BIO-RAD

White Paper

The Role of Serologic Testing in the Assessment of Immunity to Measles, Mumps, Rubella, and Varicella

Melanie L. Yarbrough, PhD, D(ABCC), D(ABMM)
Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, Missouri, USA

Introduction

The availability of vaccines for measles, mumps, rubella, and varicella-zoster (MMRV) viruses has resulted in a precipitous decline in the global incidence of these diseases. In spite of the wide availability of vaccines and nationwide efforts to increase vaccination coverage, many vaccine-preventable diseases (VPDs) have re-emerged as a threat to public health in the United States (1, 2). Reported cases of mumps have risen in recent years, with over 6,000 cases reported in 2016 (3). Outbreaks have occurred on college campuses and in other settings in which people are in close contact. Unlike mumps, local transmission of measles virus is usually the result of exposure to infected travelers. In 2015, a large, multi-state outbreak of measles resulted in 125 confirmed measles cases in the United States. At least 20% of patients were hospitalized during this outbreak (4).

The continued occurrence of outbreaks in countries with comprehensive vaccination programs indicates that these viruses remain in circulation among certain populations. Thus, susceptible individuals continue to be at risk of morbidity and mortality associated with VPDs such as measles. Knowledge of the immune status of individuals for VPDs is important to mitigate this risk. According to the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP), MMRV immunity can be established through documentation of vaccination history, prior history of laboratory-confirmed disease, or positive serologic testing (Figure 1) (5). Documentation of prior MMR vaccination supplants the results of serologic testing, and individuals who have received two doses of the MMR vaccine are presumed immune, even when negative serologic results are obtained. Thus, serologic testing is most useful in cases where no documentation of disease or vaccination history is available.

MMRV IgG assays

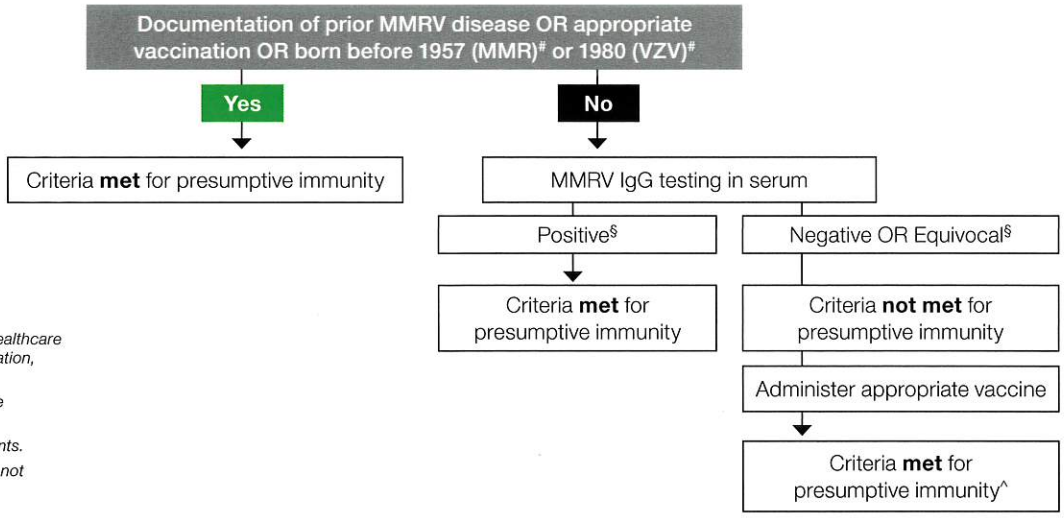
Plaque reduction neutralization tests (PRNTs) are considered the gold standard for demonstration of immunity through the assessment of neutralizing IgG antibody titers to infectious agents such as MMRV. However, these assays are labor intensive and not readily available in most clinical laboratories. Commercially available serologic assays for the detection of IgG that is specific for measles, mumps, rubella, or varicella are commonly in use. While detection of pathogen-specific IgG antibodies provides some evidence of immunity, it does not necessarily correlate with the presence of neutralizing antibodies or subsequent protection from disease, as both humoral and cellular responses may be involved and definitive serologic immune thresholds are not established for many VPDs. A 2013 study of 10,210 individuals who were tested simultaneously for measurable MMR IgG titers found seropositivity rates of 89.4%, 80.5%, and 93.4% of patients, respectively, for measles, mumps, and rubella (6). In total, only 72% of individuals were seropositive for all three viruses. Testing mumps IgG alone in this population would have resulted in missing 8.6% of individuals who lacked IgG to measles or rubella. Testing measles and mumps IgG alone, a common practice to establish immunity, failed to identify 2.6% of individuals with negative rubella IgG (6). Thus, simultaneous measurement of IgG against all 3 viruses helps ensure sufficient detection of non-immune individuals.

