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Performance of Two Commercially Available Automated Immunoassays for the Determination of Epstein-Barr Virus Serological Status

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This study evaluated the performance of two automated Vidas (V) and Liaison (L) immunoassays for Epstein-Barr virus (EBV) serology. The detection of the viral capsid antigen (VCA) IgM, the VCA/early antigen (VCA/EA) IgG, and the Epstein-Barr nuclear antigen (EBNA) IgG was assessed on 526 sera collected for routine EBV testing in immunocompetent subjects. The determination of expected EBV status (186 EBV primary infections, 183 past EBV infections, and 157 EBV-seronegative individuals) was based on results of routine laboratory enzyme immunoassays (EIAs) together with clinical data. The sensitivity and specificity of each individual marker were determined in comparison to the expected EBV status. The agreement between the V and L profiles and the expected EBV status was established through the interpretation of combinations of the different EBV markers. Statistically significant differences between the two tests were found for the specificity of the VCA IgM marker (96.2% for V versus 93.2% for L), the sensitivity of the VCA/EA IgG marker (89% for V versus 94% for L), and the specificity of the EBNA IgG marker (96.5% for V versus 74.2% for L). The results determined for the two assays with respect to overall agreement with the established expected EBV status were not significantly different (89.7% for V versus 88.2% for L), with discrepancies mainly observed in sera referenced as primary infections. These findings demonstrated the similar performances of the Vidas and the Liaison assays for the establishment of an EBV serological status using the VCA, EA, and EBNA markers.

Primary infection by Epstein-Barr virus (EBV) is the main cause of infectious mononucleosis (IM). The subsequent lifelong persistence of EBV in resting B lymphocytes, although mostly asymptomatic, is associated with several human malignancies such as endemic Burkitt lymphoma, posttransplantation lymphoproliferative disease, and undifferentiated nasopharyngeal carcinoma (NPC) (34, 35). More than 95% of adults are infected with EBV worldwide, and EBV serology is helpful for the proper clinical management of IM and some EBV-associated cancers (18, 29). The diagnosis of IM usually relies on characteristic clinical manifestations associated with the detection of heterophile antibodies. However, EBV serology is often necessary due to the lack of sensitivity of heterophile antibody detection in children and young adults and because IM-like symptoms may be related to other infectious agents such as HIV primary infection, cytomegalovirus (CMV) primary infection, or toxoplasmosis (20, 22, 26). EBV serology is also needed to determine the pretransplantation EBV serological status of the donor and the recipient in order to assess the risk of EBV-related posttransplantation lymphoproliferative disease (PTLD) (21, 36). In these two situations, antibodies against viral capsid antigen (VCA) IgG and VCA IgM and EBV nuclear antigen 1 (EBNA IgG) are the EBV serological markers most frequently sought for detection, since they allow determination of serological profiles characterizing a primary infection, a past infection, or the absence of EBV infection. Additionally, detection of IgA against VCA and EBNA and against the early antigens (EA) may have some utility for the screening of NPC (30). Other EBV antigens are less frequently used for EBV serology testing (5, 20). The indirect immunofluorescence assays (IFA) for anti-VCA IgG and -IgM detection, together with the anticomplement immunofluorescence assay (ACIF) for anti-EBNA IgG detection or certain recent immunoblot assays, are considered the gold standard for EBV serology (1, 20, 33). Nevertheless, because performing and interpreting IFA and immunoblot assays is labor-intensive and sometimes subjective, many laboratories use commercially available enzyme immunoassays (EIAs) or multiplex flow immunoassays based on native purified or recombinant proteins, fusion proteins, or synthetic peptides (6, 13, 17, 27). These assays show good agreement with gold standard assays and are easier to perform. Some of these assays are fully automated, making it even easier to perform EBV serology. The objective of this study was to assess the performance of the Vidas VCA IgM, VCA/EA IgG, and EBNA IgG assays (bioMérieux, Marcy l'Étoile, France) compared to that of another commercial automated immunoassay, the Liaison VCA IgM, VCA IgG, EA IgG, and EBNA IgG assay (DiaSorin, Saluggia, Italy) for the determination of EBV serological status of patients in the routine clinical setting.

