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Evaluation of the Bio-Rad BioPlex Measles, Mumps, Rubella, and Varicella-Zoster Virus IgG Multiplex Bead Immunoassay▽

Matthew J. Binnicker,* Deborah J. Jespersen, and Leonard O. Rollins

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota 55905

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The goal of this study was to compare the BioPlex 2200 measles, mumps, rubella, and varicella-zoster virus (MMRV) IgG multiplex assays (Bio-Rad Laboratories, Hercules, CA) to routine testing by enzyme immunoassay (EIA). Serum specimens ($n = 500$) submitted to our reference laboratory for routine MMRV IgG testing by EIA were also tested by the BioPlex assays. Following testing, the BioPlex measles, mumps, rubella, and varicella-zoster virus assays demonstrated agreements of 91.6% (95% confidence interval [CI], 88.8% to 93.7%), 94.2% (95% CI, 91.7% to 95.7%), 94.4% (95% CI, 92.0% to 96.1%), and 91.8% (95% CI, 89.0% to 93.9%), respectively, compared to the results of EIA. Timing studies showed that the BioPlex MMRV assay could provide complete analysis of 100 serum specimens in 1.7 h, compared to 5.5 h by EIA. These data indicate that the BioPlex MMRV IgG assays exhibit comparable performance (93% overall agreement [1,860/2,000 results]; $\kappa = 0.67$) to routine testing by EIA. The BioPlex assays allow for the simultaneous detection of all four analytes, thereby eliminating potential aliquot errors and reducing turnaround time.

The incidence of disease caused by measles, mumps, rubella, and varicella-zoster virus (MMRV) has been significantly reduced in developed countries due to the implementation of effective immunization programs (1, 6). However, outbreaks of disease continue to occur in the United States and worldwide due to vaccine failure, declining immunization rates, and waning immunity (2, 3, 5).

Laboratory testing for IgG class antibodies to MMRV plays an important role in the management of patients and health care workers. For example, testing for IgG class antibodies to rubella virus is routinely performed during the prenatal period (7), and detection of rubella IgG during the first trimester indicates that the mother is protected from primary infection. Furthermore, immunocompromised hosts (e.g., transplant recipients) are commonly screened for immunity to varicella, which may cause devastating disease in the immunosuppressed population if a primary infection occurs (8).

Until recently, most clinical laboratories have used methods such as indirect immunofluorescence (IFA), enzyme immunoassay (EIA), and enzyme-linked fluorescence assay (ELFA) for the detection of IgG class antibodies to MMRV. These methods have demonstrated reliable performance; however, they are labor-intensive, time-consuming, and, in the case of IFA, subjective. In addition, these conventional methods require four separate assays to test for IgG class antibodies to MMRV, thereby increasing sample volume requirements as well as hands-on time. These limitations have led to the recent development of multiplex flow immunoassay (MFI) technology, which allows for multiple analytes (e.g., antibodies) to be detected in a single reaction.

The Bio-Rad BioPlex MMRV IgG assays (Bio-Rad Laboratories, Hercules, CA) recently received FDA approval for the simultaneous detection of IgG class antibodies to MMRV in human serum or EDTA/heparinized plasma samples. The BioPlex MMRV IgG immunoassays use four distinct populations of microspheres (8-μm beads) that are coated with a capture antigen designed to bind specifically to a target antibody. After the mixture of the patient sample and assay reagents, antibodies that are bound to their respective microsphere are then detected using a fluorescently labeled reporter molecule whose emission is measured by a flow-based detector.

The goal of this study was to evaluate the performance characteristics of the BioPlex MMRV IgG multiplex immunoassays using serum specimens submitted for routine testing by EIA. Implementation of this multiplex bead immunoassay may allow clinical laboratories to meet increasing test volumes for MMRV IgG testing, while reducing hands-on time and turnaround time.

(This study was presented in part at the 2011 Clinical Virology Symposium, Daytona, FL, abstract S35.)

MATERIALS AND METHODS

Study design. Prospective, nonclinically characterized serum specimens ($n = 500$) submitted to our reference laboratory for MMRV IgG analysis were used for this study. Routine testing for measles and varicella-zoster virus IgG was performed by the Diamedix EIAs (Diamedix, Miami, FL), while routine analysis of mumps and rubella IgG was completed using the SeraQuest EIAs (Quest International, Doral, FL). In addition to routine testing by EIA, all samples were also tested in a blinded fashion using the BioPlex 2200 MMRV IgG assays. Samples showing discrepant results after initial testing were tested again by both EIA and BioPlex using the same freeze-thaw cycle of the specimen. Samples showing further discrepancies were tested by a third method (i.e., EIA or ELFA) as described below. The study was reviewed by the institutional review board at our center.

EIA. Routine testing for measles and varicella-zoster virus IgG was performed according to the manufacturer's instructions using the DiaMedix EIA kits (Diamedix; Miami, FL). For each of the Diamedix EIAs, 10 μl of serum was diluted into 1 ml of sample diluent prior to testing. The sample EIA units (EU)/milliliter were calculated by dividing the EU/milliliter assigned to the calibrator by the optical density (OD) of the calibrator. This value is then multiplied by the OD of the sample. The results were classified as negative (<15 EU/ml), equivocal (15 to 19.9 EU/ml), or positive (≥20.0 EU/ml).

