Evaluation of the ARCHITECT EBV VCA IgG, VCA IgM, and EBNA-1 IgG Chemiluminescent Immunoassays for Detecting Epstein-Barr Virus (EBV) Antibodies and Categorizing of EBV Infection Status Using Immunofluorescence Assays as the Reference Method

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ABSTRACT

Commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV), viral capsid antigens (VCA), and IgGs towards EBV nuclear antigen-1 (EBNA-1) are routinely used in combination to categorize the EBV infection status. In this study we evaluated the performance 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 anti-complement immunofluorescence assays as the reference methods for VCA IgG, IgM, and EBNA antibodies detection respectively. A total of 365 sera representing different EBV-serological profiles were included in this study. The concordance between the results obtained in the ARCHITECT CMIA and those in the reference assay was κ, 0.905; \( P = < 0.0001 \) for VCA IgM; \( \kappa, 0.889; P = < 0.0001 \) for VCA IgG; \( \kappa, 0.961; P = < 0.0001, \) for EBNA-1 IgG. The sensitivities and specificities were as follows: 91.08% and 99.48%, respectively, for VCA IgM, 99.23% and 86.27% for VCA IgG, and 96.77% and 99.16%, for EBNA-1 IgG. The sensitivity and specificity of the ARCHITECT CMIA panel for diagnosing a primary infection were 99.15% and 98.6% respectively, for diagnosing a past EBV infection they were 97.62% and 93.39%, and 92.42% and 97.82% respectively 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.
INTRODUCTION

Commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV), viral capsid antigens (VCA), and IgG towards 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 the EBV infection status. The latter is particularly relevant in solid organ transplant patients in order to assess the risk of post-transplantation lymphoproliferative disease (EBV-seronegative patients receiving an allograft from EBV-seropositive donors) (1). Abbott Diagnostics (Wiesbaden, Germany) has recently launched the ARCHITECT EBV antibody panel which includes three two-step chemiluminescent microparticle immunoassays (CMIA) to qualitatively detect VCA IgG, IgM, and EBNA-1 IgG antibodies on their automated random access platform ARCHITECT i2000SR. In this study we evaluated the performance of the ARCHITECT EBV VCA IgG, VCA IgM, and EBNA-1 IgG CMIA in EBV serological analyses using indirect immunofluorescence assays (IIF) and anti-complement immunofluorescence (ACIF) assays as the reference methods for VCA IgG, IgM, and EBNA antibodies (Ab) detection respectively (1).

MATERIALS AND METHODS

Serum specimens. A total of 365 sera 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 sera (82%) belonged to children or young adolescents (median age, 8 years; Range 1-14 years; 61% males and 39% females) with fever, rash, or clinical suspicion of IM. In our laboratory we routinely test these sera with the LIAISON VCA
IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays (CLIAs; 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 (HA; 2,3) as described below. The proportions of different EBV serological patterns included in this study do not represent the frequency 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 IFA/ACIF methods) included were the following: (i) VCA IgM-/VCA IgG-/EBNA Ab- (n = 68); (ii) VCA IgG-/VCA IgM+/EBNA Ab- (n = 25); (iii) VCA IgG+/VCA IgM+/EBNA Ab- (n = 116); (iv) VCA IgG+/VCA IgM+/EBNA Ab+ (n = 89); (v) VCA IgG+/VCA IgM-/EBNA Ab- (n = 31); (vi) VCA IgG+/VCA IgM+/EBNA Ab+ (n = 25); (vii) VCA IgG-/VCA IgM-/EBNA Ab+ (n = 11).

ARCHITECT Epstein-Barr virus chemiluminescent microparticle immunoassays. These CMIAs are two-step chemiluminescent immunoassays that use peptide-coated micro-particles (VCA p18 or EBNA-1 p72) and acridine-labeled anti-IgG or anti-IgM conjugates to qualitatively detect VCA IgM, VCA IgG, and EBNA-1 IgG antibodies. Samples were processed on a fully-automated random-access analyzer, the ARCHITECT i2000SR. The chemiluminescent signals were measured by a photomultiplier tube and expressed as relative light units (RLUs); the ARCHITECT i system calculates the result by using the ratio of the sample RLU to the cut-off RLU (S/CO). The criteria for interpretation of individual parameters were as follows: VCA IgM (negative less than 0.50, equivocal 0.50 to 1, positive 1 or higher), VCA IgG (negative less than 0.75, equivocal 0.75 to 1, positive 1 or higher) and EBNA-1 IgG.
(negative less than 0.50, equivocal 0.50 to 1, positive 1 or higher). Sera were batched and tested simultaneously over several consecutive days.