MATERIALS AND METHODS

Patient samples and determination of EBV status. This study was conducted retrospectively on samples routinely collected between 2003 and 2009 for EBV serology assessment, mostly for clinical suspicion of IM, in a reference laboratory for EBV serology testing, the Grenoble University Hospital (France) (25).

Patients (excluding HIV or transplantion patients) ranged in age from 1 to 91 years (mean, 25; median, 19). Some of the subjects were sampled

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more than once (two to five times). For 536 samples, the EBV status (i.e., primary EBV infection, past EBV infection, absence of EBV infection, or indeterminate status) was determined on the basis of the results of EBV serology routinely performed at the university laboratory and used as the reference assay: all sera were tested for EBV IgM and EBV IgG with Enzygnost anti-EBV/IgM II and Enzygnost anti-EBV/IgG reagents (Siemens Healthcare Diagnostics, Marburg, Germany) and for EBNA antibodies with BMD EBV EBNA IgG reagent (BioMedical Diagnostics, Marne la Vallée, France). Analyses were performed according to the manufacturers' recommendations. Equivocal results obtained with these EIAs were further assessed using IFA as previously described in order to detect IgM and IgG antibodies against VCA and using ACIF to detect IgG antibodies against EBNA (7). Samples negative for EBV IgM, EBV IgG, and EBNA IgG were classified as EBV seronegative ($n = 157$; median and mean ages, 12 and 5 years, respectively). Samples positive for EBV IgM and IgG and negative for EBNA IgG were classified as representing primary infection ($n = 133$). In this group, the heterophile antibody detection (Monospot latex; Meridian Bioscience, Nice, France) was positive for 68% of the cases and the mean age was 20 years (median, 19) for patients with positive heterophile antibody and 14 years (median, 7) for patients with a negative test result. Samples with concomitant detection of EBV IgG and EBNA IgG and absence of EBV IgM were classified as representing past infection ($n = 164$; median and mean ages, 43 and 42 years, respectively). The serological follow-up and/or IF testing of some of the patients allowed us to further classify 53 additional samples as representing early primary infections (positive EBV IgM, negative EBV IgG, and negative EBNA IgG with the Enzygnost and BMD assays; 47% of them were positive for heterophile antibody detection) and 19 other samples as representing past infection with a positive result only for IgG against EBV (isolated IgG EBV). Finally, 10 samples could not be classified with EIA, IFA, and clinical data and were not used for the assessment of the performance of the Vidas and Liaison assays.

Assessment of the performance of the Vidas and Liaison assays. The Vidas system combines a two-step enzyme immunoassay for the detection of EBV VCA/EA IgG and EBV EBNA IgG antibodies and an immunocapture method for the detection of EBV VCA-IgM antibodies, with a final fluorescence detection (ELFA). The Vidas EBV VCA/EA IgG and Vidas EBV EBNA IgG assays use a solid-phase receptacle coated with synthetic peptides (for VCA, p18; for EA, p54; and for EBNA, p72). The conjugate is an alkaline phosphatase-labeled mouse monoclonal anti-human IgG antibody (Fab′ fragment). The Vidas EBV VCA IgM assay uses a solid-phase receptacle coated with a mouse monoclonal anti-human IgM antibody. The anti-EBV IgM is specifically detected by the VCA p18 peptide conjugated with alkaline phosphatase. For the three tests, the substrate used is 4-methylumbelliferyl phosphate. Results are automatically calculated and expressed as an index equal to the ratio between the tested serum and a standard positive serum.

The Liaison system is a two-step immunoassay for the quantification of VCA IgM, VCA IgG, EA IgG, and EBNA IgG antibodies by the use of magnetic particles and a final chemiluminescence detection. For Liaison VCA IgG, Liaison EA IgG, and Liaison EBNA IgG assays, specific EBV antibodies are captured by VCAs (mainly p18 synthetic peptide), EA(D) 47-kDa recombinant polypeptides (produced in *Escherichia coli*), or EBNA-1 synthetic peptides coated on the magnetic particles. The specific EBV antibodies are then detected by a mouse monoclonal anti-human IgG antibody linked to an isoluminol derivative. For Liaison VCA IgM, the anti-VCA IgM antibodies in the sample are captured by VCAs (mainly p18 synthetic peptide) coated on the magnetic particles and then detected by a mouse monoclonal anti-human IgM antibody conjugated to an isoluminol derivative. During the final detection step, starter reagents are added and a flash chemiluminescence reaction is thus induced. Antibody titers are automatically calculated and expressed as units per milliliter.