* Corresponding author. Mailing address: Mayo Clinic, 200 First Street SW, SDSC 1-526, Rochester, MN 55905. Phone: (507) 538-1640. Fax: (507) 284-4272. E-mail: binnicker.matthew@mayo.edu.

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Routine testing for mumps and rubella IgG was performed according to the manufacturer's instructions using the SeraQuest EIAs (Grifols USA, Miami, FL). For each SeraQuest assay, 5 μl of serum was diluted into 250 μl of sample diluent prior to testing. For mumps IgG, results were calculated as index values and interpreted as negative (<0.9), equivocal (0.9 to 1.0), or positive (≥1.1), while the interpretive criteria for the rubella IgG assay were classified as negative (<0.9), equivocal (0.9 to 0.99), or positive (≥1.0). All testing by EIA was performed using the Triturus automated EIA analyzer (Grifols, Los Angeles, CA).

BioPlex MMRV IgG. Testing was performed according to the manufacturer's instructions using the BioPlex 2200 MMRV IgG kit on the BioPlex 2200 analyzer (Bio-Rad). The BioPlex MMRV IgG kit consists of seven different populations of dyed beads that are used during the analysis. Three of these bead sets are used for quality control purposes to generate an internal standard and verify the addition of the appropriate sample type. The remaining four bead sets are dedicated to the detection of IgG class antibodies to MMRV (e.g., one bead set per analyte). The BioPlex uses a total input volume of 5 μl of serum for all four analytes. Following flow cytometric analysis, the data are initially calculated in relative fluorescence intensity (RFI) and are then converted to a fluorescence ratio (FR) using the internal standard bead. The FR is compared to an assay-specific calibration curve to determine analyte concentration in antibody index units (AI). The interpretive criteria were established by the manufacturer, and results were defined as negative (≤0.8 AI), equivocal (0.9 to 1.0 AI), or positive (≥1.1 AI) for measles, mumps, and varicella-zoster virus IgG. For rubella IgG, the interpretive criteria are based on the World Health Organization (WHO) standards and were defined as negative (≤0.7 AI), equivocal (0.8 to 0.9 AI), or positive (≥1.0 AI).

Resolution of discordant results. Samples showing discordant results for measles and varicella-zoster virus IgG were tested by a third method (SeraQuest EIA) according to the manufacturer's instructions. Interpretive criteria were established by the manufacturer and were classified as negative (<0.9), equivocal (0.9 to 1.0), or positive (≥1.1). Discrepant samples for mumps and rubella IgG were tested by ELFA (VIDAS; bioMérieux, Inc.) according to the manufacturer's instructions. For ELFA, the test value threshold (TVT) was generated for each sample by calculating the ratio of the relative fluorescence value (RFV) of the sample to that of a standard. Mumps IgG results were classified as negative (<0.35), equivocal (0.35 to 0.49), or positive (≥0.50) based on the TVT. Rubella IgG ELFA results were calculated as an index unit (IU)/milliliter value based on WHO standards, with results being categorized as negative (<5 IU/ml), equivocal (5 to 9 IU/ml), or positive (≥10 IU/ml).

Statistical methods. All statistical analyses were performed using GraphPad Software. In addition to percent agreement and 95% confidence intervals (95% CI), kappa coefficients were also determined as an additional measure of agreement. Levels of agreement as defined by kappa values were 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) (4). Equivocal results by the BioPlex were considered negative for calculating percent sensitivity and positive for calculating percent specificity.

Analysis of turnaround time, sample throughput, and cost. The approximate turnaround time (TAT) for the testing and reporting of 100 serum samples by EIA and BioPlex was calculated using incubation and reaction times provided by the manufacturers. Estimations were made based on the use of a single instrument. The sample throughput of each assay was then calculated for a 9-h shift using the following equation: $(9/\text{TAT}) \times 100$. The cost per patient for each test was determined as the list price for reagents, as supplied by the manufacturer, and does not account for instrumentation or personnel cost associated with testing.

RESULTS

Comparison of the BioPlex MMRV IgG assay to EIA. Compared to the results of routine testing by EIA, the BioPlex MMRV IgG assays showed agreements of 91.6% (458/500 samples) for measles IgG, 94.2% (471/500 samples) for mumps IgG, 94.4% (472/500 samples) for rubella IgG, and 91.8% (459/500 samples) for varicella-zoster virus IgG. The corresponding kappa coefficients (κ) were 0.66, 0.79, 0.65, and 0.52, respectively. The sensitivity and specificity percentages of the BioPlex MMRV IgG assays were as follows: measles IgG, 94.6% and 96.4%; mumps IgG, 98.1% and 82.8%; rubella IgG, 94.9% and 100%; varicella-zoster virus IgG, 92.2% and 100% (Table 1).

