Epstein-Barr virus (EBV) is the primary agent of infectious mononucleosis (IM), a common syndrome characterized by fever, pharyngitis, and lymphadenopathy. Most individuals become infected during childhood, and it is estimated that nearly 95% of the adult population worldwide is seropositive for the virus (20). While the majority of infections result in either asymptomatic or mild disease, serious complications, including B- and T-cell lymphomas, nasopharyngeal carcinoma, and central nervous system involvement, may occur, especially in immunocompromised hosts (14).

The diagnosis of IM is made, in most cases, on the basis of characteristic clinical manifestations or the detection of heterophile antibodies (24). However, a determination of the EBV-specific antibody response may be required for young children (especially those <4 years old) (26) and for adults suspected of having heterophile-negative IM. Testing for immunoglobulin M (IgM) and IgG class antibodies to the viral capsid antigen (VCA) and for IgG class antibodies to Epstein-Barr virus nuclear antigen-1 (EBNA-1) allows for a discrimination between recent and remote infection (8, 18, 21, 22). Levels of antibodies (IgM and/or IgG) to VCA are typically elevated during the acute phase of IM (19, 22, 26), with anti-VCA IgM levels showing a steady decline 4 to 6 weeks after symptom onset (16). In contrast, anti-VCA IgG persists indefinitely, and its detection along with that of anti-EBNA-1 IgG suggests past exposure to the virus (25). The conventional methods used to detect EBV-specific antibodies include an indirect immunofluorescence assay (IFA) and an enzyme immunoassay (EIA). While sensitive and specific, these methods are labor-intensive and require separate assays for each analyte. This study evaluated the performance of a multiplex bead assay (BioPlex 2200; Bio-Rad Laboratories, Hercules, CA) for the simultaneous detection of immunoglobulin G (IgG) and IgM class antibodies to the EBV viral capsid antigen (VCA) and IgG class antibodies to Epstein-Barr virus nuclear antigen-1 (EBNA-1). Serum specimens (n = 1,315) submitted for routine EBV-specific antibody testing by EIA (Grifols-Quest, Inc., Miami, FL) were also tested by the multiplex bead assay using the BioPlex 2200 automated analyzer. Specimens showing discordant results were tested by IFA. Following IFA resolution, the BioPlex VCA IgM, VCA IgG, and EBNA-1 IgG assays demonstrated 97.9%, 91.4%, and 96.9% agreement, respectively, with the results obtained by EIA. Furthermore, the BioPlex assays showed an overall agreement of 94.1% with the EIA when the specimens were categorized by disease state (susceptible, acute, or past infection) based on the EBV-specific antibody profiles. These findings indicate that the BioPlex EBV assays demonstrate a performance comparable to that of the conventional EIA, while allowing for a more rapid (2.3 h for 100 samples versus 4.5 h by the EIA) and higher-throughput (~400 samples per 9 h versus 200 samples by the EIA) analysis of the EBV-specific antibody response.
The BioPlex assay (Grifols S.A., Barcelona, Spain). The BioPlex EBV IgG kit consists of three distinct bead sets incorporated into each reaction mixture. These internal controls verify the binding, and the performance of the detector.

According to the manufacturer’s instructions for anti-VCA IgM and IgG IFAs (Zeus Scientific, Inc., Raritan, NJ) and anti-EBNA IgG anticomplement immunofluorescence (ACIF) (Bion Enterprises, Des Plaines, IL). The Zeus VCA IFAs employ EBV-infected substrate cells, while the Bion ACIF slides consist of fixed Raji cells, which express the EBV genome but do not produce VCA or EA. For the interpretation of results, the IFA and ACIF assay were screened for characteristic patterns of fluorescence at 1:10 and 1:5 dilutions, respectively.

**Classification of disease state.** The criteria used to categorize specimens by disease state (susceptible, acute, or past infection) were based on conventional EBV-specific antibody profiles described in the literature (2, 18, 25). In brief, specimens classified as “susceptible” were negative for VCA IgM, VCA IgG, and EBNA-1 IgG. Specimens classified as “acute” were positive for VCA IgM, VCA IgG, or both but negative for EBNA-1 IgG. Specimens showing evidence of “past” infection were positive for VCA IgG and EBNA-1 IgG, with or without concomitant detection of VCA IgM. Specimens that did not meet any of these criteria were classified as “inconclusive.”

