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Performance Characteristics of a Multiplex Flow Immunoassay for Detection of IgG-Class Antibodies to Measles, Mumps, Rubella, and Varicella-Zoster Viruses in Presumptively Immune Health Care Workers

Elitza S. Theel,[a] Marisa Sorenson,[a] Crystal Rahman,[a] Dane Granger,[a] Andrew Vaughn,[b] Laura Breeher[b]

[a]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
[b]Preventive, Occupational, and Aerospace Medicine, Mayo Clinic, Rochester, Minnesota, USA

ABSTRACT Immunity to measles, mumps, rubella, and varicella-zoster viruses (VZV; MMRV) is a common condition of employment for health care workers (HCWs) to ensure compliance with national standards and state laws. When documentation of complete vaccination or laboratory-confirmed infection is not available, Advisory Committee on Immunization Practices (ACIP) criteria are used to guide vaccination or anti-MMRV IgG testing. We assessed the performance of the BioPlex 2200 MMRV IgG multiplex flow immunoassay (MFI; Bio-Rad Laboratories, Hercules, CA) and matched immunofluorescence assays (IFAs; MBL Bion, Des Plaines, IL) in 220 HCWs categorized by ACIP criteria for presumptive immunity to MMRV. Among HCWs presumptively immune to measles, mumps, rubella, and VZV, the Bio-Rad MFI was positive in 77.3, 85.4, 84.3, and 91.1% of HCWs, respectively. Comparatively, the Bion IFA was positive in 92.9, 91.1, and 93.5% of HCWs presumptively immune to measles, mumps, and VZV (a rubella IFA was unavailable). Among HCWs fully vaccinated against measles, mumps, and VZV, Bio-Rad MFI/Bion IFA positivity rates were 77.4%/93%, 84.8%/90.7%, and 54.5%/90.9%, respectively. The Bio-Rad MFI was positive in 83.7% of HCWs fully vaccinated against rubella. For HCWs whose last vaccination event occurred within 15 years of enrollment, 83.3, 93.3, and 74.2% were positive by the Bio-Rad measles, mumps, and rubella IgG MFIs, respectively. We show significantly decreased Bio-Rad MFI sensitivity for detection of anti-measles and anti-mumps IgG-class antibodies in presumptively immune or fully vaccinated HCWs. Although negative results typically prompt revaccination, failure to recognize presumptive immunity in individuals unable to receive live, attenuated vaccines may have employment implications.

KEYWORDS MMRV, multiplex immunoflow assay, serology

In 2019, more than 1,200 cases of measles and 2,600 cases of mumps were confirmed in the United States by the Centers for Disease Control and Prevention (CDC), collectively reigniting a national discussion on the importance of vaccination to stem the transmission and spread of these highly contagious viral infections (1, 2). In the health care setting, given the higher exposure risk and the significant risk of morbidity and mortality in unvaccinated or immunosuppressed patients, health care workers (HCWs) are assessed for immunity to measles, mumps, rubella, and varicella-zoster viruses (MMRV) as part of preemployment onboarding. The CDC and the Advisory Committee on Immunization Practices (ACIP) have established standardized criteria for evidence of presumptive immunity to MMRV in HCWs, which, if met, indicate that the HCW has a sufficiently high likelihood of possessing protective immunity, making them unlikely to acquire or transmit the infection (3, 4). Assessment of MMRV immunity is commonly done at the time of hire by verifying the completion of vaccinations or

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Address correspondence to Elitza S. Theel, theel.elitza@mayo.edu.

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documentation of a laboratory-confirmed infection. If proof of presumptive immunity is incomplete or not available, vaccination is typically offered. Although vaccination is a straightforward remedial action, the use of live, attenuated vaccines, such as MMRV, is contraindicated in select populations, including pregnant women and certain immunosuppressed individuals (3). Therefore, anti-MMRV IgG testing may be used in an effort to document compliance with infection control standards.

The current measles and mumps vaccines are composed of live attenuated viruses, including the Edmonston-Enders strain and the Jeryl Lynn strain, respectively. While these may be given individually, they are more frequently administered in combination, as a single vaccine, which also contains the live attenuated rubella vaccine strain (RA 27/3), or also in combination with the Oka varicella-zoster virus (VZV) live attenuated vaccine strain, generally referred to as the trivalent measles-mumps-rubella (MMR) or quadrivalent MMR-VZV (MMRV) vaccines, respectively. For otherwise immunocompetent children (over 12 months of age) and adults, the CDC and the ACIP recommend two doses of the MMR or MMRV vaccines be administered at least 4 weeks apart for optimal efficacy, which ranges from 84 to $\geq$99% (median, 97%) for measles, 66 to 95% (median, 88%) for mumps, 94 to 100% (median, 97%) for rubella (one dose), and $>$95% for VZV (3, 4). Although use of live, attenuated viral vaccines mimics natural infection, which is associated with lifelong protection, waning immunity in fully vaccinated individuals, particularly to measles and mumps, is of increasing concern (5–9).

