Qualitative Variation Among Commercial Immunoassays to Detect Measles-Specific IgG

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disclaimer
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Measurement of measles virus-specific IgG is used to assess presumptive evidence of immunity among immunocompetent individuals with uncertain immune or vaccination status. False-negative test results may lead to unnecessary quarantine and exclusion from activities such as employment, education, and travel or result in unnecessary re-vaccination. In contrast, false-positive results may fail to identify susceptible individuals and promote spread of disease by those who are exposed and unprotected. To better understand the performance characteristics of tests to detect measles IgG, we compared five widely used, commercially available measles IgG test platforms using a set of 223 well characterized serum samples. Measles virus neutralizing antibodies were also measured by in vitro plaque reduction neutralization (PRN), the gold standard method and compared to IgG test results. Discrepant results were observed for samples in the low-positive ranges of the most sensitive tests, but there was good agreement across platforms for IgG negative sera and for samples with intermediate to high levels of IgG. False negative test results occurred in approximately 11% of sera, which had low levels of neutralizing antibody.
Introduction

Routine two-dose vaccination led to the elimination of endemic measles in the United States in 2000 (1-4). However, in 2019, the U.S. experienced the highest annual number of measles cases since 1992, largely due to repeated introductions by U.S. residents traveling abroad and returning to under-vaccinated communities. Most cases (75%) were associated with two outbreaks in New York Orthodox Jewish communities that threatened the elimination status of measles in the U.S. Approximately 89% of measles patients were unvaccinated or had unknown vaccination status and 11% were vaccine recipients (5). The effectiveness of two-dose MMR vaccination for protection against measles is approximately 97% (range 67-100) (6).

Although some vaccine recipients who are exposed may develop symptomatic infection, they are less likely to transmit virus (7-10) and often have a milder clinical presentation (11-15). Measles virus-specific neutralizing IgG antibody is essential for protection against disease and a serum titer of >120 is considered protective (16). A serum titer of 120 was later extrapolated to be 200 mIU/ml using the first World Health Organization (WHO) International Serum Standard (IS) and 120 mIU/ml using the WHO second IS (17).

Effective outbreak control relies upon interrupting chains of transmission by identification and vaccination of susceptible individuals and by quarantine of those who are likely to spread disease following exposure. Importantly, measles vaccination can provide effective post-exposure prophylaxis (PEP) if given within 72 hours of initial exposure (18).

While it may be reasonable to vaccinate most individuals with uncertain measles immune status during an outbreak, vaccination is contraindicated for certain medical conditions (19).
In an outbreak setting, when immune status is uncertain, timely and accurate determination of measles IgG seropositivity can provide important guidance for individual healthcare decisions. There are a variety of commercial measles IgG test platforms that differ by specific test antigen(s) and method of detection. Accordingly, the performance characteristics among tests differ with respect to sensitivity and specificity. Although relatively infrequent, discrepant IgG test results have been observed among individuals who have received one or more MMR vaccinations. During outbreaks there is an increase in measles IgG testing volumes due to case-contact investigations and healthy individuals seeking evidence of presumptive immunity. The purpose of this study was to define the performance characteristics of five commercially available measles IgG test platforms that are in common use among commercial and public health laboratories and to compare these results with results from the standard reference assay for determining measles immunity, plaque reduction neutralization (PRN) (16).

Materials and methods
Sera
A set of 223 previously characterized de-identified sera were chosen for this study based on available volumes and previous measles IgG and IgM test ELISA results. The CDC Human Research Protections Office determined that this study was exempt from Institutional Review Board (IRB) review.
Kits description

The five measles IgG test platforms that were compared included: BioMerieux Vidas Measles IgG, Durham NC (Ref. 30219), BioRad BioPlex 2200 MMRV IgG, Hercules CA (Ref. 6652450), Diasorin Liaison Measles IgG, Cypress CA (Ref. 96652450), Trinity Biotech Captia Measles IgG, Jamestown, NY (Ref. 2326000), and Zeus Scientific Measles IgG Test System, Branchburg NJ (Ref. 9Z9271G). All sera were tested by each platform according to the manufacturers’ instructions. The Zeus and Trinity tests are manually performed ELISAs and the remaining tests are performed with manufacturer-specific automated equipment.

Of note, the Diasorin Liaison Measles IgG platform has historically been marketed in the United States and abroad with two different cut-off values for the interpretation of results. Specifically, for kits sold in the United States the interpretation of results has been as follows: <25.0 AU/ml is IgG negative, ≥25.0 AU/ml and <30.0 AU/ml is equivocal, and ≥30.0 AU/ml is positive. However, outside of the U.S., the interpretation of results has been: <13.5 AU/ml is IgG negative, ≥13.5 AU/ml and <16.5 AU/ml is equivocal, and ≥16.5 AU/ml is positive. Recently, the manufacturer lowered the interpretation of results in the U.S. to match the interpretation given for kits sold outside of the U.S. For this study, results of the Diasorin Liaison Measles IgG assay were interpreted with both sets of criteria. As described in the text below, this change in cut-off has a minor overall effect on the sensitivity and specificity of the test but could make important differences for certain individuals with low levels of IgG.
Measles In-vitro Plaque Reduction Neutralization (PRN)

Serum neutralizing antibody titers were measured by PRN as previously described (17).

