Evaluation of a Multiplex Bead Immunoassay for Determination of Immune Status to Varicella-Zoster Virus in Medical Center Students and Employees

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This study evaluated an enzyme immunoassay, a multiplex bead immunoassay (MBIA), and the anticomplement immunofluorescence (ACIF) test for detecting varicella-zoster virus IgG antibodies in sera from medical center students and employees. The agreement between methods was ≥ 95%. The MBIA was less sensitive than was the ACIF test, with a negative predictive value of 66.7%.

Documentation of evidence of immunity to varicella-zoster virus (VZV) in employees and students at medical centers is an important element of an infection control program. In spite of recommendations from the U.S. Centers for Disease Control and Prevention (CDC) that written documentation of vaccination or verification of a history of the disease is sufficient evidence of immunity (1), some health care facilities require serological evidence of immunity. Therefore, there is a need for highly sensitive anti-VZV IgG assays. While the fluorescent-antibody-to-membrane-antigen (FAMA) test is considered the gold standard for the detection of antibodies to VZV, this method is limited to research settings. The enzyme immunoassay (EIA) is commonly used by diagnostic laboratories, while latex agglutination, immunofluorescence, and the anticomplement immunofluorescence (ACIF) test are less common alternative tests. The BioPlex MMRV IgG kit (Bio-Rad Laboratories, Hercules, CA), based on a multiplex bead immunoassay (MBIA) format, was recently cleared by the U.S. Food and Drug Administration (FDA). The BioPlex provides complete automation of the immunoassay, thereby reducing labor requirements compared to those of manual or semiautomated EIAs. Good agreement was observed between the BioPlex VZV IgG assay and an EIA (2). To our knowledge, there are no published data on the performance of the BioPlex VZV assay for the determination of immunity in students and employees at health care facilities. The objective of this study was to evaluate the performances of the BioPlex MBIA, an EIA, and the ACIF test for the detection of antibodies to VZV in serum panels from students and employees at an academic medical center.

Serum specimens were submitted for routine testing by the MBIA. One hundred consecutive serum specimens from students (primarily medical students) (n = 39) and employees (n = 61) were frozen and tested in a blinded fashion by an EIA (Zeus Scientific, Somerville, NJ) and the ACIF test. The MBIA and EIA were performed according to the manufacturers’ instructions. The ACIF test was performed essentially as described by Preissner et al. (3), using slides bearing VZV-infected diploid fibroblasts purchased from MBL Bion (Des Plaines, IL), guinea pig complement from Lonza (Walkersville, MD), and fluorescein-conjugated goat IgG recognizing guinea pig C3 from MP Biomedicals (Solon, OH) (3). The serum samples were heat inactivated at 56°C for 30 min and diluted 1:4 in phosphate-buffered saline for testing; all incubations were performed at 37°C. This study was performed as part of a protocol approved by the University of Texas Medical Branch institutional review board. The percent agreement and kappa coefficients, with their 95% confidence intervals, were determined. The levels of agreement for the kappa coefficient were defined as almost perfect (0.81 to 1), substantial (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or slight (0.01 to 0.2) (2). Equivocal results by the EIA and MBIA were considered positive for calculating performance characteristics. The z test was used to assess proportions. VZV seroprevalence by the ACIF test was 93% for the entire set of 100 prospectively collected specimens. Compared to the ACIF test, the sensitivities of the MBIA were 97.1% and 96.7% for employees and students, respectively (Table 1). The agreement values were 94.9% and 96.7%, respectively. For the complete sample set, the MBIA sensitivity, specificity, positive predictive value, and negative predictive value (95% confidence interval) compared to those of the ACIF test were 90/93 (96.8% [90.9 to 98.9%]), 6/7 (85.7% [48.7 to 97.4%]), 90/91 (98.9% [94 to 99.8%]), and 6/9 (66.7% [35.4 to 87.9%]), respectively. The overall agreement between the MBIA and the ACIF test was 96%. The negative predictive value in these populations was difficult to assess, owing to the small number of specimens that tested negative for VZV IgG. We therefore identified and archived eight additional MBIA-negative specimens for analysis by the ACIF test and the EIA. Of these eight MBIA-negative specimens, five were positive by the ACIF test (four of which were also positive by the EIA), further verifying the low negative predictive value of the MBIA. One specimen was positive by the MBIA and negative by the ACIF test. However, this
The goal of this study was to compare the BioPlex MBIA to an EIA and the ACIF test for the detection of anti-VZV IgG in a population of health care workers and students, and to assess whether negative results are more likely to be true (verified by other methods) or false. In a previously published study, the sensitivity of the BioPlex VZV IgG assay (part of the MMRV kit) was 92% compared to that of a commercial EIA (Diamedix, Miami, FL) (2). However, the patient population in this study was not characterized. In the present study, the sensitivities of the MBIA and the EIA were similar and >97% among students and employees compared to those of the ACIF test. The agreement between the methods was >95%. The specificity of the MBIA compared to that of the commercial EIA was also >97%. To our knowledge, this is the first report of the sensitivity of the BioPlex VZV IgG MBIA in employees and students in a health care facility. Other immunoassays have been evaluated in various vaccinated populations, including in health care workers. In vaccinated health care workers tested 18 months after vaccination, the sensitivity of an EIA was poor (62%) compared to that of a time-resolved fluorescence immunoassay (4). In another study, the sensitivity of the same EIA in vaccinated children (1 to 6 years old) was 31% compared to that of a FAMA (with cutoff titer of 1:4) (5). In a population of recent (4 to 6 weeks) vaccinees 1 to 38 years old, three commercial EIAs showed sensitivities ranging from 45 to 96% compared to that of FAMA (6).

