

Evaluation of the Architect Epstein-Barr Virus (EBV) Viral Capsid Antigen (VCA) IgG, VCA IgM, and EBV Nuclear Antigen 1 IgG Chemiluminescent Immunoassays for Detection of EBV Antibodies and Categorization of EBV Infection Status Using Immunofluorescence Assays as the Reference Method

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Commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV), viral capsid antigens (VCA), and IgGs toward EBV nuclear antigen-1 (EBNA-1) are routinely used in combination to categorize EBV infection status. In this study, we evaluated the performances of the Architect EBV VCA IgG, VCA IgM, and EBNA-1 IgG chemiluminescent microparticle assays (CMIA) in EBV serological analyses using indirect immunofluorescence assays and anticomplement immunofluorescence assays as the reference methods for VCA IgG, VCA IgM, and EBNA-1 IgG antibody detection, respectively. A total of 365 serum samples representing different EBV serological profiles were included in this study. The κ values (concordances between the results) obtained in the Architect CMIA and those in the reference assays were 0.905 ($P < 0.0001$) for VCA IgM, 0.889 ($P < 0.0001$) for VCA IgG, and 0.961 ($P < 0.0001$) for EBNA-1 IgG. The sensitivities and specificities were, respectively, 91.08% and 99.48% for VCA IgM, 99.23% and 86.27% for VCA IgG, and 96.77% and 99.16% for EBNA-1 IgG. The sensitivities and specificities of the Architect CMIA panel were, respectively, 99.15% and 98.6% for diagnosing a primary infection, 97.62% and 93.39% for diagnosing a past EBV infection, and 92.42% and 97.82% for diagnosing the absence of an EBV infection. In summary, we demonstrated that the Architect EBV antibody panel performs very well for EBV antibody detection and correctly categorizes clinically relevant EBV infection states.

Commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV) and viral capsid antigen (VCA) and IgG antibody toward EBV nuclear antigen-1 (EBNA-1) are routinely used in combination to diagnose primary EBV infection (i.e., acute infectious mononucleosis [IM]) and to categorize EBV infection status. The latter is particularly relevant in solid-organ transplant patients in order to assess the risk of posttransplantation lymphoproliferative disease (EBV-seronegative patients receiving an allograft from EBV-seropositive donors) (1). Abbott Diagnostics (Wiesbaden, Germany) recently launched the Architect EBV antibody panel, which includes three two-step chemiluminescent microparticle immunoassays (CMIA) for qualitatively detecting VCA IgG, VCA IgM, and EBNA-1 IgG antibodies on its automated random-access platform Architect *i2000SR*. In this study, we evaluated the performances of the Architect EBV VCA IgG, VCA IgM, and EBNA-1 IgG CMIA in EBV serological analyses using indirect immunofluorescence (IIF) assays and anticomplement immunofluorescence (ACIF) assays as the reference methods for VCA IgG, VCA IgM, and EBNA-1 antibody (Ab) detection, respectively (1).

MATERIALS AND METHODS

Serum specimens. A total of 365 serum samples representing different EBV serological profiles commonly encountered in clinical practice (as determined by IIF and ACIF) were included in this study. These specimens were selected from sera submitted to our laboratory between January 2010 and March 2012 for routine EBV-specific antibody testing. Most of the serum samples (82%) belonged to children or young adolescents (median age, 8 years; range, 1 to 14 years; 61% male and 39% female) with fever, rash, or clinical suspicion of IM. In our laboratory, we routinely test

these samples with the Liaison VCA IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays (CLIA) (DiaSorin, Saluggia, Italy) (2, 3). In addition, sera from patients with a high suspicion of EBV-related IM are screened for the presence of heterophilic antibodies (HAs) as described below (2, 3). The proportions of different EBV serological patterns included in this study do not represent the frequencies at which they are observed in routine laboratory EBV testing (1). The serum sample aliquots used were stored at -20°C immediately after separation. The specimens were retrieved for EBV antibody testing by IIF and by the Architect EBV antibody panel. The EBV antibody profiles of the sera (according to IIF/ACIF methods) included (i) VCA IgG-negative (IgG⁻)/VCA IgM⁻/EBNA-1 Ab⁻ ($n = 68$), (ii) VCA IgG⁻/VCA IgM⁺/EBNA-1 Ab⁻ ($n = 25$), (iii) VCA IgG⁺/VCA IgM⁺/EBNA-1 Ab⁻ ($n = 116$), (iv) VCA IgG⁺/VCA IgM⁻/EBNA-1 Ab⁺ ($n = 89$), (v) VCA IgG⁺/VCA IgM⁻/EBNA-1 Ab⁻ ($n = 31$), (vi) VCA IgG⁺/VCA IgM⁺/EBNA-1 Ab⁺ ($n = 25$), and (vii) VCA IgG⁻/VCA IgM⁻/EBNA-1 Ab⁺ ($n = 11$).