Routine preemployment laboratory testing of HCWs to document IgG seroconversion following vaccination or to confirm prior infection is not recommended due to various limitations associated with available serologic assays. The reference methods for determining immunity remain based on detection of neutralizing antibodies using plaque reduction neutralization tests (PRNTs), hemagglutination inhibition (HI), or fluorescent antibody-to-membrane antigen (FAMA) assays, and titers consistent with protective immunity have been established for measles (PRNT titer $\geq$1:120 mIU/ml), rubella (HI titer $\geq$1:8 [prior to 1996], or 10 IU/ml using an enzyme immunoassay [EIA] calibrated to the World Health Organization [WHO] anti-rubella immunoglobulin [Ig] standard) and VZV (FAMA titer $\geq$1:4) (10–15). A cutoff titer for protective immunity against mumps has not yet been determined. Despite these defined levels, PRNT, HI, and FAMA are generally not available outside select public health laboratories or the CDC due to their labor-intensive and technically challenging nature and prolonged turnaround times. As an alternative, commercially available EIAs were developed for detection of IgG-class antibodies to these vaccine-preventable diseases (VPDs). However, compared to neutralization testing, the sensitivities of EIA-based methods in fully vaccinated individuals are notably lower and varied across assays (11, 16). As a result of these findings, the ACIP indicates that documented vaccination or prior infection supersedes negative or equivocal IgG serologic results for these VPDs and that testing for IgG against MMRV is only recommended for HCWs who lack other evidence of immunity (3, 4).

In our laboratory, testing for IgG to MMRV is performed on the BioPlex 2200 platform, a multiplex flow immunoassay (MFI), using the MMRV IgG kit (Bio-Rad Laboratories, Hercules, CA). Although previous evaluation studies have shown comparable results of the Bio-Rad MMRV IgG assays to respective virus-specific EIAs, these comparison studies were done using uncharacterized clinical samples (i.e., no knowledge of vaccination status, history of disease, patient immune status, etc.), limiting the ability to draw conclusions regarding the clinical accuracy of these assays in individuals considered presumptively immune by the CDC/ACIP criteria (17). Here, we use well-characterized sera from HCWs to assess the sensitivity of the Bio-Rad MMRV IgG assays and individual anti-measles, mumps, and VZV IgG immunofluorescence assays (IFAs; MBL Bion, Des Plaines, IL) in comparison to evidence of presumptive immunity as defined by the CDC/ACIP, individual vaccination status, and years after the last vaccination event for each HCW.

MATERIALS AND METHODS

Study design. During October 2018, 220 adult HCWs at a large tertiary academic medical center in the midwest United States consented to participate in this study. The HCWs had to be 18 years or older, born after 1967 (i.e., following the introduction of the mumps vaccine) and must not have received intravenous immunoglobulin or a blood transfusion in the past 6 months. Participants provided 2 ml of blood, collected in a serum separator tube, and were required to complete a study questionnaire which included the following questions: (i) have you had a history of adverse vaccine reaction; (ii) do you have an autoimmune disorder, and, if yes, indicate what the disorder is; (iii) have you had a stem cell or solid organ transplant; (iv) are you currently on any immunosuppressive therapy to prevent organ rejection or to treat an autoimmune or inflammatory disease; and (v) do you have a history of having employment opportunities affected by immunization requirements.

Participant demographics were documented and occupational vaccination records were reviewed to establish presence or absence of presumptive immunity to measles, mumps, rubella, and VZV based on current CDC/ACIP criteria (3, 4, 18). Evidence of immunity to measles or mumps viruses in this HCW population includes either (i) documentation of vaccination with two doses of live measles or mumps virus-containing vaccine or (ii) laboratory evidence of immunity or confirmation of disease. Evidence of HCW immunity to rubella virus includes either (i) documentation of vaccination with at least one dose of live rubella virus-containing vaccine or (ii) laboratory evidence of immunity or confirmation of disease. Finally, evidence of HCW immunity to VZV includes any one of the following: (i) documentation of vaccination with two doses of live VZV-containing vaccine, (ii) laboratory evidence of immunity or confirmation of diseases, or (iii) diagnosis or verification of a history of varicella disease or herpes zoster by a health care provider.

All serum samples were tested upon receipt to the laboratory, in a blinded fashion, for the presence of IgG-class antibodies to measles, mumps, rubella, and VZV using the BioPlex 2200 MMRV multiplex flow immunoassay (MFI; Bio-Rad, Hercules, CA) and by immunofluorescence assays (IFAs), with the exception of rubella, from MBL Bion (Des Plaines, IL). The results of both assays were compared against each other and to evidence of immunity to measles, mumps, rubella, and VZV as defined by current CDC/ACIP criteria. This study was approved by the Mayo Clinic Institutional Review Board.