**Statistics.** Statistical analyses were performed using JMP software, version 7 (SAS Institute, Inc., Cary, NC). In addition to percentages of agreement, kappa coefficients were calculated as a secondary measure of agreement. Levels of agreement of results by kappa values are categorized as near-perfect (0.81 to 1.0), substantial (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), slight (0 to 0.2), or poor (<0.0) (15). Equivocal results by the BioPlex assay were considered negative for sensitivity calculations and positive for specificity calculations.

**RESULTS**

**Agreement between the EIA and the BioPlex assay.** To measure agreement, the results obtained by the EIA and BioPlex assay were compared following testing of 1,315 serum specimens. The BioPlex VCA IgM, VCA IgG, and EBNA-1 IgG assays demonstrated agreements of 96.2%, 89.4%, and 92.4%, respectively, with the results obtained by EIA (Table 1). Kappa coefficients showed substantial agreement for the VCA IgM (κ = 0.8) and IgG (κ = 0.74) assays and near-perfect agreement for the EBNA-1 IgG assay (κ = 0.84) (Table 1). Specimens showing discordant results after repeat testing were analyzed by IFA. Among the specimens showing discordant VCA IgM results, 18/39 (46.2%) were resolved in favor of the BioPlex assay by IFA analysis. For specimens with discordant VCA IgG or EBNA-1 IgG results, IFA resolved 19/123 (15.4%) and 45/81 (55.6%), respectively, in favor of the BioPlex assay (Table 1). Following IFA resolution, the BioPlex assay was analyzed by IFA. Among the specimens showing discordant VCA IgM results, 18/39 (46.2%) were resolved in favor of the BioPlex assay by IFA analysis. For specimens with discordant VCA IgG or EBNA-1 IgG results, IFA resolved 19/123 (15.4%) and 45/81 (55.6%), respectively, in favor of the BioPlex assay (Table 1). Following IFA resolution, the BioPlex assay was analyzed by IFA. Among the specimens showing discordant VCA IgM results, 18/39 (46.2%) were resolved in favor of the BioPlex assay by IFA analysis. For specimens with discordant VCA IgG or EBNA-1 IgG results, IFA resolved 19/123 (15.4%) and 45/81 (55.6%), respectively, in favor of the BioPlex assay (Table 1). Following IFA resolution, the BioPlex assay was analyzed by IFA. Among the specimens showing discordant VCA IgM results, 18/39 (46.2%) were resolved in favor of the BioPlex assay by IFA analysis. For specimens with discordant VCA IgG or EBNA-1 IgG results, IFA resolved 19/123 (15.4%) and 45/81 (55.6%), respectively, in favor of the BioPlex assay (Table 1). Following IFA resolution, the BioPlex assay.
TABLE 2. Correlation of disease state based on EBV-specific antibody profiles after repeat testing by the EIA and BioPlex assay*  

| BioPlex EBV profile | No. of specimens with indicated EIA EBV profile |
|---------------------|------------------------------------------------|
|                     | Acute | Past* | Susceptible | Inconclusive | Total |
| Acute               | 139   | 0     | 3           | 0            | 142   |
| Past                | 73*   | 755   | 3           | 28           | 859   |
| Susceptible         | 24*   | 0     | 259         | 6            | 289   |
| Inconclusive        | 3     | 14    | 3           | 5            | 25    |
| Total               | 239   | 769   | 268         | 39           | 1,315 |

* The overall profile agreement after repeat testing by the EIA and BioPlex assay was 88.1% (κ = 0.78). The overall profile agreement following IFA resolution of discordant results was 94.1%.

a Includes results suggestive of past, convalescent, or reactivated infection.

b One of these three specimens was positive for anti-EBNA IgG by IFA.

c All three of these specimens were positive for anti-VCA IgG by IFA.

d Forty-two of these 73 specimens were positive for anti-EBNA IgG by IFA.

