False-Positive Results for Immunoglobulin M Serologic Results: Explanations and Examples

Charles R. Woods
Pediatric Infectious Diseases, University of Louisville School of Medicine, Kentucky

Corresponding Author: Charles R. Woods, 571 S Floyd St, Ste 321, Louisville, KY 40202. E-mail: charles.woods@louisville.edu.

Scenario*

A measles outbreak occurred last month among a cluster of unvaccinated children in a suburban area in your referral region. Two weeks ago, an adolescent who received two doses of measles-containing vaccine in childhood developed a febrile illness with rash. This patient tested positive for measles IgM in a commercial assay. Subsequent testing by the Centers for Disease Control and Prevention demonstrated seronegativity for measles IgM. (A low positive concentration of measles IgG was detected.) The initial IgM test result has been labeled as false positive. The family of the patient is upset about the disruption of life experienced due to the false positive IgM result. The pediatrician who ordered the test has called to ask you how this result could have happened. What are potential explanations for this?

The false-positive rate of any diagnostic test is a function of the specificity of the test and prevalence of the disease in the population represented by the patient. For serologic assays, the presence of antibodies that cross-react with microbial antigens used in the assay or interfering substances that interact with assay components can also lead to false-positive results. Technical performance issues such as over-reading of weakly reactive bands on immunoblots can also lead to false-positive serologic test results for microbes for which these assays are used (eg, Borrelia burgdorferi) [1]. Thus, positive IgM assay results can require cautious interpretation—consideration of clinical course compatibility and epidemiological factors—and/or confirmation by other serological or molecular testing methods. The issue of reactivation of IgM production against herpes viruses is beyond the scope of this review.

Cross-reacting Antibodies

Cross-reacting antibodies have been described for many infections. The association of acute human parvovirus infection with false-positive measles IgM was first described in Alaska in 1994 [2]. Subsequently, human parvovirus, rubella, and human herpesvirus (HHV) 6 infections have been described as causes of false-positive IgM test results for measles [3–7]. Human parvovirus infection has also been implicated in false-positive IgM assays for Epstein-Barr virus (EBV), herpes simplex virus, and HHV 6 virus infections [6, 8]. False-positive IgM serologic results for EBV (capsid antigen) and cytomegalovirus may occur in approximately 3% of patients with acute human immunodeficiency virus infection and 30% of patients with acute hepatitis A infection [9]. West Nile virus (WNV) is known to cross-react with St. Louis encephalitis virus and other flaviviruses [10].

This issue is not limited to viral infections. In the past, cross-reactivity of rickettsial antibodies with Proteus OXK antigens was the basis of the Weil-Felix test used in the past for diagnosis of Rocky Mountain Spotted Fever and other rickettsial diseases [11, 12]. False-positive Brucella antibody test results can be caused by cross-reactivity of antibodies, predominantly of IgM class, due to infections by Escherichia coli O157, Francisella tularensis, Moraxella phenylpyruvica, Yersinia enterocolitica, and certain Salmonella species [13]. A positive IgM
serology for *Brucella* in a patient in Florida in 2005 prompted expensive investigations of dairy farms in 2 other states where the patient had recently traveled and consumed implicated food products [14]. Subsequent testing excluded brucellosis.

Administration of type III group B streptococcal polysaccharide as a vaccine induces antibodies that cross-react with *Streptococcus pneumoniae* type 14 and are capable of mediating opsonophagocytosis in vitro [15]. Cross-reactivity among pneumococcal serotypes may both confound serodiagnosis assays and provide useful cross-protection against infection in some instances [16]. The polysaccharide capsules of *Neisseria meningitis* group B and *E coli* K1 (and *Pasteurella haemolytica*, and *Moraxella nonliquefaciens*) have nearly identical molecular structures [17]. Other examples of actual and potential bacterial serologic cross-reactivity abound.

**Interference: Rheumatoid Factor and Other Examples**

Assay interference occurs when a serum component interacts with a test component to generate a false-positive or false-negative assay result. A common mechanism of interference is binding of heterophile antibody in patient serum to the “reagent” antibodies used for detection of target antibodies captured by the test-specific antigen(s) used in modern serologic assays. Rheumatoid factor (RF) is the best characterized interfering factor. Rheumatoid factor is a heterogeneous group of auto-antibodies that recognize epitopes on Fc regions of IgG molecules [18]. These are found in approximately 70% of patients with rheumatoid arthritis (RA) but also up to 10% of adults without RA [19].

Rheumatoid factor-type antibodies can also be generated during multiple infections, including leprosy, infective endocarditis, tuberculosis, trypanosomiasis, visceral larva migrans, infectious mononucleosis, cytomegalovirus, influenza A, and hepatitis A [20]. Human antibodies against epitopes on animal-derived antibodies used as components of serologic assays can also lead to interference [18]. These may be present without prior host exposure to specific animal IgGs.

Sample dilution and other chemical or heat treatments can be used in some assays in an effort to reduce false-positive test results due to interfering antibodies. Repeat testing using assays based on different detection antibodies or different assay antigens may be required in some instances to evaluate whether a positive result is true or false [18, 21, 22]. Interference is also an issue in nonserologic tests that use antibodies as reagents. Falsely elevated tacrolimus concentrations are an example [23]. Antibody depletion steps can overcome this issue when detection of a serum antibody is not the purpose of the test.