Both assays were performed in accordance with the manufacturers' recommendations without the knowledge of the results of the reference status determined with the reference tests. The Vidas analysis was performed at the university laboratory of Grenoble, and the Liaison analysis was performed at the Biomnis Laboratory (Lyon, France). For the Vidas VCA IgM and Liaison VCA IgM assays, the sensitivity was assessed with sera classified by the reference assay as primary infections and early primary infections and the specificity with sera classified as seronegative and past infections. For the Vidas VCA/EA IgG and the Liaison VCA IgG + EA IgG assays, sensitivity was assessed with sera classified as primary infections (excluding early primary infection) and as past infections. The specificity was assessed with seronegative sera. For the Vidas EBNA IgG and Liaison EBNA IgG assays, the sensitivity was assessed with sera classified as past infections (excluding isolated VCA IgG profiles) and the specificity with sera classified as primary infections and with seronegative sera.

The agreement between Vidas and Liaison profiles and the expected EBV status was established in accordance with the criteria of the respective manufacturers, through the combined interpretation of the three Vidas EBV markers or the four Liaison EBV markers. Of note, to establish these profiles, the Liaison assay used different threshold values for the VCA IgM test (20 and 40 U/ml) and for the EBNA IgG test (5 and 20 U/ml).

Statistical analysis. The sensitivity and specificity of each assay were determined in comparison to those of the EBV reference assays. Equivocal results obtained with one of the assays were considered negative for sensitivity calculations and positive for specificity calculations. Equivocal results obtained with both assays (Vidas and Liaison) for a given sample were not taken into account for the performance determination.

Confidence intervals for the differences between specificity or sensitivity values of the Vidas and Liaison assays were calculated using Newcombe's method to compare the two assays for each parameter in each population (8).

Ethical considerations. The study was conducted using samples left over from clinically motivated EBV testing of patients who had given consent with respect to their potential use for research and development.

RESULTS

Sensitivity and specificity of the Vidas and Liaison assays. The sensitivities of both the Vidas and Liaison assays for the detection of VCA IgM were calculated with sera classified as early primary infections and primary infections and were not statistically different: 86.7% and 89%, respectively (Table 1). With the Vidas VCA IgM assay, 24 samples were classified as falsely negative (21 negative and 3 equivocal). With the Liaison IgM VCA assay, 20 samples were considered falsely negative (7 negative and 13 equivocal). The sensitivity values of the Vidas and Liaison VCA assays were also not statistically significantly different among samples collected during the early phase of primary infections (74.5% and 76.5%, respectively) or among the samples collected later, during primary infection (91.5% and 93.9%, respectively). The specificity of the Vidas VCA IgM test, calculated with sera referenced as past infection or EBV seronegative, was significantly higher than that of the Liaison VCA IgM test: 96.2% versus 93.2% (Table 1). With the Vidas VCA IgM assay, 13 samples were considered falsely positive (8 positive and 5 equivocal). With the Liaison IgM VCA assay, 23 samples were falsely positive (8 positive and 15 equivocal). Thus, the difference in specificity between the two assays was due to a larger number of equivocal VCA IgM results with the Liaison assay.

Table 2 shows the sensitivity and the specificity of the Vidas VCA/EA IgG and Liaison VCA IgG + EA IgG assays. The sensitivities of the two assays were not significantly different with sera classified as past EBV infection: 96.2% and 94.5%, respectively. The sensitivity of the Vidas assay was significantly lower than that of the Liaison assay with sera classified as primary infection: 89% and 94%, respectively. Of the 53 cases of early primary infection (positive only for EBV IgM with the reference assay), 25 were VCA/EA IgG positive with the Vidas assay and 22 were VCA or EA IgG positive with the Liaison assay. The specificity values of the

TABLE 1 Sensitivities and specificities of the Vidas and Liaison VCA IgM assays[a]

