Virology

Detection of IgG-class antibodies to measles, mumps, rubella, and varicella-zoster virus using a multiplex bead immunoassay☆

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Abstract

Serologic testing for measles, mumps, rubella, and varicella (MMRV) IgG is traditionally performed by immunofluorescence assay or enzyme immunoassay (EIA). Although sensitive and specific, these methods are labor intensive, time consuming, and require separate assays for each analyte. This study evaluated the performance of the MMRV IgG AtheNA Multi-Lyte® assay using nonclinically characterized serum specimens submitted to our laboratory for routine MMRV IgG testing. Mumps (n = 492) or rubella (n = 500) IgG were initially tested by enzyme-linked fluorescent antibody (ELFA), whereas measles (n = 494) or varicella (n = 497) were analyzed by EIA. Each sample was also tested by the AtheNA Multi-Lyte assay. Discordant results were retested by the predicate method and the multiplex assay, with further discrepancies being arbitrated by a third test. Compared to EIA/ELFA for MMRV IgG, the AtheNA assay demonstrated an overall agreement of 97.4%, 98.2%, 97.6%, and 100%, respectively. Use of this multiplex assay allows for the simultaneous detection of MMRV IgG, potentially decreasing cost, sample volume requirements, aliquot errors, and hands-on testing time.

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1. Introduction

Measles, mumps, rubella, and varicella (MMRV) are viral syndromes that have historically affected children and young adults. Immunization programs using combination MMRV vaccines have significantly reduced the incidence of disease associated with these viruses in the United States, Europe, and other developed countries (Czajka et al., 2009; Schuster et al., 2008). However, cases of acute disease continue to occur worldwide as a result of primary and secondary vaccine failure, nonimmunization, or waning immunity (Doshi et al., 2009; Kancherla and Hanson, 2006; Mulholland, 2006).

Serologic testing for IgG-class antibodies to MMRV is a useful tool for measuring the response to vaccination and determining immune status. For example, testing for IgG-class antibodies to rubella virus is routinely performed during prenatal screening (Tamer et al., 2009), and its detection indicates immunity and protection from primary infection. In addition, an assessment of prior exposure to varicella-zoster virus is critical in the management of immunocompromised hosts and pregnant women (World Health Organization [WHO], 1998). The conventional methods of antibody detection to MMRV include indirect immunofluorescence (IFA) and enzyme immunoassay (EIA). Although these methods are sensitive and specific, they are labor intensive, time consuming, and the case of IFA, subjective. Furthermore, IFA and EIA require separate assays for the detection of IgG-class antibodies to MMRV. This may increase the volume of sample required for testing, as well as the cost due to increased technologist time, instrument time, and reagents used.

Multiplex antibody detection methods have been described for the serologic diagnosis of several infectious diseases (Binnicker et al., 2008; Mahony et al., 2009). Recently, the AtheNA Multi-Lyte MMRV IgG assay (Zeus

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Scientific, Raritan, NJ) received Food and Drug Administration approval for the simultaneous detection of IgG-class antibodies to MMRV in human serum. The AtheNA Multi-Lyte test is a multiplex bead immunoassay that operates using a principle similar to conventional EIA. This technology uses microspheres (5–6 μm in diameter) with a unique spectral identification based on the red/infrared content. The microspheres are coated with a capture reagent (e.g., antigen) that binds a specific analyte. The analyte is then detected using a fluorescently labeled reporter molecule whose emission is measured by a flow-based detector. The MMRV AtheNA Multi-Lyte assay is fully automated allowing for a high-throughput analysis of the MMRV IgG status.

The goal of this study was to evaluate the performance characteristics of the MMRV IgG AtheNA Multi-Lyte assay using serum specimens submitted for routine testing by EIA or enzyme-linked fluorescent antibody (ELFA) analysis. Implementation of this multiplex bead immunoassay may allow clinical laboratories to meet increasing test volumes for MMRV IgG analysis in a cost- and time-efficient manner.

2. Materials and methods

2.1. Study design

Prospective, nonclinically characterized serum specimens (n = 1983 total sera) submitted to our reference laboratory for routine serologic analysis of MMRV IgG were used for this evaluation. Specimens submitted for measles (n = 494) or varicella (n = 497) IgG were initially tested by EIA (DiaMedix, Miami, FL) using the Triturus automated EIA analyzer (Grifols, Los Angeles, CA). Sera submitted for mumps (n = 492) or rubella (n = 500) IgG were tested by ELFA (BioMerieux, Durham, NC) on the VIDAS instrument (BioMerieux). All samples were also tested in a blinded fashion using the Athena Multi-Lyte (Zeus Scientific) MMRV IgG assay on the Automated Immunoassay Multiplexing System (AIMS) instrument (Inverness Medical, Waltham, MA). The study was reviewed and approved by the institutional review board of the Mayo Clinic, Rochester, MN.