Turnaround time, sample throughput, and reagent cost. The BioPlex MMRV IgG assays were estimated to yield a TAT of ~1.7 h for analysis of 100 samples and reporting of all four analytes. The approximate hands-on time for testing 100 samples by the BioPlex was ~48 min. In contrast, testing by EIA using a single Triturus instrument required ~5.5 h for analysis and reporting of all four analytes, with an estimated hands-on time of ~220 min. These TAT calculations translated into an approximate sample throughput of 530 samples by BioPlex and 163 samples by EIA during a 9-h shift. The list-fee reagent cost (cost per patient) for analysis of all four analytes was $40 ($10/analyte) by BioPlex versus $12.55 ($3.14/analyte) by EIA; however, these values do not account for instrumentation or associated personnel costs.

DISCUSSION

The data presented in this report indicate that the BioPlex MMRV IgG assays show substantial agreement (93% [1,860/2,000 results] overall; $\kappa = 0.67$) to routine testing by EIA. However, despite comparable overall performance, there were differences in test performance that should be discussed. Most importantly, the BioPlex assays showed lower sensitivity than EIA, especially for varicella-zoster virus IgG (92.2%) and measles IgG (94.6%). Among the 18 samples with discordant (BioPlex-negative, Diamedix EIA-positive) varicella-zoster virus IgG results, all 18 (100%) tested positive by a third method (SeraQuest varicella-zoster virus IgG EIA). Furthermore, among the 10 samples that were positive for measles IgG by EIA but negative by BioPlex, all 10 (100%) samples were positive by the third method (SeraQuest EIA). We reviewed the numerical (index) EIA values for the BioPlex-negative, EIA-positive samples ($n = 38$) and found that 60.5% (23/38) were marginally positive (e.g., within 20% of the assay cutoff) by EIA, suggesting that low levels of antibody were present in these samples. Whether or not the "marginally positive" EIA results reflect patients with protective levels of antibody or waning immunity is not known. Future studies should be focused on determining whether the BioPlex MMRV assays truly demonstrate lower sensitivity, or if patients with low levels of antibodies (e.g., marginally positive by EIA and negative or equivocal by BioPlex) should be considered for reimmunization.

This study has several limitations. First, we did not have access to clinical information or vaccination records for the patients tested in this study. Therefore, our ability to arbitrate discordant results was limited, despite the fact that samples with discrepant results were tested by a third method. Second, the results of the BioPlex MMRV IgG assays were compared only to the EIAs used for routine MMRV testing in our laboratory. Because of this, laboratories using other methods (e.g., IFA, ELFA, or different EIAs) should revalidate the performance characteristics of the BioPlex MMRV IgG assays, as the sensitivity and specificity percentages may differ depending on the predicate device.

Despite these limitations, the BioPlex MMRV IgG assays possess several advantages over EIA testing, including a higher throughput (530 versus 163 samples in a 9-h shift) and a reduced turnaround time (1.7 h versus 5.5 h for 100 samples). In addition,

TABLE 1. Comparison of the BioPlex MMRV IgG assays to routine testing by EIA

Assay and result	No. of samples by EIA that were:			% sensitivity (95% CI)	% specificity (95% CI)	% agreement (95% CI)	Kappa value
	Positive	Negative	Equivocal				
BioPlex measles IgG (against Diamedix EIA)				94.6 (92.1, 96.4)	96.4 (80.8, 100)	91.6 (88.8, 93.8)	0.66
Positive	420	1[a]	0				
Negative	10[b]	27	17				
Equivocal	14	0	11				
BioPlex mumps IgG (against SeraQuest EIA)				98.1 (96.2, 99.1)	82.8 (70.9, 90.6)	94.2 (91.8, 96.0)	0.79
Positive	412	4[c]	8				
Negative	3[d]	48	3				
Equivocal	5	6	11				
BioPlex rubella IgG (against SeraQuest EIA)				94.9 (92.5, 96.6)	100.0 (83.1, 100)	94.4 (92.0, 96.1)	0.65
Positive	446	0	0				
Negative	7[e]	23	4				
Equivocal	17	0	3				
BioPlex varicella-zoster virus IgG (against Diamedix EIA)				92.2 (89.4, 94.3)	100.0 (82.5, 100)	91.8 (89.0, 93.9)	0.52
Positive	436	0	0				
Negative	18[f]	22	4				
Equivocal	19	0	1				

[a] This sample tested negative by the SeraQuest IgG EIA.
[b] All 10 samples tested positive by the SeraQuest IgG EIA.
[c] All four of these samples tested positive by Vidas mumps IgG ELFA.
[d] One of these three samples tested negative by Vidas mumps IgG ELFA.
[e] Six of these seven samples tested as equivocal by Vidas rubella IgG ELFA.
[f] All 18 samples tested positive by the SeraQuest IgG EIA.

the BioPlex MMRV assays are performed simultaneously and therefore allow for custom ordering and efficient test add-ons if requested. Furthermore, the BioPlex assays include internal controls which verify the addition of sample and enhance quality assurance. Finally, the ability to perform multiplex analysis using a single system may reduce errors associated with aliquoting samples and performing testing on multiple platforms.

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