BioPlex 2200 MMRV IgG assay. The BioPlex MMRV IgG assays (Bio-Rad Laboratories) are approved by the U.S. Food and Drug Administration for simultaneous detection of IgG-class antibodies to MMRV in sera using an MFI. Testing was performed on serum samples in accordance with manufacturer instructions and as described previously (17). Briefly, on the BioPlex 2200 instrument (Bio-Rad), a patient sample is mixed with seven different types of dyed beads, three of which are used for control purposes (i.e., serum verification bead, internal standard bead and reagent blank bead) and four of which are used for the detection of IgG-class antibodies to each of the four MMRV analytes. The presence or absence of antibodies is determined using flow cytometry, with an initial relative fluorescence intensity determined, followed by calculation of a fluorescence ratio (FR) based on the internal standard control bead. The antibody index (AI) value is subsequently established for each analyte by comparison of the analyte-specific FR to an assay-specific calibration curve. The qualitative interpretive criteria, as established by the manufacturer, for measles, mumps, and VZV are as follows: positive, $\geq$1.1 AI; equivocal, 0.9 to 1.0 AI; and negative, $\leq$0.8 AI. According to the manufacturer, negative results indicate that the patient is presumed to not have had prior exposure to measles, mumps, or VZV, whereas positive results "... may indicate that the patient was exposed to measles, mumps, or VZV through infection or vaccination." The interpretive criteria for the Bio-Rad anti-rubella IgG MFI component, which is calibrated against the WHO First International anti-rubella Ig standard (RUBI-1-94), are as follows: positive, $\geq$1.0 AI; equivocal, 0.8 to 0.9 AI; and negative, $\leq$0.7 AI. Positive results for rubella indicate that "IgG antibody levels are at a level considered to indicate positive immunity" (19).

MBL Bion measles, mumps, and VZV IFAs. Detection of IgG-class antibodies to measles, mumps and VZV was also performed using *in vitro* diagnostic reagents and slides from MBL Bion (Des Plaines, IL). Briefly, patient sera were diluted in phosphate-buffered saline (PBS) and doubling dilutions thereafter. Diluted sera were subsequently applied to 12-well slides with either measles virus-, mumps virus-, or VZV-infected and fixed cells, followed by incubation in a moist chamber at room temperature (35 to 37°C) for 30 min. Slides were subsequently rinsed with PBS and incubated with fluorescein isothiocyanate-labeled goat antihuman IgG conjugate (with Evans blue counterstain) for 30 min in a moist chamber at room temperature (35 to 37°C). Finally, slides were washed with PBS and evaluated for fluorescence intensity using a fluorescence microscope. The fluorescence intensity is characterized using the manufacturer-recommended grading scale, with "1+ fluorescence," defined as very dim and subdued fluorescence, considered the minimum level of fluorescence necessary to determine a sample as positive for the presence of antibodies to the analyte of interest. The endpoint titer for each sample was defined as the final dilution at which a 1+ fluorescence intensity is observed. Samples with a fluorescence intensity of less than 1+ at the 1:10 dilution were considered negative for IgG antibodies to the virus of interest.

Statistics. Sensitivity, specificity, agreement, Cohen's kappa, and 95% confidence intervals (CIs) were determined using GraphPad QuickCalcs software (La Jolla, CA). This software was also used to perform McNemar's test to compare sensitivities of the BioPlex MFI and Bion IFA anti-measles, anti-mumps, and anti-VZV IgG assays in all presumptively immune HCWs according to CDC/ACIP criteria and separately, in fully vaccinated HCWs. *P* values of $<$0.05 were considered statistically significant. For statistical analysis, equivocal results by the Bio-Rad MMRV IgG assay were considered "negative" in accordance with ACIP recommendations (3). Kappa values of $<$0.20, 0.21 to 0.40, 0.41 to 0.60, 0.61 to 0.80, or 0.81 to 1.0 were considered to indicate poor, fair, moderate, good, or very good inter-rater agreement, respectively.

TABLE 1 Demographics and vaccination status of enrolled HCWs ($n = 220$)[a]

Parameter	Result
Age range, yr (median)	21–51 (37)
No. (%) female	150 (68.2)
No. (%) of HCWs with an autoimmune disorder	22[d] (10)
GI tract autoimmune disease[b]	8
Hashimoto's thyroiditis	5
Rheumatoid arthritis	4
Idiopathic thrombocytopenic purpura	2
Other[c]	8
No. of HCWs on immunosuppressive treatment	10
No. (%) of HCWs meeting CDC/ACIP criteria for presumptive immunity to:	
Measles	211 (96)
Mumps	205 (93)
Rubella	216 (98)
VZV	124 (56)

[a]Abbreviations: HCWs, health care workers; VZV, varicella-zoster virus; CDC, Centers for Disease Control and Prevention; ACIP, Advisory Committee on Immunization Practices.
[b]These include Crohn's disease ($n = 1$), celiac disease ($n = 1$), irritable bowel disease ($n = 3$), and ulcerative colitis ($n = 3$).
[c]The category "Other" includes autoimmune inner ear disease ($n = 1$), cutaneous lupus ($n = 1$), eczema ($n = 1$), Raynaud's disease ($n = 1$), type I diabetes mellitus ($n = 1$), Sjogren's syndrome ($n = 1$), and undifferentiated autoimmune condition ($n = 2$).
[d]Some individuals were counted twice because they had multiple comorbidities.