Expected EBV status in referenced assay	Vidas VCA IgM			Liaison VCA IgM			Liaison minus Vidas (%) (95% CI)
	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	
Early primary infection ($n = 53$)	74.5 (62.8–86.2)	13	0	76.5 (65.1–87.9)	6	6	2.0 (−10.29 to 14.19)
Primary infection ($n = 133$)	91.5 (86.8–96.2)	8	3	93.9 (89.8–98.0)	1	7	2.4 (−3.24 to 8.13)
Total ($n = 186$)	86.7 (81.8–91.6)	21	3	89.0 (84.5–93.5)	7	13	2.3 (−2.88 to 7.41)
Past infection ($n = 183$)	95.0 (91.8–98.2)	5	4	92.9 (89.2–96.6)	5	8	−2.1 (−6.57 to 1.90)
EBV seronegative ($n = 157$)	97.5 (95.1–99.9)	3	1	93.6 (89.8–97.4)	3	7	−3.9 (−8.70 to 0.46)
Total ($n = 340$)	96.2 (94.2–98.2)	8	5	93.2 (90.5–95.9)	8	15	−3.0 (−5.99 to −0.16)*

[a] False neg or pos, false negative (with early primary and primary infection samples) or false positive (with past infection or EBV-seronegative samples); no., number of samples; Eq, equivocal (considered false positive for specificity and false negative for sensitivity); CI, confidence interval; *, statistically significant.
[b] Percentages for samples from patients with past infections and EBV-seronegative samples are percent specificities. Percentages for the other samples are percent sensitivities.

Vidas VCA/EA IgG assay and the Liaison VCA IgG + EA IgG assay calculated with EBV-seronegative samples were not statistically different: 99.4% and 100%, respectively.

Table 3 shows the performance of the Vidas and Liaison assays for the detection of EBNA IgG. With sera classified as past infections (excluding isolated VCA IgG), the sensitivity of the Vidas EBNA IgG assay was significantly higher than that of the Liaison EBNA IgG assay: 95.7% and 92%, respectively. The specificity of the Vidas EBNA IgG assay was significantly higher than that of the Liaison EBNA IgG assay with sera referenced as primary infections and EBV seronegative: 96.5% versus 74.2%, respectively. As shown in Table 3, this difference was due to a large number of false-positive or equivocal EBNA IgG results of the Liaison EBNA IgG assay with sera referenced as primary infections and not with sera referenced as seronegative. Of the 19 cases of isolated VCA IgG referenced as past infection with the reference EIAs, three were EBNA IgG positive with the Vidas assay and five were EBNA IgG positive with the Liaison assay.

Agreement between the Vidas and Liaison EBV profiles and reference EBV status. Agreement between the expected EBV status obtained with the reference assays and the EBV profiles determined with the Vidas or Liaison assays is shown in Table 4. Overall, this agreement was 89.7% with the Vidas assay and 88.2% with the Liaison assay. For both assays, this agreement was better with EBV-seronegative sera and sera referenced as past infections compared to specimens classified as primary infections.

The details of the differences between the expected EBV status with the EIA reference assays and the profiles obtained with the Liaison and Vidas assays are shown in Tables 5 and 6. With the Liaison assay applied to sera referenced as primary infection, the most frequently reported discrepant profile was "transition phase" (i.e., VCA IgG, VCA IgM, and EBNA IgG positive) (22/186; 11.8%) followed by the profile "EBV seronegative" (5/186; 2.7%) (Table 5). In sera referenced as past infection, the two most frequently reported discrepant profiles were "isolated IgG VCA" (8/164; 4.9%) and "indeterminate" (8/164; 4.9%), always due to the detection of isolated EBNA IgG. In the 157 sera referenced as EBV seronegative, the Liaison assay reported 10 cases (6.4%) of "primary infection."

With the Vidas assay (Table 6), 15 of the 186 samples (8.1%) referenced as primary infections were reported as indeterminate and 9 (4.8%) were reported as EBV seronegative. Among the 164 sera referenced as past infections, eight (4.8%) were reported as "indeterminate" profiles and five (3.1%) were reported as "isolated VCA/EA IgG." In the 157 sera referenced as EBV seronegative, the Vidas assay reported four cases of primary infection (2.5%) and three "indeterminate" profiles (1.9%).