2.2. Resolution of discordant results

All samples that had discrepant results were repeated by the predicate method (EIA or ELFA) and the Athena Multi-Lyte assay using the same freeze/thaw cycle of the specimen. Samples showing further discrepancies for measles and mumps IgG were tested by SeraQuest EIA (Grifols/USA, Miami, FL) on the Triturus instrument. Rubella discrepant samples were further analyzed using the Vitros/Eci/ECiQ Rubella IgG Immunodiagnostic System (Ortho-Clinical Diagnostics, Raritan, NJ). There were no varicella discrepant samples, so no further testing was required.

2.3. Enzyme immunoassay

Routine EIA testing for measles and varicella IgG antibodies was performed according to the manufacturers’ instructions using the DiaMedix EIA kits (DiaMedix). The sample EIA unit (EU)/mL values were calculated by dividing the EU/mL 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 (15–19.9 EU/mL), or positive (≥20.0 EU/mL).

2.4. Enzyme-linked fluorescent Immunoassay (ELFA)

Routine IgG testing for mumps and rubella was carried out by ELFA (VIDAS, BioMerieux), according to manufacturer’s instructions. For the mumps assay, the Test Value Threshold (TVT) was generated for each sample by calculating the ratio of the relative fluorescence value of the sample to that of a standard. The results were classified as negative (<0.35), equivocal (0.35 to <0.50), or positive (≥0.50) based on the TVT. Rubella IgG results were also based on the TVT, with results being categorized as negative (<0.40), equivocal (0.40 to <0.50), or positive (≥0.50).

2.5. AtheNA Multi-Lyte MMRV

In addition to testing by the routine methods, each specimen was also tested by the AtheNA Multi-Lyte MMRV IgG assay (Zeus Scientific) using the AIMS instrument following the manufacturer’s instructions. Briefly, each of the 4 antigens (measles, mumps, rubella, or varicella) is covalently coupled to different polystyrene microsphere sets that are color coded with varying ratios of fluorescent dyes. Appropriately diluted specimen along with positive and negative controls are added to a reaction well and allowed to incubate with the multiplex bead set. If specific antibody is present, it will bind to the immobilized antigen on the coated microspheres. After incubation and washing, a reporter antibody conjugated to phycoerythrin (PE) is added, and the reaction product is analyzed using the AtheNA Multi-Lyte instrument. During analysis, the beads are aspirated from each microwell and passed through a flow-based detector. The flow analysis occurs at a rate of up to 20 000 beads per second. Lasers striking each bead determine which assay is being measured based on the internal dye composition of the bead and whether specific antibody is present based on the fluorescence emitted from any bound PE-conjugated secondary antibody. The data are first calculated as the relative fluorescence intensity and then converted to a fluorescence ratio using an internal bead standard. A set of 5 microspheres in the multiplex bead mix are used for intrawell calibration, standard curve calibration, and internal quality control. The fluorescence ratio is compared with the assay-specific calibration curve, and the analyte concentration is calculated in Athena units (AU). For the measles, mumps, and varicella assays, results are classified as negative (<100 AU/mL),
equivocal (100–120 AU/mL), or positive (>120 AU/mL). For the rubella component of the assay, results are classified as negative (0–9 AU/mL), equivocal (9–10.99 AU/mL), or positive (≥11 AU/mL).

2.6. Vitros ECiQ rubella IgG

All samples showing discrepant results for rubella IgG were further analyzed using the Vitros/Eci/ECiQ Rubella IgG Immunodiagnostic System (Ortho-Clinical Diagnostics) according to the manufacturer’s instructions. The results were classified as negative (≤9.99 IU/mL), equivocal (10–14.9 IU/mL), or positive (≥15.0 IU/mL).

2.7. Statistical methods

All statistical analyses were performed using JMP software, version 7 (SAS Institute, Cary, NC). In addition to percentage agreement, \( \kappa \) coefficients were also determined as an additional measure of agreement. Levels of agreement as defined by \( \kappa \) values were categorized as near perfect (0.81–1.0), substantial (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4), slight (0–0.2), or poor (<0) (Landis, 1977). Equivocal results by the AtheNA multiplex assay were considered negative for calculating percentage sensitivity and positive for calculating percentage specificity.

2.8. Cost and timing analyses

For cost comparisons, the list reagent fees necessary to test for IgG-class antibodies to MMRV by EIA/ELFA were compared to the AtheNA Multi-Lyte assay. Cost analyses did not include cost associated with technologist time or instrumentation. For timing analyses, the time necessary to complete testing of 90 serum samples for IgG-class antibodies to MMRV using a single EIA/ELFA instrument was calculated and compared to the time required using a single AIMS multiplex system.

3. Results

3.1. Agreement of the AtheNA Multi-Lyte MMRV IgG assay to EIA/ELFA

After initial testing, the AtheNA Multi-Lyte MMRV assay showed 100% (497/497) agreement for varicella IgG (\( \kappa = 1.0 \)). For measles, mumps, and rubella the AtheNA assay demonstrated agreements of 97.4% (481/494), 98.2% (483/492), and 97.6% (488/500) when compared to the results of routine testing by EIA/ELFA. The corresponding \( \kappa \) coefficients were 0.83, 0.91, and 0.82, respectively, indicating near-perfect agreement for each analyte (Table 1).