RESULTS

HCW demographics. Consenting HCWs ranged in age from 21 to 51 years old (median, 37 years), and 68.2% (150/220) were female (Table 1). Among the 220 HCWs, 22 (10%) reported an autoimmune disorder for which five were on an immunosuppressive regimen at the time of specimen collection. A review of employee health records indicated that CDC/ACIP criteria for HCW presumptive immunity to measles, mumps, rubella, and VZV were met for 211 (96%), 205 (93%), 216 (98%), and 124 (56%) HCWs, respectively (Table 1). A total of 197 HCWs had a documented history of receiving two doses of the measles and mumps vaccines, either individually or combined, and at least one dose of the rubella vaccine. Of the remaining 23 HCWs, 7 had prior laboratory documentation of immunity to measles, mumps, and rubella; 14 had mixed immunity to the measles, mumps, and/or rubella; and 2 were considered nonimmune to all three. Among the 124 HCWs who met the CDC/ACIP criteria for immunity to VZV, 93 had prior positive anti-VZV IgG titers, 3 had documentation of a single VZV vaccination event and a positive anti-VZV IgG result, and 11 had received two doses of the VZV vaccine. Of the 96 HCWs who did not meet the criteria for VZV immunity, 91 self-reported a prior VZV infection, which, alongside a negative prior IgG result for VZV, is not acceptable evidence of presumptive immunity according to the current CDC/ACIP guidelines. Five HCWs did not report past infection and were negative on prior anti-VZV IgG testing.

Performance of the Bio-Rad MFI and Bion IFA methods in HCWs for detection of IgG against disease. (i) Measles. Among the 211 HCWs who met current CDC/ACIP criteria for presumptive immunity to measles, the Bio-Rad MFI was positive in 163 individuals, with an overall sensitivity of 77.3% (163/211) (Table 2). In comparison, the Bion IFA showed a significantly higher sensitivity of 92.9% (196/211) for the detection of anti-measles IgG in these same HCWs ($P = <0.001$). Of the 199 employees with a documented history of at least two measles vaccine doses, the Bio-Rad MFI and Bion IFA methods were positive in 154 (77.4%) and 185 (93%) HCWs, respectively (Table 3). A head-to-head comparison of the Bio-Rad MFI and Bion IFA in these fully vaccinated HCWs showed positive, negative, and overall agreement of 79.5% (147/185), 50% (7/14), and 77.4% (154/199), respectively, with a kappa value of 0.15, indicating poor inter-rater agreement (see Table S1 in the supplemental material).

TABLE 2 Performance of the Bio-Rad MFI and Bion IFA methods for detection of IgG to measles, mumps, rubella and VZV in HCWs with and without evidence of presumptive immunity to these viruses as defined by the 2018 CDC/ACIP criteria[a]

Test and disease antibody	Test result	No. of HCWs with presumptive immunity to measles, mumps, rubella, or VZV		% Sensitivity or agreement (95% CI)	
		Yes	No	Sensitivity	Overall agreement
Bio-Rad MFI					
Measles IgG	Positive	163	7	77.3[f] (71.1–82.4)	75 (68.9–80.3)
	Negative	43	2		
	Equivocal	5	0		
Mumps IgG	Positive	175	11	85.4[g] (79.8–89.6)	81.4 (75.7–86)
	Negative	26	4		
	Equivocal	4	0		
Rubella IgG	Positive	182	4	84.3 (78.8–88.6)	82.7 (77.2–87.2)
	Negative	17	0		
	Equivocal	17	0		
VZV IgG	Positive	113	89[d]	91.1[h] (84.7–95.1)	54.1 (47.5–60.6)
	Negative	8	6		
	Equivocal	3	1		
Bion IFA					
Measles IgG	Positive	196	9	92.9[f] (88.5–95.7)	89.1 (84.2–92.6)
	Negative	15	0		
Mumps IgG[b]	Positive	184	13	91.1[g] (86.3–94.4)	85.7 (80.4–89.8)
	Negative	18	2		
VZV IgG[c]	Positive	115	91[e]	93.5[h] (87.5–97.2)	54.6 (48–61.1)
	Negative	8	4		

[a]Abbreviations: VZV, varicella-zoster virus; CI, confidence interval; CDC, Centers for Disease Control and Prevention; ACIP, Advisory Committee on Immunization Practices. Evidence of health care worker (HCW) immunity to measles, mumps, rubella, or VZV as defined in Materials and Methods according to CDC/ACIP criteria. Per CDC recommendations, equivocal results were interpreted as negative for this evaluation.
[b]Three samples gave nonspecific fluorescence by the IFA and were not included in the analysis.
[c]Two samples gave nonspecific fluorescence by the IFA and were not included in the analysis.
[d]A total of 85 of these HCWs self-reported prior VZV infection (i.e., chickenpox or shingles). Self-reported VZV infections do not meet current criteria for evidence of HCW immunity to VZV.
[e]A total of 87 of these HCWs self-reported prior VZV infection (i.e., chickenpox or shingles). Self-reported VZV infections do not meet current criteria for evidence of HCW immunity to VZV.
[f]$P = 0.0005$.
[g]$P = 0.031$.
[h]$P = 0.58$.