TABLE 2 Sensitivities and specificities of the Vidas VCA/EA IgG and Liaison VCA IgG + EA IgG assays[a]

Expected EBV status in referenced assay	Vidas VCA/EA IgG			Liaison VCA IgG + EA IgG			Liaison minus Vidas (%) (95% CI)
	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	
Past infection ($n = 183$)	96.2 (93.4–99.0)	5	2	94.5 (91.2–97.8)	2	8[c]	−1.7 (−5.33 to 1.70)
Primary infection ($n = 133$)	89.0 (83.7–94.3)	5	11	94.0 (90.0–98.0)	7	1[c]	5.0 (0.30 to 12.27)*
Total ($n = 316$)	92.7 (89.8-95.6)	10	13	94.3 (91.7–96.9)	9	9	1.6 (−2.66 to 3.76)
EBV seronegative ($n = 157$)	99.4 (98.2–100.0)	0	1	100 (100.0–100.0)	0	0	0.6 (−1.81 to 3.52)

[a] False neg or pos, false negative (with past infection and primary infection samples) or false positive (with EBV-seronegative samples); no., number of samples; Eq, equivocal (considered false positive for specificity and false negative for sensitivity); CI, confidence interval; *, statistically significant.
[b] Percentages for samples from patients with EBV-seronegative samples are percent specificities. Percentages for the other samples are percent sensitivities.
[c] Equivocal only in the EA IgG test.

TABLE 3 Sensitivities and specificities of the Vidas and Liaison EBNA IgG assays[a]

Expected EBV status in referenced assay	Vidas EBNA IgG			Liaison EBNA IgG			Liaison minus Vidas (%) (95% CI)
	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	Sensitivity or specificity (%)[b] (95% CI)	False neg or pos (no.)	Eq (no.)	
Past infection[c] ($n = 164$)	95.7 (92.6–98.8)	5	2	92.0 (87.8–96.2)	8	5	−3.7 (−7.76 to −0.44)*
Primary infection ($n = 186$)	95.6 (92.6–98.6)	5	3	56.8 (49.7–63.9)	24	55	−38.8 (−46.12 to −31.15)*
EBV seronegative ($n = 157$)	97.5 (95.1–99.9)	2	2	94.3 (90.7–97.9)	0	9	−3.2 (−8.29 to 1.57)
Total ($n = 343$)[d]	96.5 (94.5–98.5)	7	5	74.2 (69.5–78.5)	24	64	−22.3 (−27.48 to −17.31)*

[a] False neg or pos, false negative (with past infection samples) or false positive (with primary infection and EBV-seronegative samples); no., number of samples; Eq, equivocal (considered false positive for specificity and false negative for sensitivity); CI, confidence interval; *, statistically significant.
[b] Percentages for samples from patients with primary infection and EBV-seronegative samples are percent specificities. Percentages for the other samples are percent sensitivities.
[c] For past infection data, samples with isolated VCA IgG antibodies were not included.
[d] Totals for Primary infection and EBV seronegative results.

DISCUSSION

Although IFA and/or immunoblot assays are considered the gold standard for EBV serology, various more versatile new automated assays for EBV serology have recently been launched on the market and need to be analyzed by independent evaluators. To our knowledge, this was the first study to assess the performance of the Vidas system in comparison with the Liaison assay for the determination of EBV serology on a large number of EBV primary infection sera.

In this study, the overall proportions of agreement with the expected EBV status of the EBV profiles (i.e., primary infection, past infection, or absence of infection) determined either with the three Vidas markers or with the four Liaison markers were similar and close to 89%. In the literature, the agreement or concordance of the EBV profiles obtained with chemiluminescent immunoassays or enzyme-linked immunoassays in comparison with gold standard EBV assays (mostly IFA) ranges from 74% to 95% (4, 9, 15, 24, 31).