After resolution of discrepant results, the AtheNA mumps IgG assay showed a sensitivity and specificity of 100% (438/438) and 84.6% (44/52), respectively. In addition, the adjusted sensitivity and specificity of the AtheNA measles IgG assay was 98.5% (449/456) and 93.3% (28/30), whereas the rubella IgG assay demonstrated values of 97.8% (452/462) and 100% (36/36), respectively. The AtheNA varicella IgG assay showed 100% sensitivity and specificity in comparison to routine testing by EIA (Table 1).

4. Discussion

The efficacy of most currently licensed vaccines, including MMRV, is determined by the ability of the vaccine to provide protection from disease (Plotkin, 2001, 2008).

<table>
<thead>
<tr>
<th>Result by AtheNA Multi-Lyte</th>
<th>No. of specimens with indicated result by EIA/ELFA</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% Agreement (95% CI)</th>
<th>( \kappa )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles IgG</td>
<td>Positive</td>
<td>449</td>
<td>98.5 (96.8–99.3)</td>
<td>93.3 (77.6–99.2)</td>
<td>97.4 (95.5–98.5)</td>
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<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>2</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mumps IgG</td>
<td>Positive</td>
<td>438</td>
<td>100 (98.9–100)</td>
<td>84.6 (72.2–92.3)</td>
<td>98.2 (96.5–99.1)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rubella IgG</td>
<td>Positive</td>
<td>452</td>
<td>97.8 (96.0–98.9)</td>
<td>100 (88.5–100)</td>
<td>97.6 (95.8–98.7)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7</td>
<td>36</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Varicella IgG</td>
<td>Positive</td>
<td>469</td>
<td>100 (99.0–100)</td>
<td>100 (85.0–100)</td>
<td>100 (99.1–100)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CI = confidence interval.

\(^{a}\) Equivocal results by the AtheNA multiplex assay were considered negative for calculating percentage sensitivity and positive for calculating percentage specificity.
Serologic testing is a convenient approach for determining immune status and assists in making important decisions regarding patient management, treatment, and potential revaccination. Serology is also instrumental in monitoring vaccine coverage, failures, and waning immunity in epidemiological surveys for global disease eradication initiatives (Kancherla and Hanson, 2006; Mulholland, 2006; WHO, 2009). In recent years, many clinical laboratories have used commercially available EIA or ELFA assays to measure the IgG-class antibody response against the components of MMRV. These methods require that each analyte be tested using a separate assay, which likely increases cost, sample volume requirements, as well as instrument and technologist time.

To our knowledge, this is the first evaluation of a multiplex bead immunoassay for the determination of IgG-class antibodies to MMRV. Our study showed excellent performance of the MMRV IgG Athena Multi-Lyte assay when compared to the results of routine testing, demonstrating near-perfect agreement for measles (κ = 0.83), mumps (κ = 0.91), and rubella (κ = 0.82), and perfect agreement for varicella (κ = 1.0). In addition to evaluating analytic performance, we also compared the sample volume requirements for EIA/ELFA versus the AtheNA multiplex assay. Taking into account the dead-volume requirements for automated testing, the measurement of IgG-class antibodies to MMRV by EIA/ELFA requires approximately 600 μl of serum. In contrast, the AtheNA Multi-Lyte assay requires a dead volume of 200 μl, thereby reducing the total volume of sample needed by approximately 3-fold.

We also conducted preliminary cost and timing analyses for EIA/ELFA versus the AtheNA multiplex assay. Based on list reagent fees, and excluding technologist time, the overall cost for MMRV IgG testing by EIA/ELFA is $19.45. In contrast, the total cost to measure all 4 analytes by the AtheNA multiplex assay is $15.80 (approximately 19% cost reduction compared to EIA/ELFA). When comparing total test time, we estimated that a single EIA and ELFA automated analyzer can provide complete MMRV IgG results of 90 samples in 11.4 h, versus 3.5 h by the AtheNA Multi-Lyte assay. This translates into a approximately 69% reduction in total analytic time when testing is performed on the multiplex platform.

This study has several limitations. First, our study used nonclinically characterized serum samples that were submitted to our reference laboratory for MMRV IgG testing. Therefore, percentage agreement and sensitivity/specificity calculations were performed by comparison to a predicate/reference method (e.g., EIA or ELFA) with samples showing discordant results being further evaluated by a third assay. Second, multiplex testing was performed using 1 AIMS instrument and a single lot of reagents, so the potential for inter- and intralaboratory variability was not assessed.

Despite these limitations, the MMRV IgG Athena Multi-Lyte assay has several advantages over current methods, including a higher throughput, lower sample volume requirement, and lower cost. In addition, the AtheNA MMRV multiplex assay includes internal controls that verify the addition of sample and enhance quality assurance. The AtheNA Multi-Lyte system also has the capability to report individual or multiple results on a single sample, allowing for customization of ordering. Additionally, the AtheNA multiplex software allows the laboratory to retrospectively retrieve results if a test “add-on” is later requested. Finally, the capacity to perform multiplex analysis using a single system may reduce errors associated with aliquoting samples and performing testing on multiple platforms.

Acknowledgments

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References