**Immunofluorescence assays.** IIF for VCA IgG and IgM were performed with the Merifluor EBV VCA IgG and VCA IgM IFA assays (Meridian Bioscience Inc.). The Merifluor EBV VCA IgM and EBV VCA IgG IFA methods are qualitative indirect IF assays. EBV-infected lymphocytes from Burkitt lymphoma are incubated with patient serum. After washing, cells complexed with bound anti-VCA antibodies are incubated with either anti–human IgM or anti–human IgG labelled with fluorescein. The sample is considered to be positive if approximately 10% to 20% of the cells in each field show green-apple fluorescence upon observation. ACIF was used to detect EBNA 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 minute 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 appear as 20 to 30% of the cells exhibiting bright apple-green fluorescence against a background of counterstained EBNA negative control cells. These procedures were conducted and interpreted following the manufacturer’s instructions. IFAs were read by a single person (IC). The reader was blinded to CLIA results.

**Detection of heterophilic antibodies.** HA 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-IgG- profile corresponds to an EBV-seronegative status. VCA IgG+/VCA IgM-/EBNA IgG- and VCA IgG+/VCA IgM+/EBNA IgG-/HA+ patterns were both interpreted as compatible with a primary EBV infection. The VCA IgG+/VCA IgM-/EBNA IgG+ profile was deemed to represent 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 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 sera (n = 341) yielded S/CO values either above or below the established cut-off for the respective assay. Twenty-four sera 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 concordance between the results obtained in the ARCHITECT CMIA and those in the reference assay was good for all three parameters (κ, 0.905; P = < 0.0001 for VCA IgMs; κ, 0.889; P = < 0.0001 for VCA IgGs; κ, 0.961; P = < 0.0001, for EBNA-1 IgGs). The single-parameter performance of the ARCHITECT CMIA in comparison with the reference assay is shown in Tables 1 and 2. The EBNA-1 IgG assay best matched the IF assay (ACIF), with only 6 discordant results, thus yielding 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. Next, 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 concordance between the EBV antibody profiles obtained with the ARCHITECT panel and those with the reference assay were 97.4% for EBV primary infection, 97.5% for EBV past infection, and 92.4% for EBV ‘seronegativity’. The sensitivity and specificity of the ARCHITECT CMIA panel for diagnosing a primary infection were 99.15% and 98.6% respectively, for diagnosing a past EBV infection they were 97.62% and 93.39%, and 92.42% and 97.82% respectively 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%). Of interest, (i) two VCA IgM+/VCA IgG-/EBNA-1 IgG-/HA- sera (n = 8) were categorized as primary EBV infections by the ARCHITECT panel. These sera belonged to two children (two and three years old) with a high-level suspicion of EBV-related IM. One of the 8 sera samples tested negative for all three parameters in the ARCHITECT panel. This serum was obtained from an adult patient with persistent fever and absence of clinical signs compatible with IM; (ii) concordant results between IF and the ARCHITECT CMIAs were obtained for 23 of 25 sera with an isolated VCA
IgG profile. The remaining two sera were interpreted by the ARCHITECT panel as compatible with a past infection. None of the 25 sera tested positive in the HA assay; (iii) 11 of 21 sera displaying a VCA IgM+/VCA IgG+/EBNA-1 IgG+/HA- profile were interpreted as past EBV infections by the ARCHITECT panel. Most of these sera (n = 8) were obtained from children and young adults with a low-level suspicion (persistent fever) of IM; (iv) the ARCHITECT panel interpreted one of three VCA IgM+/VCA IgG+/EBNA-1 IgG+/HA+ sera as corresponding to a primary EBV infection; (v) 4 of 11 isolated EBNA IgG profile sera 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 been historically considered the gold standard for EBV antibody testing, despite the fact that the reading of IF results is subjective. Thus, some authors advocate the use of immunoblot assays as the reference method (1). Regarding the choice of IF assays as the gold standard methods, one must bear in mind that while these methods are considered to be more specific than EIAs, their sensitivity has occasionally been reported to be lower than that of EIAs, specially for VCA IgM and IgG antibody detection (1).

We performed a single-parameter analysis and also 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’, both in immunocompetent and transplant patients, 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%) in comparison with that reported for other chemiluminescent assays (30% for the DiaSorin CLIA and 4% for the Immulite 2000 CLIA; 4). The frequency of sera which gave S/COs within the GZ in the VCA IgG (1.10%) and 4.66%) in the VCA IgM assays was comparable to that 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 has been assessed in two previous studies (4,5). The data reported in these 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. With respect to the ARCHITECT VCA IgG assay, its sensitivity was superior to that 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, or serum dilutions.

In this study, the overall agreement between the expected EBV status, as determined by IF assays, and that obtained with the ARCHITECT antibody panel, was excellent (97.4% for EBV primary infection, 97.5% for EBV past infection, and 92.4% for the EBV ‘seronegativity’). The most frequent EBV staging discrepancy between the ARCHITECT antibody panels and 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 false positives, we cannot rule out that they...
As stated above, it has been reported that EIAs might display a better sensitivity than IF assays for VCA antibody detection (1). The above figures are in the upper range (74 to 95%) reported for several commercially available CLIA or EIAs that were compared with IFs, western blots, or line blot assays as reference assays (4-10).