Despite the substantial agreement of both tests with the expected EBV status, this study showed differences in the performance of the individual EBV markers that affected the antibody profile results. In comparison with the expected EBV status, the majority of discrepant EBV profiles obtained with the Vidas and the Liaison assays concerned sera collected during EBV primary infection. In this situation, the most frequently reported discrepant profiles with the Liaison and Vidas assays were "transition phase" and "indeterminate," respectively, corresponding to the concomitant positivity of VCA IgM, VCA/EA IgG, and EBNA IgG. Overall, in this study, the concomitant presence of these three markers was determined at 3.0% of all the sera for the Vidas assay and 4.5% of all the sera for the Liaison assay. This particular profile was associated with a primary infection in 69% of the cases for the Vidas assay and in 91% of the cases for the Liaison assay. Nystad and Myrmel reported the presence of the three markers in 6.4% of 668 patients with suspected IM and demonstrated that 42% of these cases corresponded to the end of a primary infection and 49% to a past infection whereas the last 9% of cases could not be clearly classified (28). More recently, with a multiplex flow immunoassay, Klutts et al. demonstrated that 4.6% of 1,757 samples subjected to routine EBV tests harbored these three markers and that those results, upon retrospective clinical review, corresponded mostly to past infections (24). This study found a similar frequency of the simultaneous detection of the three markers, but this situation was mainly associated with a primary infection. This could have been the consequence of the larger proportion of primary infections included in the present study, but this result could also stem from differences in the performance of the EBV markers. This study revealed the more frequent detection of EBNA IgG with the Liaison assay in sera collected during primary infection compared with the Vidas EBNA IgG assay. This concern about the specificity of the Liaison EBNA IgG assay has already been reported in a study conducted by de Ory et al. (10). In the clinical setting, the definitive interpretation of the presence of the three markers is difficult and obviously depends on clinical data (e.g., age, stage of the illness) but often requires further EBV testing (i.e., heterophile antibody detection or gold standard IFA or immunoblot assay, avidity testing) or a serological follow-up in order to clearly differentiate primary infection from past infection.

The second most common discrepant profile reported with both assays, performed with sera classified as primary infection, was "EBV seronegative." Nine of 186 (4.8%) samples referenced as primary infections were reported as seronegative with the Vidas assay, as were 5 of 186 (2.6%) with the Liaison assay. This was, in part, due to the fact that nearly one-third of the primary infections

TABLE 4 Agreement between the Vidas and Liaison EBV serological profiles and the reference EBV status

Expected EBV status in reference assay	Vidas EBV status agreement (%)	Liaison EBV status agreement (%)	Liaison minus Vidas (%) (95% CI)[a]
Primary infection ($n = 186$)	82.8	83.9	1.1 (−8.05 to 5.89)
Past infection ($n = 164$)	92	87.8	−4.2 (−0.87 to 9.71)
EBV seronegative ($n = 157$)	95.5	93.6	−1.9 (−3.06 to 7.08)
Total ($n = 507$)	89.7	88.2	−1.5 (−4.93 to 1.75)

[a] CI, confidence interval.

TABLE 5 Characteristics of discrepant profiles with the Liaison assay

Expected EBV status in reference assay	Liaison EBV status					
	EBV seronegative	Primary infection	Past infection	Isolated VCA IgG	Transition phase	Indeterminate
Primary infection ($n = 186$)	5		2	1	22	0
Past infection ($n = 164$)	1	1		8	2	8[a]
EBV seronegative ($n = 157$)		10	0	0	0	0

[a] Data represent 8 sera with isolated EBNA IgG.

were sampled at the very beginning of the disease, with IgM detectable only with the reference EIA and defined as early primary infection. In about 25% of these cases, both the Vidas and Liaison assays detected neither VCA IgM nor VCA IgG. Of note, some of these cases were retested during serological follow-up and most became positive for VCA IgM and VCA IgG with both tests (data not shown). Both the Vidas and Liaison VCA IgG and VCA IgM tests use the VCA p18 antigen. Other EIA methods use recombinant gp125 or P23 antigens for VCA antibody detection, which may increase sensitivity during early primary infection (3, 4, 24). Thus, in the case of a strong clinical suspicion of IM at the beginning of the disease, these results suggest that a serological follow-up can be helpful when the first serological result is entirely negative for EBV markers.

With sera reported as representing past infection with the reference assays, most of the discrepant EBV profiles with the Liaison assay were reported as "isolated VCA IgG" or "indeterminate" with isolated EBNA IgG. In one case of past infection, the Liaison interpretation was "EBV seronegative." With the Vidas assay, no past infection was misclassified as EBV seronegative and the discrepant EBV profiles were reported as "isolated VCA IgG" or "indeterminate." These indeterminate profiles corresponded to either the presence of isolated EBNA IgG or the presence of the three VCA IgG, VCA IgM, and EBNA markers. Overall, in this study, isolated VCA IgG cases were detected in 1.7% and 2.5% of cases with Liaison and Vidas, respectively, while isolated EBNA IgG cases were reported in 1.5% and 0.95% of cases with the Liaison and Vidas assays, respectively.