The results of our evaluation demonstrated that the BioPlex VCA (IgM and IgG) and EBNA-1 IgG assays showed substantial agreement (κ > 0.61) with routine testing by EIA. Furthermore, the BioPlex assays showed 94.1% agreement with EIA and IFA when specimens were categorized as susceptible, acute, or past infection based on the antibody profile results. Our findings are similar to those described in a recent report by Klutts et al. (13), in which they assessed the prototype BioPlex EBV assays using 167 nonconsecutive serum samples. We have extended their observations by evaluating the recently FDA-cleared BioPlex EBV assays in a large, prospective study using IFA to resolve discordant results. Our evaluation showed higher concordance between the BioPlex assay and the EIA for VCA IgM (96.2%) but lower concordance for EBNA-1 (92.4%) and VCA IgG (89.4%) than the concordance values of 92.0%, 97.0%, and 92.0%, respectively, reported in the previous study (13). Furthermore, we observed that the BioPlex assay had a higher sensitivity for VCA IgM (94.1%) but a lower specificity for EBNA-1 IgG (84.0%) than the values of 85.7% and 96.3%, respectively, reported by Klutts et al. (13). These differences are likely due to the increased number of specimens tested in our evaluation and the different EIAs used as the comparative method in these studies.

Despite substantial agreement between the BioPlex assay and the EIA, there are differences in the performance of the individual assays that may affect antibody profile results. In our evaluation, the major difference was in patients whose condition was categorized as “acute infection” by the EIA but “past infection” by the BioPlex assay (Table 2). This is most likely due to the increased detection of anti-EBNA-1 IgG by the BioPlex assay in comparison to the EIA. IFA analysis of specimens showing discordant anti-EBNA-1 IgG results revealed that 45/81 (55.5%) BioPlex assay-positive, EIA-negative specimens were truly positive for anti-EBNA-1 IgG (Table 1). This indicates that the BioPlex EBV-1 IgG assay may be more sensitive than the EIA. A second notable difference was observed for patients classified as having “acute infection” by the EIA but as “susceptible” by the BioPlex assay (Table 2). This group was positive for anti-VCA IgG by the EIA but negative by the BioPlex assay and was negative for anti-EBNA-1 IgG by both tests. IFA analysis confirmed the presence of anti-VCA IgG in 19/24 (79.2%) of these patients (Table 2). These findings correlated with our overall observation that the BioPlex VCA IgG assay was less sensitive than the EIA (Table 1). This may be due, in part, to the different capture antigens used by the assays, with recombinant VCA p18 for the BioPlex assay and recombinant gp125 and whole-cell lysate for the EIA. We should also emphasize that the presence of VCA IgG alone was classified as “acute infection” in this study; however, this profile may be observed in subacute infection or in certain patients (e.g., immunocompromised hosts, transplant patients) with past infection who fail to develop detectable anti-EBNA-1 IgG (5, 23). Additional laboratory or clinical information may be required for these patients in order to accurately categorize the disease state. Despite the differences in performance between the BioPlex assay and the EIA, our evaluation showed closer correlation between these methods than past studies comparing conventional tests, such as the EIA and IFA (6–8, 22).

This study has several additional limitations. First, the conclu-
sions that can be drawn regarding the clinical sensitivity and specificity of the BioPlex assay are limited by the lack of available clinical information. Second, this report does not describe the performance of the BioPlex heterophile and EA assays. As mentioned, heterophile tests are commonly performed as point-of-care tests and are infrequently carried out at large reference laboratories. Tests for EA may be useful in cases of acute IM, but the detection of antibodies to EA is often transient and variable (3, 12). Testing for anti-EA IgG is generally of significant benefit only under select clinical circumstances, including the differentiation of primary and reactivated viral infections (26) and the serologic evaluation of patients with EBV-associated malignancies (e.g., nasopharyngeal carcinoma) (10). It will be of interest in future studies to evaluate the BioPlex heterophile and EA assays in order to determine their use in these clinical settings and their performance in comparison to conventional methods.

In conclusion, we have demonstrated that the BioPlex EBV assays show a performance comparable to that of routine testing by EIA while offering several advantages. First, the BioPlex assay has the capacity to test for as many as five EBV serologic markers using only two aliquots of sample. This may reduce both sample volume requirements and aliquot errors. Second, the BioPlex assay incorporates three internal controls into each reaction, allowing for an assessment of specimen addition, nonspecific binding, and detector performance. Finally, the BioPlex assay allows for a more rapid (2.3 h for 100 samples, compared to 4.5 h by the EIA) and higher-throughput (~400 samples per 9 h, compared to 200 samples by the EIA) analysis of the EBV serologic response. This may prove beneficial for high-volume clinical laboratories experiencing significant increases in the number of specimens submitted for EBV serologic testing.

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