(ii) Mumps. The Bio-Rad MFI had a sensitivity of 85.4% (175/205) for detection of anti-mumps IgG among the 205 HCWs who met CDC/ACIP criteria for presumptive immunity to mumps, which was significantly lower than the 91.1% (184/202) sensitivity achieved by the Bion IFA ($P = 0.031$; Table 2). A total of 197 HCWs had documentation

TABLE 3 Detection of anti-measles IgG by the Bio-Rad MFI and Bion IFA methods compared to documented measles vaccination history ($n = 220$)

Method	Result	No. of HCWs		
		Completed two-dose measles vaccine ($n = 199$)	Incomplete measles vaccination record	
			One dose ($n = 12$)	No vaccination ($n = 9$)[d]
Bio-Rad MFI	Positive	154	9	7
	Negative	40	3	2
	Equivocal	5	0	0
Bion IFA	Positive	185[a]	11[b]	9[c]
	Negative	14	1	0

[a]The IFA titer range for positive samples was 1:10 to ≥1:320, with a median titer of 1:40.
[b]The IFA titer range for positive samples was 1:10 to 1:80, with a median titer of 1:20.
[c]The IFA titer range for positive samples was 1:10 to 1:160, with a median titer of 1:40.
[d]The HCWs were born between 1967 and 1988. Seven HCWs had a previously documented anti-measles IgG positive result.

TABLE 4 Detection of anti-mumps IgG by the Bio-Rad MFI and Bion IFA methods compared to documented mumps vaccination history ($n = 220$)

Method	Result	No. of HCWs		
		Completed two-dose mumps vaccine ($n = 197$)	Incomplete mumps vaccination	
			One dose ($n = 11$)	No vaccination ($n = 12$)[e]
Bio-Rad MFI	Positive	167	8	11
	Negative	26	3	1
	Equivocal	4	0	0
Bion IFA[a]	Positive	176[b]	10[c]	11[d]
	Negative	18	1	1

[a]A total of 197 HCWs had documented two-dose mumps vaccination; however, IFA yielded nonspecific fluorescence results for three HCWs. These three samples were not included in the analysis.
[b]The IFA titer range for positives was 1:10 to ≥1:320 with a median titer of 1:40.
[c]The IFA titer range for positives was 1:10 to 1:80 with a median titer of 1:40.
[d]The IFA titer range for positives was 1:10 to 1:80 with a median titer of 1:20.
[e]The HCWs were born between 1967 and 1988. Five HCWs had a previously documented anti-mumps IgG positive result.

of a two-dose mumps vaccination history; of these, 84.8% (167/197) were positive by the Bio-Rad MFI assay, and 90.7% (176/194) were positive by the Bion IFA (the Bion IFA showed nonspecific fluorescence in 3 of these 197 HCWs which were excluded) (Table 4). Direct comparison of the Bio-Rad MFI and Bion IFA methods in the 194 HCWs with valid results by both assays showed positive, negative, and overall percent agreements of 89.2% (157/176), 61.1% (11/18), and 86.1% (167/194), respectively, with a kappa value of 0.37, suggesting fair inter-rater agreement (Table S2).

(iii) Rubella. Presumptive immunity to rubella was established for 216 of the enrolled HCWs and the Bio-Rad MFI was positive for 182 of these employees, for an overall sensitivity of 84.3% (Table 2). Among the 209 HCWs with at least one documented rubella vaccination event, the Bio-Rad MFI assay was reactive in 83.7% of employees (Table 5). IFA for anti-rubella IgG was not available at the time of this study.

(iv) VZV. Among the 124 HCWs who met the CDC/ACIP criteria for immunity to VZV, the Bio-Rad MFI was positive in 113 employees, leading to a sensitivity of 91.1%, similar to the 93.5% (115/123) sensitivity observed by the Bion IFA ($P = 0.58$; Table 2). Only 11 of the 220 HCWs had documentation of two VZV vaccine doses, and among these employees, the Bio-Rad VZV IgG MFI was positive in 6 (54.5%) HCWs, while the Bion IFA was positive in 10 (90.9%) (Table 6). Comparison of the Bio-Rad MFI to the Bion IFA in all HCWs with valid results by both assays ($n = 218$) showed positive, negative, and overall percent agreements of 94.2% (194/206), 50% (6/12), and 91.3% (199/218), respectively, and a kappa value of 0.33, which is indicative of fair inter-rater agreement (Table S3).