The BioPlex MBIA produces an AI between 0.2 and 8.0. Specimens with AIs of <0.9 are reported as negative, 0.9 to 1.0 are reported as equivocal, and >1.0 are reported as positive. Although the manufacturer makes no quantitative claims, we found a good level of correlation between the BioPlex MBIA AI and EIA index values. We then examined the AIs of specimens that were negative by MBIA to determine if the AI value could distinguish between a likely true-positive and a true-negative specimen. Among specimens negative by both the MBIA and the ACIF test, the MBIA AIs ranged from <0.2 to 0.6 (mean, 0.28). Among MBIA-negative/

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**TABLE 1 Performances of MBIA and EIA versus the ACIF test**

| Test and population | No. detected/total no. (%; 95% CI) | Sensitivity | Specificity | Agreement | Kappa (95% CI) |
|---------------------|-----------------------------------|-------------|-------------|-----------|---------------|
| **MBIA**            |                                   |             |             |           |               |
| Employees           | 34/35 (97.1; 85.5, 99.5)          | 3/4 (75; 30.1, 95.4) | 37/39 (94.9; 83.1, 98.6) | 0.72 (0.35, 1) |
| Students            | 56/58 (96.6; 88.3, 99)            | 3/3 (100; 43.9, 100) | 59/61 (96.7; 88.8, 99.1) | 0.73 (0.37, 1) |
| **EIA**             |                                   |             |             |           |               |
| Employees           | 35/35 (100; 90.1, 100)            | 3/4 (75; 30.1, 95.4) | 38/39 (97.4; 86.8, 99.5) | 0.84 (0.54, 1) |
| Students            | 57/58 (98.3; 90.9, 99.7)          | 3/3 (100; 43.9, 100) | 60/61 (98.4; 91.3, 99.7) | 0.84 (0.55, 1) |

*ACIF, anticomplement immunofluorescence; EIA, enzyme immunoassay; MBIA, multiplex bead immunoassay.

* 95% CI, 95% confidence interval.

* A single false-positive specimen was positive by EIA and MBIA.