Key points to remember

- **Knowledge of immune status is important to mitigate the risk of transmission of vaccine preventable diseases.**
- **Serologic evidence for immunity is not necessary in individuals with documentation of prior MMRV immunization or disease history.**
- **Multiplex MMRV IgG assays are highly specific to limit the chance of false positive results and thus presumed immunity.**
- **At-risk individuals with negative or equivocal MMRV IgG results are considered non-immune and should receive vaccination.**

Assessment of MMRV serologic status is ideally accomplished with simultaneous measurement of multiple analytes using only one blood sample. One recent study that compared the Bio-Rad BioPlex 2200 MMRV multiplex assay to routine testing by enzyme immunoassays (EIA) in 500 prospectively collected samples sent to a reference laboratory found that the BioPlex 2200 MMRV assay demonstrated positive agreement with the EIA of 94.6%, 98.1%, 94.8%, and 92.2% for MMRV, respectively (7). The majority of the discrepant results fell in the equivocal zone for the EIA. Negative agreement between the MMRV assay and the EIA was 96.4%, 82.8%, 100%, and 100%, respectively, for MMRV IgG. The low negative agreement for mumps was due to four positive results on the BioPlex 2200 assay that tested negative using the comparator EIA method. Notably, repeat testing using a different method was positive for mumps on all four specimens, suggesting that the positive results on the BioPlex 2200 assay were true positives (7). An evaluation of the BioPlex 2200 assay for determination of varicella-zoster virus (VZV) immunity in 100 healthcare workers (HCWs) found

Figure 1. Assessment of evidence of MMRV immunity*



Footnotes:

* *Applies to routine vaccination and healthcare personnel. For more detailed information, see references 5, 12, and 13.*

\# *Criteria does not apply to healthcare personnel for rubella or VZV.*

§ *Result applies to individual viral agents.*

^ *Post-vaccination serologic testing is not necessary or recommended.*

that the sensitivity and specificity of the BioPlex 2200 MMRV assay were 96.8% and 85.7%, respectively, for VZV IgG when compared to gold standard (8). Interestingly, 1 of 3 false positive results also tested positive with a separate EIA, suggesting that the "false positive" on the BioPlex 2200 MMRV assay was actually a true positive. Together, these studies demonstrate that multiplex assays are highly specific and of comparable sensitivity to single-analyte EIA counterparts.

Current role of MMRV serologic testing

The clinical utility of MMRV screening assays has changed over time. Older assays were tuned to confirm a presumptive diagnosis of infection; hence, sensitivity was prioritized over specificity for these assays. In the post-vaccine era, assays that maximize specificity are now desirable to avoid the potential devastating consequences of a false positive result, in which a susceptible individual was presumed immune. On an individual level, this person would be at risk for acquiring MMRV infection after exposure to one of these viruses and transmitting the disease to other susceptible individuals. Risks are especially high for nonimmune HCWs, who have an increased chance of exposure to these diseases in hospital settings. Susceptibility may also contribute to nosocomial transmission by the HCW. In fact, during a 2008 outbreak of 14 confirmed measles cases in the United States, exposure to measles occurred in a healthcare setting in 7 (50%) cases (9). False positive rubella serology during routine prenatal screening may lead to misclassification of a pregnant woman as immune. Subsequent rubella infection could put the fetus at risk of congenital rubella syndrome, with potential debilitating consequences such as deafness, heart defects, and developmental delay. False seropositivity also has potential consequences for public health through decreased herd immunity, putting those too young to be vaccinated or with contraindications to vaccination at risk for disease.

New multiplex MMRV serologic assays were designed for the assessment of immune status and thus are necessarily highly specific. This may come at the expense of some sensitivity. With appropriate use of serologic testing, the risk of a false negative that spurs unnecessary vaccination may be minimized. Individuals, including HCWs, who have documentation of prior immunization or history of disease are presumed to be immune, regardless of the results of serologic testing (Figure 1). Thus, testing for immunity is unnecessary in this population. In fact, evaluations of serologic screening methods indicated that historical IgG assays for measles and varicella have low sensitivity in previously vaccinated populations (10, 11). Additionally, testing for evidence of virus-specific antibodies following vaccination is a practice that should be avoided and is not recommended by the ACIP, as many commercially available assays are not sensitive enough to detect IgG antibody seropositivity for up to 8-12 weeks after vaccination (12, 13). This may be a reflection of lower virus-specific IgG titers that are observed with immunity induced by vaccination rather than natural infection (14).

Negative MMRV IgG results may also be an indication of waning immunity in previously vaccinated individuals or the absence of prior vaccination. Mumps antibodies are the quickest to wane after vaccination, which is supported by the lower seropositivity rates seen for mumps virus compared to measles and rubella (6, 15). Additionally, in the absence

of documentation, there is poor correlation between an individual's self-reported vaccination history and the presence of IgG antibodies (16). Thus, the ACIP recommends that negative or equivocal serologic results are not supportive of immunity and that vaccination should be initiated in these individuals (12). If HCWs are reluctant to undergo vaccination, an additional option is to measure seropositivity using the gold standard PRNT method. However, PRNT is unavailable at most clinical laboratories, and testing would likely need to be performed via a reference laboratory, at increased cost and time to result. In practice, vaccination is more straightforward, is generally well-tolerated, and definitively resolves the issue.