In this study, the WHO second IS anti-measles serum (IS, coded 66/202, supplied by the National Institute for Biological Standards and Control, South Mimms, United Kingdom) was included to calculate the reciprocal of the 50% endpoint titer by the Kärber method (20). A dilution of the WHO second IS was included in each assay and end point titers for the internal standards did not differ by more than 20%. With the validation of the second WHO standard serum, PRN titers were expressed in mIU/ml. A titer of 8 was equivalent to a concentration of 8 mIU/ml. PRN titers were used as the gold-standard for comparison of the various IgG test platforms. Receiver-operator curves were determined for each IgG test platform using PRN titers of 8, 40, 60, and 120 mIU/ml as the reference. A PRN titer >120 was considered protective for measles (16) and is equivalent to 120 mIU/ml based on use of the WHO second international standard.

Measles IgM capture ELISA

Sera were tested for measles IgM using a capture ELISA as previously described (21). Because measles-specific IgM antibody can neutralize virus and contribute to the PRN titer, sera that were IgM positive were analyzed as a separate group in the platform comparison as described below.
Results

A set of 223 sera was examined by measles in vitro plaque reduction neutralization (PRN), measles-specific IgM capture ELISA, and by 5 different commercially available measles IgG tests. The methods and antigens used by the various IgG test platforms may or may not detect neutralizing antibody. However, we chose to directly compare each test to PRN titer since ≥120 mIU/ml is considered protective (16). In addition, because it is not practical to perform PRN for routine testing, IgG test methods that best correlate with PRN would be the most useful in determining measles immunity.

Sera containing high levels of measles-specific IgM can contribute detectable neutralizing activity to the PRN assay. To avoid confounding results, ELISA to PRN comparisons were performed using only IgM negative specimens (n=146). We performed receiver-operating characteristic (ROC) analysis for each IgG test using the PRN results as the reference standard. Inherent variability in the PRN assay can be 2 to 3 fold between each test run for individual sera (17). Previous reports used >8 mIU/ml as a seropositive PRN result and >120 mIU/ml as a seroprotective result. We used ≥8, ≥40, ≥60, and ≥120 mIU/ml PRN cut-offs to determine the effects on sensitivity and specificity for each comparison (Table 1). A PRN titer of ≥40 mIU/ml gave the optimal cut-off for overall agreement, sensitivity, and specificity for all tests. Although these IgG immunoassay tests are not intended to specifically detect neutralizing antibody, an in vitro plaque neutralization titer of 40mIU/ml generally discriminates between sera that are IgG negative versus positive based on these data. The results of the ROC analysis are summarized in Table 1. Compared to PRN, the Zeus test had the greatest overall agreement (92.5%), sensitivity (91.7%), and specificity (100%). At a PRN cut-off
of ≥40 mIU/ml, the remaining commercial assays had moderate overall agreement with PRN (82.2-87.7%). Although all the other tests had excellent specificities ranging from 96.0-100%, the sensitivity was lower and ranged from 78.3-88.2%. Finally, the results from each test platform for the entire set of 223 sera, including 77 measles IgM positive sera, were compared against the Zeus assay (Table 1, red font). The overall agreement of the other platforms with the Zeus test ranged from 88.3-92.4%. The ranges for sensitivity and specificity were 87.1-93.3% and 94.4-100% respectively. Except for PRN, there did not appear to be any effect of measles-specific IgM on the performance of these commercial IgG tests platforms relative to each other.

Figure 1 shows the comparison between PRN titer and IgG results for 223 individual sera across the 5 IgG platforms. As mentioned previously, because some measles-specific IgM antibody can have neutralizing activity, sera that were IgM negative (n=146, panels A, C, E, G, I) were analyzed separately from IgM positive (n=77, panels B, D, F, H, J). Most of the commercial assays evaluated, demonstrated good correlation with PRN in the lowest ranges (true negative) and highest ranges (strong positive) of the assays. The majority of discrepant results occurred with sera that yielded low-positive PRN results (>8 and <120 mIU/ml) and were typically discrepant across multiple platforms. Individual results highlighted in red in Figure 1 A and B indicate samples that were discrepant between the Zeus test and at least one other IgG platform. Results highlighted in red in Figure 1 C-J were discrepant between the indicated test as compared with the Zeus IgG result.

