IgM assay. A number of sera ($n = 12$) were also tested in the *B. burgdorferi* sensu lato IgM assay from DiaSorin and found to be negative.

In the study by Berth and Bosmans (1), 84% of the parvovirus B19 IgM-positive sera were found to be reactive in the EBV IgM assay, 90% in the HSV IgM assay, 20% in the CMV IgM assay, and 22% in the *B. burgdorferi* sensu lato IgM assay. Furthermore, a number of sera from patients with acute rubella virus infection also reacted nonspecifically in the DiaSorin EBV IgM assay. In our setting, contrarily, no false-positive reactivities were found in the EBV IgM and *B. burgdorferi* sensu lato IgM assays, and a low percentage of sera (10%) yielded an unspecific positive result in the HSV IgM assay. Our data are thus strikingly different from those previously reported (1). To our knowledge, neither the EBV IgM test nor the HSV IgM assay has been recently modified in light of the reported findings (1) (personal communication from an authorized source at DiaSorin), so that the explanation for the discrepancy between our data and those of Berth and Bosmans (1) is uncertain. Although speculative, it might be that the antigen density on coated beads varied between the batches of reagents employed in the two studies. In this sense, in our study, chemical blocking with PVP and PVA, both polymers known to compete with the nonspecific adsorption of proteins to the solid phase (2), was found to eliminate HSV IgM false reactivities, suggesting a suboptimal antigen coating. Nevertheless, the occurrence of false-positive HSV IgM results was not restricted to the DiaSorin assay, as 7 of the 55 sera assayed tested positive in the Vircell test. Interestingly, two of these seven sera tested negative in the DiaSorin HSV IgM test. In summary, our results support the specificity of the EBV and HSV IgM assays from DiaSorin performed on the Liaison platform with sera from patients displaying acute parvovirus B19 infection; nevertheless, further studies are warranted to resolve the discrepancy between our data and those reported in reference 1.

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