Preservation of population or "herd" immunity is necessary to prevent outbreaks of infectious diseases such as MMRV, and many vulnerable members of the population depend upon herd immunity to avoid infection. Although some unnecessary immunization may occur as a result of negative serologic testing, the consequences of a false positive result may be dire. Additionally, investigation and containment of an outbreak is associated with high costs to healthcare facilities (9, 17). Prevention through vaccination in seronegative individuals is a cost-effective approach to boost immunity of both HCWs and the general population to prevent future outbreaks and maintain effective herd immunity.

References

1. *Clemmons NS, Wallace GS, Patel M, Gastanaduy PA. 2017. Incidence of Measles in the United States, 2001-2015. JAMA 318:1279-1281.*
2. *Phadke VK, Bednarczyk RA, Salmon DA, Omer SB. 2016. Association Between Vaccine Refusal and Vaccine-Preventable Diseases in the United States: A Review of Measles and Pertussis. JAMA 315:1149-1158.*
3. *Centers for Disease Control and Prevention. Mumps Cases and Outbreaks. https://www.cdc.gov/mumps/outbreaks.html. Accessed December 19, 2017.*
4. *Zipprich J, Winter K, Hacker J, Xia D, Watt J, Harriman K, Centers for Disease C, Prevention. 2015. Measles outbreak--California, December 2014-February 2015. MMWR Morb Mortal Wkly Rep 64:153-154.*
5. *Advisory Committee on Immunization P, Centers for Disease C, Prevention. 2011. Immunization of health-care personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 60:1-45.*
6. *Shirts BH, Welch RJ, Couturier MR. 2013. Seropositivity rates for measles, mumps, and rubella IgG and costs associated with testing and revaccination. Clin Vaccine Immunol 20:443-445.*
7. *Binnicker MJ, Jespersen DJ, Rollins LO. 2011. Evaluation of the Bio-Rad BioPlex Measles, Mumps, Rubella, and Varicella-Zoster Virus IgG multiplex bead immunoassay. Clin Vaccine Immunol 18:1524-1526.*
8. *Loeffelholz MJ, Prince HE. 2015. Evaluation of a multiplex bead immunoassay for determination of immune status to varicella-zoster virus in medical center students and employees. Clin Vaccine Immunol 22:351-353.*
9. *Chen SY, Anderson S, Kutty PK, Lugo F, McDonald M, Rota PA, Ortega-Sanchez IR, Komatsu K, Armstrong GL, Sunenshine R, Seward JF. 2011. Health care-associated measles outbreak in the United States after an importation: challenges and economic impact. J Infect Dis 203:1517-1525.*
10. *Cohen BJ, Doblas D, Andrews N. 2008. Comparison of plaque reduction neutralisation test (PRNT) and measles virus-specific IgG ELISA for assessing immunogenicity of measles vaccination. Vaccine 26:6392-6397.*
11. *Sauerbrei A, Wutzler P. 2006. Serological detection of varicella-zoster virus-specific immunoglobulin G by an enzyme-linked immunosorbent assay using glycoprotein antigen. J Clin Microbiol 44:3094-3097.*
12. *McLean HQ, Fiebelkorn AP, Temte JL, Wallace GS, Centers for Disease C, Prevention. 2013. Prevention of measles, rubella, congenital rubella syndrome, and mumps, 2013: summary recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 62:1-34.*
13. *Marin M, Guris D, Chaves SS, Schmid S, Seward JF, Advisory Committee on Immunization Practices CfDC, Prevention. 2007. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 56:1-40.*
14. *Francis BH, Thomas AK, McCarty CA. 2003. The impact of rubella immunization on the serological status of women of childbearing age: a retrospective longitudinal study in Melbourne, Australia. Am J Public Health 93:1274-1276.*
15. *Davidkin I, Jokinen S, Broman M, Leinikki P, Peltola H. 2008. Persistence of measles, mumps, and rubella antibodies in an MMR-vaccinated cohort: a 20-year follow-up. J Infect Dis 197:950-956.*
16. *Koivisto K, Puhakka L, Lappalainen M, Blomqvist S, Saxen H, Nieminen T. 2017. Immunity against vaccine-preventable diseases in Finnish pediatric healthcare workers in 2015. Vaccine 35:1608-1614.*
17. *Parker AA, Staggs W, Dayan GH, Ortega-Sanchez IR, Rota PA, Lowe L, Boardman P, Teclaw R, Graves C, LeBaron CW. 2006. Implications of a 2005 measles outbreak in Indiana for sustained elimination of measles in the United States. N Engl J Med 355:447-455.*

For more information, please visit www.bio-rad.com