Performance of the Bio-Rad MFI and Bion IFA MMRV IgG detection methods relative to last vaccination event. Fully vaccinated HCWs (i.e., at least two-dose vaccination documented for measles and mumps and at least one-dose vaccination

TABLE 5 Detection of anti-rubella IgG by the Bio-Rad MFI assay compared to documented rubella vaccination history ($n = 220$)

Bio-Rad MFI result	No. of HCWs	
	Received ≥1 dose of rubella vaccine ($n = 209$)[a]	No record of rubella vaccination ($n = 11$)
Positive	175	11[b]
Negative	17	0
Equivocal	17	0

[a]A total of 14 employees had documented evidence for one dose of MMR vaccine; all 14 were found to be positive by the BioPlex 2200 Rubella IgG assay.
[b]The HCWs were born between 1967 and 1988. Seven HCWs had a previously documented anti-rubella IgG positive result.

TABLE 6 Detection of anti-VZV IgG by the Bio-Rad MFI and Bion IFA methods compared to documented VZV vaccination history ($n = 220$)

Method	Result	No. of HCWs		
		Completed two-dose varicella vaccine ($n = 11$)	Incomplete varicella vaccination	
			One-dose varicella vaccine ($n = 3$)	No varicella vaccine ($n = 206$)[a]
Bio-Rad MFI	Positive	6	2	194
	Negative	4[b]	1	9
	Equivocal	1[c]	0	3
Bion IFA[d]	Positive	10[e]	3[f]	193[g]
	Negative	1	0	11

[a]A total of 93 employees had a previously positive VZV IgG test on record, including 88 (94.6%) and 85 (91.4%) determined to be positive by the Bio-Rad MFI and IFA VZV IgG assays, respectively.
[b]All four samples were positive by the Varicella IgG IFA with a titer range of 1:10 to 1:40 (median, 1:10).
[c]This sample was negative by the varicella IgG IFA.
[d]Two samples showed nonspecific fluorescence and were not included in this evaluation.
[e]Varicella IgG titers ranged from 1:10 to 1:80, with a median titer of 1:20.
[f]Varicella IgG titers ranged from 1:10 to 1:40, with a median titer of 1:40.
[g]Varicella IgG titers ranged from 1:10 to ≥1:320, with a median titer of 1:40.

documented for rubella) were divided into three groups based on date of last vaccination event: group A, last vaccination dose administered within 15 years of enrollment (date range, 2003 to 2018); group B, last vaccination dose received 16 to 25 years prior to enrollment (date range, 1993 to 2002); and group C, complete vaccination occurred >25 years prior to enrollment (date range, 1977 to 1992). Sensitivity of the Bio-Rad MFI to detect anti-measles IgG in fully vaccinated HCWs trended downward, ranging from 83.3% (25/30) in group A to 75.4% (43/57) in group C (Table 7). The Bion anti-measles IgG IFA was positive in 93.3% (28/30), 93.8% (105/112), and 91.2% (52/57) of HCWs in groups A, B, and C, respectively, and was significantly more sensitive than the Bio-Rad MFI in group B and C HCWs who completed two-dose measles vaccinations 16 to 25 years ($P < 0.001$) and ≥26 years ($P = 0.026$) prior to enrollment (Table 7). The sensitivity of the Bio-Rad MFI to detect anti-mumps IgG was higher in group A HCWs (93.3%; 28/30) than in group B (82.1%; 92/112) and group C (85.5%; 47/55) employees, while the Bion IFA anti-mumps IgG showed consistently higher sensitivity (88.3 to

TABLE 7 Performance of the Bio-Rad MFI and Bion IFA for detection of IgG-class antibodies to measles, mumps, and rubella in fully vaccinated HCWs relative to years after the final vaccination event[a]

Patient category	% positive HCWs (95% CI)		*P*
	Bio-Rad MFI	Bion IFA	
Last vaccination event occurring ≤15 yrs prior to enrollment (2003–2018)			
Measles IgG ($n = 30$)	83.3 (66–93)	93.3 (77.6–99.2)	0.37
Mumps IgG ($n = 30$)	93.3 (77.6–99.2)	96.7 (81.9–100)	1.00
Rubella IgG ($n = 31$)	74.2 (56.5–86.5)	NA	NA
Last vaccination event occurring 16 to 25 yrs prior to enrollment (1993 to 2002)			
Measles IgG ($n = 112$)	76.8 (68.1–83.7)	93.8 (87.4–97.2)	0.0005
Mumps IgG ($n = 111$)	82.1 (73.9–88.2)	88.3 (80.9–93.2)	0.21
Rubella IgG ($n = 112$)	84.8 (76.9–90.4)	NA	NA
Last vaccination event occurring ≥26 yrs prior to enrollment (1977 to 1992)			
Measles IgG ($n = 57$)	75.4 (62.8–84.9)	91.2 (80.6–96.6)	0.026
Mumps IgG ($n = 53$)	84.9 (72.7–92.4)	94.3 (84.0–98.7)	0.14
Rubella IgG ($n = 55$)	83.6 (71.5–91.4)	NA	NA

[a]VZV was excluded from this analysis since there were only 11 enrolled HCWs with documentation of a two-dose VZV vaccine. NA, not applicable.

