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.

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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 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 ELISA) 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. Interpretive criteria were based on the World Health Organization (WHO) standards and were defined as negative (<0.7 IU/ml), equivocal (0.8 to 0.9 IU/ml), or positive (≥1.0 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 0.80), moderate (0.41 to 0.60), 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 manufacturer. Estimations were made based on the use of a single instrument needed to analyze the sample on January 8, 2021 by guest http://cvi.asm.org/ Downloaded from
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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REFERENCES