De Paschale et al. reported that 8% of 2,442 samples from immunocompetent patients, routinely screened for EBV serology using a Diasorin EIA, displayed an isolated VCA IgG profile and demonstrated that 81% of these cases corresponded to past infection and 19% to a primary infection (11, 12). In the study conducted by Klutts et al., only 2.4% of the samples harbored an isolated VCA IgG profile and 66% of them corresponded to a past infection (24). In the present study, an isolated VCA IgG profile corresponded to past infection in 89% and 38.4% of the cases with the Liaison and the Vidas assays, respectively. Concerning the detection of isolated EBNA IgG, García et al. reported this profile in 5.3% of 1,612 sera tested with a commercial EIA; half of these cases were associated with a past infection whereas the other half of the cases were not clearly classified (14). Klutts et al. found this profile in 1.4% of the sera; half of them corresponded to a past infection, and the other half could not be classified (24).

The misclassification of samples reported as EBV seronegative with the reference assays was the least frequent situation with both tests. With the Liaison assay, this situation was always due to the presence of isolated VCA IgM antibodies detected as positive or equivocal, leading to a diagnosis of primary infection in 6.4% of the EBV-seronegative sera. The specificity of the VCA IgM of that assay has already been extensively studied by Berth and Bosmans, who demonstrated that the presence of an isolated VCA IgM profile could not be reliably associated with IM (2, 3). The frequency of misclassification with the Vidas assay was not statistically different (4.5%), and the reported profiles were either "primary infection" or "indeterminate."

This study has several limitations. First, we did not use the gold standard IFA as the reference test to establish the EBV profiles. Our reference test was a polyantigenic EIA for detection of IgG and IgM antibodies against a mixture of EBV antigens completed with an EIA for specific EBNA IgG detection. The polyantigenic EIA has demonstrated good correlation with IFA (19, 23, 32), and indeterminate profiles with this EIA were reassessed with IFA in our study. Nevertheless, since a definitive clinical diagnosis could not be obtained each time or be retrospectively related to the serology results, we could not exclude some misclassifications with the reference test. Another limitation of the study was that cross-reactive sera collected during acute infections, such as CMV primary infection and hepatitis A virus, hepatitis E virus, and acute parvovirus B19 infections, or sera collected during the course of autoimmune diseases were not tested. These clinical situations could have an impact on the specificity of the VCA IgM assay (3, 16). In addition, some of the sensitivity and specificity values presented in this study could appear to be relatively low compared to those determined in other studies, because we deliberately classified equivocal results as false-negative or false-positive results in the statistical analysis. We also included a significant number of early primary infections, which could not be the routine popula-

TABLE 6 Characteristics of discrepant profiles with the Vidas assay

Expected EBV status in reference assay	Vidas EBV status				
	EBV seronegative	Primary infection	Past infection	Isolated VCA/EA IgG	Indeterminate
Primary infection ($n = 186$)	9		0	8	15[a]
Past infection ($n = 164$)	0	0		5	8[b]
EBV seronegative ($n = 157$)		4	0	0	3[c]

[a] Data represent 4 sera with isolated equivocal VCA/EA IgG and 11 positives for all three markers.
[b] Data represent 5 positives for all three markers and 3 sera with isolated EBNA IgG.
[c] Data represent 1 serum sample with isolated equivocal VCA/EA IgG and 2 with isolated EBNA IgG.

tion for EBV testing. Finally, we did not perform a real cost-effectiveness analysis for the two assays; we can report only that, in our hands, the turnaround times of the two assays were similar (the EBV status was obtained after 35 and 40 min for the Liaison and the Vidas assays, respectively) and that the Liaison assay had a higher throughput.

In conclusion, this study demonstrated the similar performances of the Vidas and the Liaison assays for the convenient and clear differentiation of a primary EBV infection from a past infection or the absence of infection. This study also underlines that, since differences in the sensitivities and specificities of each single EBV marker in a given commercial assay may be observed, the concomitant use of at least three EBV markers is necessary to establish an accurate EBV profile. These performance differences within a commercial assay have to be known for a proper interpretation of the serology and could suggest the use of more than one automated assay to resolve certain difficult EBV profiles.

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