Architect Epstein-Barr virus chemiluminescent microparticle immunoassays. These CMIA are two-step chemiluminescent immunoassays that use peptide-coated microparticles (VCA p18 or EBNA-1 p72) and acridine-labeled anti-IgG or anti-IgM conjugates for qualitatively detecting VCA IgM, VCA IgG, and EBNA-1 IgG antibodies. Samples were processed on a fully automated random-access analyzer (the Architect

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i2000SR). The chemiluminescent signals were measured by a photomultiplier tube and expressed as relative light units (RLU); the Architect *i* system calculates each result by using the ratio of the sample RLU to the cutoff RLU (S/CO). The criteria for interpretation of individual parameters were as follows: for VCA IgM, <0.50 RLU was considered negative, 0.50 to 1 RLU equivocal, and ≥ 1 RLU positive; for VCA IgG, <0.75 RLU was considered negative, 0.75 to 1 RLU equivocal, and ≥ 1 RLU positive; and for EBNA-1 IgG, <0.50 RLU was considered negative, 0.50 to 1 RLU equivocal, and ≥ 1 RLU positive. Sera were batched and tested simultaneously over several consecutive days.

Immunofluorescence assays. IIF assays for VCA IgG and IgM were performed with the Merifluor EBV VCA IgG and VCA IgM IIF assays (Meridian Bioscience Inc.). The Merifluor EBV VCA IgM and EBV VCA IgG IIF assay methods are qualitative. EBV-infected lymphocytes from Burkitt lymphoma were incubated with patient serum. After being washed, cells complexed with bound anti-VCA antibodies were incubated with either anti-human IgM or anti-human IgG labeled with fluorescein. The sample is considered to be positive if approximately 10% to 20% of the cells in each field show apple-green fluorescence upon observation. ACIF was used to detect EBNA-1 antibodies (Merifluor EBV nuclear antigen test; Meridian Bioscience Inc.). Heat-inactivated patient serum was applied to the fixed antigens (EBV-infected lymphocytes from Burkitt lymphoma) on glass wells of a microscope slide. Following a washing step, guinea pig complement was added to react with any antigen-antibody complexes. After a 30-min incubation, the slides were washed. Fluorescein-conjugated goat antibody against the C3 component of guinea pig complement was added to react with the antigen-complement complexes. Positive reactions appeared as 20 to 30% of the cells exhibiting bright apple-green fluorescence against a background of counterstained EBNA-1 negative-control cells. These procedures were conducted and interpreted following the manufacturer's instructions. IIF assays were read by a single person (I.C.). The reader was blinded to the CLIA results.

Detection of heterophilic antibodies. HAs were detected by a differential agglutination assay (I.M. kit; Microgen, Surrey, Great Britain).

Interpretation of Epstein-Barr virus serostatus. The criteria used to define the EBV serostatus were based on consensus EBV-specific antibody profiles (1). The VCA IgG⁻/VCA IgM⁻/EBNA-1 IgG⁻ profile corresponded to an EBV-seronegative status. The VCA IgG⁺/VCA IgM⁺/EBNA-1 IgG⁻ and VCA IgG⁻/VCA IgM⁺/EBNA-1 IgG⁻/HA⁺ patterns were interpreted as compatible with a primary EBV infection. The VCA IgG⁺/VCA IgM⁻/EBNA-1 IgG⁺ profile represented a past EBV infection. The remaining EBV-specific antibody profiles were considered indeterminate and were analyzed separately.

Statistical analysis. Kappa (κ) statistics were used to evaluate the degree of consensus between the results of the Architect EBV assays and those of the immunofluorescence (IF) assays. These analyses were performed with the aid of the SPSS statistical package (version 20.0).