Indeterminate, unresolved EBV antibody profiles occur relatively 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 younger than five years), or a false positive result. In our series, 2 of 13 sera 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 obtained using the reference method (1).

The presence of an isolated VCA IgG profile occurs in 2-8% 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 sera with this EBV antibody profile, none of which tested positive in the HA assay. Two of these 25 sera also tested positive for EBNA-1 IgGs in the ARCHITECT panel. These two sera were drawn from two adult patients with a low-level suspicion of IM, meaning that these 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, cross-reaction with CMV.
IgMs in the setting of a primary CMV infection, or to a state of polyclonal stimulation by a heterologous infectious agent (1,13,14). In this study, 11 of 21 sera with a VCA IgM+/VCA IgG+/EBNA-1 IgG+/HA- profile were interpreted as 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, and thus these might have been true negative VCA IgM results. In addition, one out of three VCA IgM+/VCA IgG+/EBNA-1 IgG+/HA+ sera 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 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 classical texts consider this to be implausible, in most cases it has been shown to correspond to past infections (8,15). In this study, 4 of 11 sera giving an isolated EBNA IgG profile with IF were EBNA-1 IgG and VCA IgG positive in the ARCHITECT panel, again suggesting a plausible possibility of these in fact being true positives for VCA IgG.

This study has several limitations. First, no follow up specimens were available from patients. Second, further testing (VCA IgG avidity or real-time PCR assays) was not performed with sera displaying unresolved EBV antibody profiles. Third, definitive clinical diagnosis was not available for most patients. Fourth, sera giving discordant results in the index and reference assays were not re-assayed using a second reference method (western or line blot assays) to resolve these discrepancies. Fifth, potentially cross-reactive sera such as those obtained during primary CMV or HHV-6 infection or Parvovirus B19 infection (16,18) 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...
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 to ascertain the actual clinical value of the antibody panel. Studies addressing this issue are underway. Despite these limitations, the data presented indicate that the ARCHITECT EBV antibody panel performs very well for EBV antibody detection and correctly categorizes clinically relevant EBV infection states.

ACKNOWLEDGMENTS

Abbott Diagnostics provided the reagent kits for serological analysis free of charge and David Navarro received honoraria from Abbott Diagnostics for attendance to two conferences. None of the other authors has a commercial or other association that might represent a conflict of interest.

REFERENCES


proteins (Immunoquick(®) filtration EBV M) for the diagnosis of heterophile antibody-


5. Gärtner BC, Gärtner BC, Hess RD, Bandt D, Kruse A, Rethwilm A, Roemer K,
virus enzyme immunoassays with an immunofluorescence assay as the reference

Evaluation of a multiplex flow immunoassay for detection of epstein-barr virus-specific

three ELISA techniques and an indirect immunofluorescence assay for the serological

for interpretation of Epstein-Barr virus serological patterns. J. Clin. Microbiol. 47:
3204–3210.

Performance of two commercially available automated immunoassays for the
934.


<table>
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<th>Result</th>
<th>ARCHITECT EBV antibody panel</th>
<th>Parameter (no. of sera)</th>
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<td>VCA IgG</td>
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<td>2</td>
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</table>

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.
TABLE 2. Performance of three commercially available chemiluminescent immunoassays, including the ARCHITECT EBV panel (evaluated in the current study), in detecting VCA IgG, and IgM, and EBNA-1 IgG antibodies in comparison with immunofluorescence assays as a reference.

<table>
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<th>Parameter</th>
<th>Immunoassay (Antibody type: VCA IgM/VCA IgG/EBNA-1-IgG)</th>
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<tr>
<td></td>
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<tr>
<td>Sensitivity</td>
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<td></td>
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\(^a\) 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 performance of the LIAISON VCA IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays (CLIA; DiaSorin, Saluggia, Italy) was evaluated using the IF assays from Meridian (like in the current study) as a reference (4).
The performance of the LIAISON VCA IgM, VCA IgG, and EBNA-1 IgG chemiluminescent assays was evaluated using “in house” IF assays as a reference (5).

The Immulite 2000 CLIA (Siemens, Germany) was evaluated using the IF assays from Meridian (like in the current study) as a reference (4).
TABLE 3. Performance of the ARCHITECT EBV chemiluminescent immunoassay in categorizing sera with several EBV serological profiles, as determined by immunofluorescence reference methods.

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<th>ARCHITECT panel EBV antibody profile (VCA IgM/VCA IgG/EBNA-1 IgG)</th>
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aSera testing positive for heterophilic antibodies (HA).
bSera testing negative for heterophilic antibodies (HA).