96.7%) across all three groups, although these differences were not statistically significant (Table 7). Finally, sensitivity of the Bio-Rad anti-rubella IgG MFI was lowest in group A HCWs (74.2%; 23/31) and was higher in groups B (84.8%; 95/112) and C (83.6%; 46/55).

DISCUSSION

Assessment of immunity to VPDs, such as MMRV, is a necessary component of preemployment onboarding for HCWs. For individuals born after 1957, this is established by providing vaccination records or documentation of prior, laboratory-confirmed clinical illness. For those unable to provide evidence of immunity, remedial vaccination can be performed, unless contraindicated, in which case evaluation for IgG-class antibodies to MMRV is pursued, typically using readily accessible assays (e.g., EIA-, IFA-, or MFI-based methods) in the clinical laboratory. Among these assays, the Bio-Rad MFI-based anti-MMRV IgG assay was previously shown to perform equivalent to analyte-matched EIAs, with overall agreement ranging from 91 to 94% for detection of anti-MMRV IgG in non-clinically characterized serum samples (17). Here, we have expanded on these findings by evaluating performance of the Bio-Rad MMRV IgG MFI assay in sera from HCWs classified as presumptively immune according to the CDC/ACIP criteria, including HCWs with documented receipt of at least two MMR or MMRV vaccine doses.

Overall, we show that among the four individual components of the Bio-Rad MMRV IgG MFI assay, the VZV IgG component had the highest sensitivity (91%) for the detection of anti-VZV IgG in HCWs who met the CDC/ACIP criteria for presumptive immunity, and this was similar to the sensitivity achieved by the Bion anti-VZV IgG IFA (94%). In contrast, among HCWs presumptively immune to measles, the Bio-Rad anti-measles IgG MFI showed significantly lower sensitivity, yielding a positive result in only 77% of sera, compared to a positivity rate of 93% by the Bion anti-measles IgG IFA. The Bio-Rad anti-measles IgG positivity rate was notably lower than the previously reported 90% seropositivity rate in HCWs born between 1960 and 1995 in the Netherlands and was also lower than the seropositivity rate (86%) documented for all adults tested for measles IgG at a national reference laboratory in the United States (16, 20). Although the populations tested differ between our study and the latter (i.e., HCWs versus any adult), the median ages of the tested individuals were similar, 37 versus 34 years, minimizing the possibility that waning immunity due to age discrepancies may account for the difference in positivity rates. Overall, the sensitivity of the Bio-Rad anti-mumps IgG MFI among presumptively immune HCWs was 85% in our study, which was also significantly lower than the Bion IFA positivity rate (91%) in this group and yet was higher than that previously reported (79%) using alternative EIAs across the general population (20). Also, although a matched IFA for anti-rubella IgG was unavailable, the Bio-Rad anti-rubella MFI was positive in 84% of presumptively immune HCWs, which is somewhat lower than that documented among adults (91%) in prior studies (20). Collectively, these data suggest lower sensitivity rates for the Bio-Rad MMRV IgG MFI and notable performance differences across the various methods available for detection of IgG-class antibodies to these VPDs.

The majority of enrolled HCWs (90 to 95%) met the CDC/ACIP criteria for presumed MMR immunity due to documented receipt of at least two doses of the MMR or MMRV vaccines. We therefore assessed performance of the Bio-Rad anti-measles, mumps, and rubella IgG MFIs in this subset of fully vaccinated HCWs. The highest overall sensitivity, irrespective of time since last vaccination event, was achieved by the anti-mumps and anti-rubella IgG MFIs at approximately 85 and 84%, respectively, compared to 77% sensitivity for the anti-measles IgG MFI. Previous postvaccination studies show that 10 to 15 years following the second MMR vaccine dose, despite overall declining antibody titers, approximately 95 to 100%, 74 to 95%, and 83 to 100% of individuals remain seropositive for anti-measles, mumps, and rubella IgG-class antibodies, respectively (7, 21–23). To evaluate whether time between the final documented vaccination date and testing for this study impacted the sensitivity of the Bio-Rad MFI MMRV IgG assays in

fully vaccinated HCWs, we assessed the performance of this method relative to years after the last vaccination event. Among HCWs who received their final MMR or MMRV dose within 15 years of enrollment in our study, 93% were determined to be positive by the Bio-Rad anti-mumps IgG MFI, whereas only 83 and 74% were determined to be positive by the anti-measles and anti-rubella IgG MFIs, respectively, within this same time frame. Similar to published studies, we observed downward trending of anti-measles and rubella IgG positivity rates by the Bio-Rad MFI among HCWs with a final vaccination event occurring 16 to 25 years or >25 years prior to study enrollment.