RESULTS

Single-parameter performance of the Architect immunoassays. Most of the sera ($n = 341$) yielded S/CO values either above or below the established cutoff for the respective assay. Twenty-four serum samples gave S/CO values within the gray zone (GZ) in one or two EBV antibody assays: 17 (4.66%) in the VCA IgM assay, 4 (1.10%) in the EBV IgG assay, and 4 (1.10%) in the EBNA-1 IgG assay. Sera giving GZ S/CO values were excluded from the analyses described below. The concordances between the results obtained in the Architect CMIA and those in the reference assay were good for all three parameters ($\kappa = 0.905$ and $P < 0.0001$ for VCA IgM; $\kappa = 0.889$ and $P < 0.0001$ for VCA IgG; $\kappa = 0.961$ and $P < 0.0001$ for EBNA-1 IgG). The single-parameter performances of the Architect CMIA compared with those of the reference assay are shown in Tables 1 and 2. The EBNA-1 IgG assay best matched the IF assay (ACIF assay), with only 6 discordant results, thus yielding

TABLE 1 Single-parameter performances of the Architect EBV chemiluminescent immunoassay in comparison with those of the reference immunofluorescence methods

Result ^a	Results for Architect EBV antibody panel (no. of serum samples with indicated result) ^b		
	VCA IgM	VCA IgG	EBNA-1 IgG
True positive	143	257	120
True negative	190	88	235
False positive	1	14	2
False negative	14	2	4

^a Sera tested by the Architect EBV panel were categorized as true or false positives/negatives on the basis of their agreement or disagreement, respectively, with immunofluorescence assay results.

^b Sera displaying values within the gray zone of the respective assay were excluded from the analyses.

excellent sensitivity and specificity values (Table 2). The VCA IgG assay gave the lowest specificity, whereas the VCA IgM assay gave the lowest sensitivity.

Performance of the Architect assays for diagnosing clinically relevant Epstein-Barr virus infection statuses. The ability of the Architect assays to correctly identify the absence of a previous EBV infection or evidence of either a primary or a past EBV infection was assessed. Sera that displayed an isolated VCA IgM⁺ profile were considered to correspond to a primary EBV infection only when they also tested positive for HA; the raw data are shown in Table 3. The concordances between the EBV antibody profiles obtained with the Architect panel and those with the reference assay were 97.4% for primary EBV infection, 97.5% for past EBV infection, and 92.4% for EBV seronegativity. The sensitivities and specificities of the Architect CMIA panel were, respectively, 99.15% and 98.6% for diagnosing a primary infection, 97.62% and 93.39% for diagnosing a past EBV infection, and 92.42% and 97.82% for diagnosing the absence of an EBV infection.

Performance of the Architect assays with indeterminate Epstein-Barr virus serological profiles. We also evaluated the performance of the Architect assays with sera displaying unresolved EBV serological profiles ($n = 73$; Table 3). The overall agreement between the Architect panel and the reference method for these sera was modest (65.7%). The following findings were of particular interest. (i) Two VCA IgM⁺/VCA IgG⁻/EBNA-1 IgG⁻/HA⁻ serum samples ($n = 8$) were categorized as primary EBV infections by the Architect panel. These sera belonged to two children (2 and 3 years old) with a high-level suspicion of EBV-related IM. One of the 8 serum samples tested negative for all three parameters in the Architect panel. This serum was obtained from an adult patient with persistent fever and an absence of clinical signs compatible with IM. (ii) Concordant results between the IF assays and the Architect CMIA were obtained for 23 of 25 serum samples with an isolated VCA IgG profile. The remaining two samples were interpreted by the Architect panel as compatible with a past infection. None of the 25 serum samples tested positive in the HA assay. (iii) Eleven of 21 serum samples displaying a VCA IgM⁺/VCA IgG⁺/EBNA-1 IgG⁺/HA⁻ profile were interpreted as corresponding to past EBV infections by the Architect panel. Most ($n = 8$) of these serum samples were obtained from children and young adults with a low-level suspicion of IM (persistent fever). (iv) The Architect panel interpreted one of three VCA IgM⁺/VCA IgG⁺/EBNA-1 IgG⁺/HA⁺ serum samples as corresponding to a primary

TABLE 2 Performances of three commercially available chemiluminescent immunoassays in detecting VCA IgG, VCA IgM, and EBNA-1 IgG antibodies compared with those of immunofluorescence assays as reference

Immunoassay	Sensitivity (%)			Specificity (%)		
	VCA IgM	VCA IgG	EBNA-1-IgG	VCA IgM	VCA IgG	EBNA-1-IgG
Architect ^a	91.0	99.2	96.7	99.4	86.2	99.1
DiaSorin						
Meridian ^b	92.2	86.9	90.6	98.0	94.4	32.2
In-house ^c	94.4	90.2	95.9	76.9	95.8	83.3
Siemens ^d	77.9	94.4	93.8	95.8	100	76.6

^a Sera tested by the Architect EBV panel were categorized as true or false positives/negatives on the basis of their agreement or disagreement with immunofluorescence assay results. Sera displaying values within the gray zone of the respective assay were excluded from the analyses.