**Specificity, Prevalence, and Predictive Value**

*Specificity* is the characteristic of a diagnostic test that indicates how well the test performs among patients who do not have the disease, ie, those who should test negative. It is the proportion of tests performed in patients who do not have the disease in question (as defined by a reference standard) that yield negative results. False-positive results can occur with any diagnostic test with less than 100% specificity, and this applies essentially to all serologic tests. Specificity, like sensitivity, is a stable characteristic of test performance when the test is used in populations similar to that in which specificity has been ascertained.

The **positive predictive value** (PPV) of a diagnostic test indicates how often a positive result represents a true positive (conversely, negative predictive value indicates how often a negative result represents a true negative). Predictive values vary by disease prevalence. In situations of low disease prevalence, such as that of measles in the United States (despite recent small outbreaks), a substantial proportion of positive test results will be false positives even when a test with very high specificity is used. This variance of PPV with prevalence is depicted in a theoretical population in the Table. Positive predictive value is improved when testing is done and patients are “stratified” into higher prevalence subgroups based on history.

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<th>Disease Prevalence</th>
<th>True-Positive Results</th>
<th>False-Negative Results</th>
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*aCalculated based on prevalence in a theoretical population of 10,000 persons.  

bPositive Predictive Value = True-Positive Results/(True-Positive Results + False-Positive Results).  

False-Positive Rate = False-Positive Results/(True-Positive Results + False-Positive Results).
of potential exposure to an infection and presence of symptoms or signs, in appropriate sequence, suggestive of the etiology.

The False-Positive Measles IgM Scenario

The need to take both prevalence and cross-reacting antibodies into account was demonstrated in an adult case similar to that in the case scenario described above, which occurred in Maine in early 2012 [3]. This patient had fever, headache, and rash for 2 to 3 days followed by coryza and cough. The patient had had measles as a child and there was no recent exposure to any known measles cases. There was contact with a grandson with human parvovirus infection 1 to 2 weeks before onset of the illness. In this case, epidemiological history and clinical course suggested low risk for measles. Further investigation demonstrated a high titer of parvovirus IgM in the patient.

Recent experience in Latin America provides further corroboration. Endemic transmission of measles was absent in Latin America in 2003 and had decreased before this. In the State of Sao Paulo, Brazil, from 2000 to 2004, 109 (66%) of 166 positive measles IgM results among persons who had not been recently vaccinated against measles were determined to be false-positive results based on absence of subsequent IgG seroconversion [4]. Other viral infections (ie, human parvovirus, rubella, HHV 6) were associated with 98 of these with no clear explanation for the remaining 11 cases. These could just be part of the low rate of false positives intrinsic to the test: specificities of 3 commercial measles IgM assays in 1 study were 94.6%–98.7% [24]. This further highlights the need for thorough investigation when patients who have viral exanthemata potentially caused by other viruses test positive for measles IgM—PPV falls with declining prevalence of the infection [4, 5].

West Nile Virus and Coccidioidomycosis Examples

Commercially available kits for testing for IgM antibodies against WNV have also had problems in the past. One lot of a kit distributed in the United States in 2008 was associated with 119 (72%) of 166 false-positive IgM results compared with repeat test results at the Centers for Disease Control and Prevention (CDC) [25]. A ratio method to account for nonspecific reactivity due to RF, heterophile antibodies, and other interfering substances has been described for improved utility of an IgM capture enzyme-linked immunosorbent assay (ELISA) for WNV IgM test [26]. Four WNV IgM ELISA kits from different manufacturers are currently approved by the U.S. Food and Drug Administration in the United States, but all positive results with any of these kits should be confirmed by additional testing at the CDC or a state health department laboratory [27]. Although a high index of suspicion is required to make the diagnosis of WNV in many cases, especially presentations without signs of encephalitis, the risk of false-positive results in nonspecific illnesses prompted a CDC recommendation that commercial kits “not be used to test specimens from persons without compatible illness” [25].

In a recent study of 17 patients with positive results for IgM and negative results for IgG by a commercially available enzyme immunoassay (EIA) test for Coccidioides, only 3 had clinical disease consistent with Coccidioides infection [28]. Fourteen (82%) of the IgM results were deemed false positives. The clinical laboratory had performed 2139 serologic tests for Coccidioides during the study year and had a total of 104 (5%) of tests with positive IgM and negative IgG. This suggested a total of 85 false-positive test results for the year. The basis for the false-positive results was not certain in these cases and may have been caused by antibody cross-reactivity or other assay limitations that are exposed when testing patients from populations with low prevalence of disease (which applies to coccidioidomycosis even in endemic areas). There were 12 true-positive results (positive IgM and IgG and compatible clinical findings) during the year.

False-Negative Results

False-negative results for IgM (and IgG or IgA) serologic tests can occur as well. In addition to the reality that test sensitivities are typically <100%, false-negative serologic results can occur due to immunocompromised states that prevent a patient from mounting a response, insufficient time elapsed since onset of infection for production of IgM to begin or exceed the antibody detection threshold of the test, or presence of interfering substances in host serum that block assay function.

*This scenario is based in part on the description of a case of an adult in Maine in February 2012 [3].

References