The observed discrepancy in seropositivity rates among fully vaccinated HCWs evaluated within 15 years of the last vaccination event between our study and others may be related to multiple factors, including differences in study populations (i.e., adult HCWs versus fully vaccinated children), the size of the cohort, and differences related to the assay methods and thresholds used, all warranting further discussion. First, the differences in sensitivity between the Bio-Rad MMRV IgG MFIs and prior studies using neutralization assays may be related to the different viral antigens targeted by the assays; while PRNT-based assays detect neutralizing antibodies targeting surface glycoproteins of live viruses, commercially available assays are frequently designed to detect total IgG, typically against recombinant, purified, or lysate antigenic material (the antigen source for the Bio-Rad MMRV IgG MFI is not disclosed). Second, while thresholds for protective immunity have been established for anti-measles and anti-rubella IgG levels, an anti-mumps IgG threshold associated with protective immunity has not yet been defined (22). Therefore, while the higher anti-mumps IgG positivity rates observed for the Bio-Rad MFI and Bion IFA are consistent with the documented completion of a full MMR/MMRV vaccine series in our HCW cohort, whether these antibody levels are sufficient to provide protective immunity against reinfection is unknown. In addition, prior studies showing high anti-measles IgG seropositivity levels 10 years or longer postvaccination utilized neutralization assays or assays calibrated against a WHO anti-measles immunoglobulin standard, whereas the BioPlex anti-measles IgG MFI is not currently calibrated to a WHO standard, potentially impacting result accuracy. Notably, in a recent study using the third anti-measles immunoglobulin WHO standard, Hatchette et al. calibrated the BioPlex anti-measles IgG MFI assay, converting the qualitative MFI to provide a quantitative output, and showed excellent agreement with anti-measles IgG PRNT titers, enabling differentiation between patients with protective versus nonprotective immunity and identifying individuals who require additional testing (e.g., via PRNT) for confirmation (24). Finally, it is noteworthy that while the anti-rubella IgG component of the Bio-Rad MMRV MFI is the only assay calibrated against a WHO standard, it was associated with the lowest positivity rate (74%) among fully vaccinated HCWs tested within 15 years of the last vaccination event, a rate that is notably lower than previously reported rates (>90%) during this same time frame (7, 23). Interestingly, despite calibration of commercially available anti-rubella IgG assays against the current WHO anti-rubella immunoglobulin standard, significant variability in results has been documented across platforms, limiting the reliability of these assays to differentiate between immune and nonimmune status (25).

Our study has a number of limitations. The sensitivity of the Bio-Rad MMRV IgG MFI was not thoroughly evaluated either in immunosuppressed HCWs or in those with autoimmune disorders. Only 22 HCWs affirmed an autoimmune condition, 5 of whom were on an immunosuppressive regimen, and although 14 (64%) of these individuals had detectable IgG by the Bio-Rad MMRV MFI to all four analytes (data not shown), definitive conclusions regarding sensitivity in this subset of HCWs cannot be made. In addition, some HCWs may have had prior, undocumented vaccination events, which may have led to inaccurate categorization as nonimmune. Also, the distribution of HCWs across years after the last vaccination event was uneven, with only 30 HCWs falling within the ≤15-year group. Finally, due to lack of accessibility, reference standard neutralization studies were not performed during this evaluation, limiting our ability to state whether HCWs across the spectrum of years after the last vaccination event maintained protective antibody levels against these VPDs.

Overall, our findings suggest decreased sensitivity of the Bio-Rad MMRV MFI to detect IgG-class antibodies to a number of these VPDs in presumptively immune or fully vaccinated HCWs. Notably, there are multiple advantages to using the fully automated, open-access Bio-Rad BioPlex 2200 instrument to perform such testing compared to manual IFA methods, including shorter assay time (45 min versus 2 to 3 h), and an objective result output versus the visual and subjective nature of IFA fluorescence intensity interpretation by technologists. The implications of the observed lower sensitivity of the Bio-Rad MMRV MFI are not insignificant, however, and vary depending on how these assays are utilized. For epidemiologic studies, testing using this method may underestimate true seroprevalence rates, potentially leading to unnecessary public health alarm. If used for otherwise healthy individuals undergoing preemployment occupational health evaluation who require anti-MMRV IgG testing due to an inability to provide alternative evidence of presumptive immunity, the implications of lower assay sensitivity are generally minimal; in the event of a negative result, these individuals would be revaccinated. Since vaccination is both safe and cost-effective, revaccination of these HCWs would boost individual immunity and contribute to maintaining high levels of herd immunity in the health care setting. Undercalling presumptive immunity by any anti-MMRV IgG assay, however, becomes problematic in individuals for whom revaccination with the live, attenuated MMR or MMRV vaccine is contraindicated, including in pregnant women and in certain immunosuppressed individuals (26). For these HCWs, negative results may require additional testing or may adversely impact employment opportunities.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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