^b The performances of the Liaison VCA IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays (CLIAs) (DiaSorin, Saluggia, Italy) were evaluated using the IF assays from Meridian (like in the current study) as a reference (4).

^c The performances of the Liaison VCA IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays were evaluated using in-house IF assays as a reference (5).

^d The Immulite 2000 CLIA (Siemens, Germany) was evaluated using the IF assays from Meridian (like in the current study) as a reference (4).

EBV infection. (v) Four of 11 isolated EBNA-1 IgG profile serum samples tested positive for both EBNA-1 IgG and VCA IgG in the Architect panel.

DISCUSSION

In this study, we evaluated the performance of the Architect EBV panel for EBV antibody detection using IF assays as a reference. IF assays have historically been considered the gold standard for EBV antibody testing, despite the fact that the reading of the assay results is subjective. Thus, some researchers advocate for the use of immunoblot assays as the reference method (1). Regarding the choice of IF assays as gold standard methods, one must bear in mind that while these methods are considered to be more specific than enzyme immunoassays (EIAs), their sensitivities have occasionally been reported to be lower than those of EIAs, especially for VCA IgM and VCA IgG antibody detection (1).

We performed a single-parameter analysis and assessed the ability of the Architect antibody panel to correctly categorize clinically relevant EBV infection staging (primary infection to diagnose EBV IM, past infection to exclude EBV IM and to ascertain previous viral exposure in transplant recipients or donors, and EBV seronegativity in both immunocompetent and transplant pa-

tients to show EBV susceptibility) (1). Finally, we evaluated the performance of the Architect panel with sera displaying unresolved EBV serological profiles. Interestingly, we obtained a remarkably low rate of indeterminate results (within the GZ of the assay) using the EBNA-1 IgG CMIA (1.10%) compared with those reported for other chemiluminescent assays (30% for the DiaSorin CLIA and 4% for the Immulite 2000 CLIA) (4). The frequencies with which sera gave S/COs within the GZ in the VCA IgG assay of 1.10% and in the VCA IgM assay of 4.66% were comparable to those of the above-mentioned CLIAs (4).

Single-parameter analyses revealed very good concordance between the Architect EBV assays and the reference methods, which translated into excellent sensitivities and specificities for all three assays. The performance of several commercially available CLIAs compared to an IF assay as the reference method was assessed in two previous studies (4, 5). The data reported in those studies are summarized in Table 2. In this context, the Architect VCA IgM and EBNA-1 IgG assays appeared to perform better than the CLIAs from DiaSorin and Siemens. The sensitivity of the Architect VCA IgG assay was superior to those reported for the other two CLIAs (described above), whereas its specificity was slightly lower (4, 5). These differences could be attributable to several factors, including the use of different antigen preparations, solid phases, IF assays, and serum dilutions.

In this study, the overall agreements between the expected EBV status, as determined by the IF assays, and that obtained with the Architect antibody panel were excellent (97.4% for primary EBV infection, 97.5% for past EBV infection, and 92.4% for EBV seronegativity). The most frequent EBV staging discrepancy between the Architect antibody panels and the IF assays was the occurrence of an isolated VCA IgG pattern in 5 patients with an expected EBV-seronegative profile. Although we assumed these to be Architect assay-specific false positives, we cannot rule out the possibility of them being true positives because follow-up specimens from these patients were not available. As stated above, it has been reported that EIAs might display better sensitivities than IF assay for VCA antibody detection (1). The above figures are in the upper range (74 to 95%) reported for several commercially available CLIAs or EIAs that were compared with immunofluorescence, Western blot, or line blot assays as reference assays (4–10).