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**TABLE 2 Performance of MBIA versus that of EIA**

| Population | No. detected/total no. (%; 95% CI) | Sensitivity | Specificity | Agreement | Kappa (95% CI) |
|------------|-----------------------------------|-------------|-------------|-----------|---------------|
| Employees  | 35/36 (97.2; 85.8, 99.5)          | 3/3 (100; 43.9, 100) | 38/39 (97.4; 86.8, 99.5) | 0.84 (0.54, 1) |
| Students   | 55/57 (96.3; 88.1, 99)            | 3/4 (75; 30.1, 95.4) | 58/61 (95.1; 86.5, 98.3) | 0.64 (0.24, 1) |

* EIA, enzyme immunoassay; MBIA, multiplex bead immunoassay.

* 95% CI, 95% confidence interval.

* MBIA false-positive specimen was also positive by the ACIF test.

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The goal of this study was to compare the BioPlex MBIA to an EIA and the ACIF test for the detection of anti-VZV IgG in a population of health care workers and students, and to assess whether negative results are more likely to be true (verified by other methods) or false. In a previously published study, the sensitivity of the BioPlex VZV IgG assay (part of the MMRV kit) was 92% compared to that of a commercial EIA (Diamedix, Miami, FL) (2). However, the patient population in this study was not characterized. In the present study, the sensitivities of the MBIA and the EIA were similar and >97% among students and employees compared to those of the ACIF test. The agreement between the methods was >95%. The specificity of the MBIA compared to that of the commercial EIA was also >97%. To our knowledge, this is the first report of the sensitivity of the BioPlex VZV IgG MBIA in employees and students in a health care facility. Other immunoassays have been evaluated in various vaccinated populations, including in health care workers. In vaccinated health care workers tested 18 months after vaccination, the sensitivity of an EIA was poor (62%) compared to that of a time-resolved fluorescence immunoassay (4). In another study, the sensitivity of the same EIA in vaccinated children (1 to 6 years old) was 31% compared to that of a FAMA (with cutoff titer of 1:4) (5). In a population of recent (4 to 6 weeks) vaccinees 1 to 38 years old, three commercial EIAs showed sensitivities ranging from 45 to 96% compared to that of FAMA (6).

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ACIF-positive specimens, the MBIA AIs ranged from 0.4 to 0.8 (mean, 0.63). These results suggest that specimens negative by the BioPlex MBIA with AIs between 0.7 and 0.9 may represent false negatives, and these would be candidates for follow-up testing by another method. Additionally, we observed four specimens with equivocal results by the BioPlex MBIA (AI, 0.9 to 1). All four were positive by the ACIF test. It is important to reiterate that the manufacturer of the BioPlex MBIA makes no quantitative performance claims.

Our study has several limitations. The sample set was small and from a single site. The reference standard will affect the observed sensitivity of any test method being evaluated. We used the ACIF test as the reference method in this study. The ACIF test is a reasonable gold standard, with a caveat: in two studies, the sensitivity of any test method being evaluated. We used the ACIF test compared to that of the FAMA was 93% in one study (3) and 100% in the other (7) for determining immune status after natural infection (8). In conclusion, three methods used to detect anti-VZV IgG showed similar performances and high levels of agreement for university medical center students and employees with high seroprevalence. The BioPlex MBIA failed to detect some VZV IgG titers detected by the ACIF test, with a negative predictive value of 66.7%, which was verified by testing additional MBIA-negative specimens. The MBIA AI values correlated with the index values of a commercial EIA and may distinguish between the absence of IgG and its presence at levels below the manufacturer’s cutoff.

**TABLE 3** MBIA index values for 17 serum samples negative for VZV IgG by MBIA in relation to ACIF test resultsᵃ

| ACIF result | BioPlex AI (no. of specimens)b | Mean AI (SD) |
|-------------|-------------------------------|-------------|
| Negative    | ≤0.2 (2), 0.2 (4), 0.3 (1), 0.4 (1), 0.6 (1) | 0.28 (0.13) |
| Positive    | 0.4 (1), 0.5 (1), 0.6 (2), 0.7 (3), 0.8 (1) | 0.63 (0.12) |

ᵃ MBIA, multiplex bead immunoassay; ACIF, anticomplement immunofluorescence.
ᵇ AI, antibody index.

**FIG 1** Correlation between MBIA and EIA indices.

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