Thirty samples with sufficient volume were chosen for reproducibility testing across all test platforms (data not shown). Qualitative results were compared and none of the platforms
had more than two discrepant results (range 0-6.7%). All discrepant results were near the cut-offs for the negative-indeterminate or indeterminate-positive ranges.

Discussion

We compared five measles IgG test platforms in common use among commercial and public health laboratories in the United States; IgG results were compared with neutralizing antibody titers, taking into consideration the presence of measles-specific IgM and the effects of IgM on virus neutralization. Although none of the IgG tests are designed to specifically measure neutralizing antibody, all of them demonstrated a good overall correlation with PRN (Figure 1). Furthermore, measles IgM did not appear to cause interference with any of the IgG tests that were examined since a wide range of IgG values were detected among IgM positive samples across all test platforms.

The results demonstrate differences in the sensitivity and specificity of individual IgG tests. In general, tests that are based on a manual ELISA format (Zeus and Trinity) were more sensitive than the automated high-throughput platforms (BioPlex 2200, Diasorin Liaison, and BioMerieux Vidas). However, all the commercial platforms demonstrated good agreement of qualitative results. Similar to previous reports, up to 11% of samples gave discordant results when compared on the most sensitive versus least sensitive platforms (22, 23). The discrepant results were in the low-positive, equivocal, and high-negative ranges for all platforms.

In an outbreak setting, there is often an increased level of IgG testing. Since most commercial assay cut-offs are set to identify true negatives increased testing will result in an increase in the total number of potentially false-negative results. Previously vaccinated
individuals with low levels of IgG may have false-negative results depending on the test that is used. This may lead to either unnecessary vaccination, additional laboratory testing or unnecessary restriction of activities as well as a waste of public health resources.

The PRN test is considered the gold standard laboratory method for determining measles immunity because it measures functional neutralizing antibody. From a public health perspective, it is not practical or necessary to determine PRN titers for every individual that requires testing (16). Compared to IgG testing by ELISA, PRN is labor intensive, time consuming, costly, has a long turnaround time, and therefore, is not practical for routine testing.

In a measles elimination setting, a conservative approach for an IgG test would be to have an appropriately high cut-off that may err on the side of providing a small fraction of false-negative results. This approach would allow the identification of individuals who may not be sufficiently protected and who should consider (re)vaccination. By contrast, an IgG test with a cut-off that is too low would result in false-positive results, which would prevent the identification and vaccination of susceptible individuals. Additional testing guidance is available at the CDC website (https://www.cdc.gov/measles/hcp/index.html#lab) and the American Public Health Laboratories (APHL) website (https://www.aphl.org/programs/infectious_disease/_layouts/15/WopiFrame.aspx?sourcedoc=/programs/infectious_disease/Documents/APHL%20statement%20measles%20IgG%20final.docx&action=default&DefaultItemOpen=1). The data presented here are intended to raise awareness regarding the limitations and utility of measles IgG testing in general, and to provide an estimate of the performance characteristics of the specific tests described. Individuals that have negative or equivocal results for measles IgG should be
vaccinated or revaccinated (6). In some cases, vaccination is not possible and testing with a second line diagnostic assay may be necessary to determine immune status.

Figure 1

Comparison of quantitative IgG test results with PRN. Measles IgM negative samples are shown in panels A, C, E, G and I. Measles IgM positive samples are shown in panels B, D, F, H, and J. The Zeus test had the greatest overall agreement with PRN and was therefore used as the comparator. The platforms tested include Zeus (A, B), Trinity (C, D), BioRad (E, F), BioMerieux Vidas (G, H), and Diasorin (I, J). Horizontal dotted lines indicate cut-offs provided by the test manufacturers. For Diasorin (I, J) the black horizontal lines indicate the cut-off previously used in the US and the blue horizontal lines indicate the new cut-off used in the US and also used in Europe. Vertical dotted lines indicate PRN = 40 mIU/ml and 120 mIU/ml. Samples indicated in red in panels A and B were discrepant between Zeus and at least one other platform. Samples indicated in red in panels C-J were discrepant with the Zeus result.

Table 1

Performance of PRN vs IgG immunoassays using multiple PRN cut-offs. Please note the materials and methods section for information regarding the Diasorin Liaison measles IgG test cut-offs.
Acknowledgements

We would like to thank the New York City Department of Health and Mental Hygiene and the California Department of Public Health for providing some test specimens.
References


Table 1

Performance of PRN vs IgG immunosays using multiple PRN cut-offs and comparison of Zeus to other IgG immunosays.

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Figure 1

*Please note the materials and methods section for information regarding the Damon Laboratories IgG test cut-offs.

**AUC** under the curve; AUC provides a measure of separability and predicts the ability of model to distinguish between classes.