Indeterminate, unresolved EBV antibody profiles occur rela-

TABLE 3 Performance of the Architect EBV chemiluminescent immunoassay in categorizing sera with several EBV serological profiles, as determined by immunofluorescence reference methods

IF antibody profile (VCA IgM/VCA IgG/EBNA-1 IgG) (no. of serum samples)	Results (no. of serum samples) for the indicated Architect panel EBV antibody profile (VCA IgM/VCA IgG/EBNA-1 IgG)						
	-/-/-	+/-/-	+/+/-	+/+/+	-/+/+	-/+/-	-/-/+
-/-/- (66)	61	0	0	0	0	5	0
+/-/- (8) ^a	1	5	2	0	0	0	0
+/-/- (13) ^b	3	8	2	0	0	0	0
+/+/- (110)	0	2	108	0	0	0	0
+/+/+ (21) ^b	0	0	0	10	11	0	0
+/+/+(3) ^a	0	0	1	2	0	0	0
-/+/+ (84)	0	0	0	1	82	1	0
-/+/- (25)	0	0	0	0	2	23	0
-/-/+ (11)	2	0	0	0	4	0	5

^a Sera tested positive for heterophilic antibodies (HAs).

^b Sera tested negative for HAs.

tively frequently in routine laboratory EBV serological testing (1, 8). The presence of an isolated VCA IgM profile in the absence of HAs most commonly reflects either the presence of a primary infection (particularly in children <5 years of age) or a false-positive result. In our series, 2 of 13 serum samples with this pattern were categorized as primary infections by the Architect panel. These sera belonged to two children with a high-level suspicion of EBV-related IM. Thus, although speculative, these may have been false-negative VCA IgG results that were obtained using the reference method (1).

The presence of an isolated VCA IgG profile occurs in 2 to 8% of immunocompetent patients tested for the EBV antibody (8, 11), and most of these cases are thought to correspond to past infections. We included 25 serum samples with this EBV antibody profile, none of which tested positive in the HA assay. Two of these 25 samples also tested positive for EBNA-1 IgGs in the Architect panel. These serum samples were drawn from two adult patients with low-level suspicion of IM, meaning that the results might have represented true positives for EBNA-1 IgGs (1).

VCA IgG, VCA IgM, and EBNA-1 IgG antibodies are detected concomitantly in 3 to 6.4% of samples subjected to routine EBV testing (8, 9, 12). In most cases, this profile reflects a past EBV infection, although it may also correspond to a relatively recent EBV-related primary infection, an EBV reactivation episode, a cross-reaction with CMV IgM in the setting of a primary CMV infection, or a state of polyclonal stimulation by a heterologous infectious agent (1, 13, 14). In this study, 11 of 21 samples with a VCA IgM⁺/VCA IgG⁺/EBNA-1 IgG⁺/HA⁻ profile were interpreted as corresponding to past EBV infections by the Architect panel. Most of these sera were obtained from children and young adults with a low-level suspicion of IM, so they might have been true-negative VCA IgM results. In addition, one out of three VCA IgM⁺/VCA IgG⁺/EBNA-1 IgG⁺/HA⁺ serum samples was interpreted as corresponding to a primary EBV infection by the Architect panel, which we assume was a true negative for EBNA-1 IgG⁺, given that this serum sample belonged to a patient with clear signs and symptoms of IM.

The presence of an isolated EBNA-1 IgG profile has been reported in 1.4 to 5.3% of sera tested with commercial EIAs (8, 15). Although classic texts consider this to be implausible, it has been shown in most cases to correspond to past infections (8, 15). In this study, 4 of 11 serum samples that gave an isolated EBNA-1 IgG profile with the IF assay were EBNA-1 IgG and VCA IgG positive in the Architect panel, again suggesting a plausible possibility of these being true positives for VCA IgG.

This study had several limitations. First, no follow-up specimens were available from the patients. Second, further testing (VCA IgG avidity or real-time PCR assays) was not performed on sera displaying unresolved EBV antibody profiles. Third, a definitive clinical diagnosis was not available for most of the patients. Fourth, sera giving discordant results in the index and reference assays were not reassayed using a second reference method (Western or line blot assays) to resolve the discrepancies. Fifth, potentially cross-reactive sera, such as those obtained during primary cytomegalovirus or human herpesvirus 6 infection or parvovirus B19 infection (16, 17), were not included in our study panel. Any of these factors may have caused some misclassifications (particularly with sera displaying unresolved EBV antibody profiles) and thus might have skewed our calculations of the sensitivities and specificities. In addition, evaluation of the diagnostic accuracy of

the Architect EBV panel under routine laboratory conditions, which was not performed in the current study, seems crucial for ascertaining the actual clinical value of the antibody panel. Studies addressing this issue are under way. Despite these limitations, the data presented here indicate that the Architect EBV antibody panel performs very well for EBV antibody detection and correctly categorizes clinically relevant EBV infection states.

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