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# Cross-Reactivity of Disease-Specific Antibody Assays for the Detection of Current Infections: With Potentially Interfering Substances of Other Infections

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**Background:** Current infections are frequently diagnosed based on positive immunoglobulin (Ig) M results, although false positivity can occur. We evaluated cross-reactivity among infectious antibody (Ab) assays.
**Methods:** A total of 167 positive sera were collected for: rubella IgM and IgG, cytomegalovirus (CMV) IgM and IgG, *Toxoplasma gondii* (Toxo) IgG, human immunodeficiency virus (HIV) antigen (Ag)/Ab, hepatitis A virus (HAV) IgM and IgG, hepatitis C virus (HCV) Ab, herpes simplex virus (HSV) IgM and IgG, Epstein-Barr virus viral capsid Ag IgM, hepatitis B core (HBc) IgM, hepatitis B e Ab, and severe acute respiratory syndrome coronavirus 2 total Ab, *Treponema pallidum* IgM (each n=10) and Toxo IgM (n=7). All sera were tested with seven assays in duplicate: Architect rubella IgM, CMV IgM, Toxo IgM, HIV Ag/Ab, HAV IgM, anti-HCV (Abbott Laboratories, USA), and Elecsys HBc IgM (Roche Diagnostics GmbH, Germany). Additionally, sera showing repeatedly reactive were evaluated by following supplemental testing: Elecsys Toxo IgM, HIV Duo and HAV IgM, VIDAS rubella IgM and CMV IgM (bioMérieux SA, France); LIAISON XL CMV IgM (DiaSorin S.p.A., Italy); and HCV blot 3.0 (MP Diagnostics Inc., Philippines).
**Results:** Except Elecsys HBc IgM, six assays showed reactive for several sera, including other infectious Abs. Upon supplemental testing, Architect rubella IgM, CMV IgM, and anti-HCV showed reactive or gray zone for two sera with HSV IgM ($\kappa$=0.903), eight to fifteen with various Abs ($\kappa$=0.607–0.814), and one with HAV IgG ($\kappa$=0.960), respectively.
**Conclusions:** Architect rubella IgM, CMV IgM, and anti-HCV showed cross-reactivity with reactive sera to other infectious Abs. Considering cross-reactivity of Ab assays with other pre-existing infectious Abs, infectious Ab results should be carefully interpreted.
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**Key Words** Immunoassay, Viral antibodies, Immunoglobulin M, Immunoglobulin G, Cross-reactivity

## INTRODUCTION

Current infections are frequently diagnosed based on positive immunoglobulin (Ig) M results. However, false-positive reactions can occur due to interference by pre-existing antibodies (Abs) against other infectious diseases, leading to a cascade of unnecessary tests and treatments [1]. For example, a 39-year-old woman with myalgia, low-grade fever, chills, headache, and polyarthralgia tested positive for both cytomegalovirus (CMV) IgM and Epstein-Barr virus (EBV) viral capsid antigen (VCA) IgM. After unnecessary hospitalization, testing, and consultations, the true etiology of this case was confirmed as CMV by highly positive CMV IgM, low positive CMV IgG, positive CMV polymerase chain reaction (PCR), and negative EBV PCR results [2]. A recent report found that infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause false-positive results for dengue virus Ab, as the clinical presentation of these two conditions is sometimes indistinguishable [3]. Chemiluminescent immunoassay (CLIA), which is commonly used for the detection of infectious Abs, appears to result in high false-positive rates (37.3%, 134 of 359 positive samples on syphilis screening by CLIA); thus, the identification of characteristics to predict false-positive results remains challenging [4].

Package inserts supplied by the manufacturer usually inform the user of the potential for cross-reactivity of the assay reagents, but the details are often insufficient. Moreover, it is difficult to fully determine the authenticity of Abs (especially IgM) test results among individual laboratories because interfering Abs are difficult to recognize and eliminate [5]. Therefore, the present study aimed to assess cross-reactivity among Ab assays commonly used to diagnose current infections in clinical laboratories.

## MATERIALS AND METHODS

A total of 167 reactive serum samples and 17 types of infectious Abs (numbers of sample) were collected using the Alinity *i* immunoassay system (Abbott Laboratories, Lake Bluff, IL, USA) for detection of rubella IgM (n=10), rubella IgG (n=10), CMV IgM (n=10), CMV IgG (n=10), *Toxoplasma gondii* (Toxo) IgM (n=7), Toxo IgG (n=10), human immunodeficiency virus (HIV) antigen (Ag)/Ab (n=10), hepatitis A virus (HAV) IgM (n=10), HAV IgG (n=10), and hepatitis C virus (HCV) Ab (n=10), the Liaison XL system (DiaSorin S.p.A., Saluggia, Italy) for detection of herpes simplex virus (HSV) IgM (n=10) and EBV VCA IgM (n=10); the Cobas e801 system (Roche Diagnostics GmbH, Mannheim, Germany) for detection of HSV IgG (n=10), hepatitis B core (HBc) IgM (n=10), hepatitis B envelope Ab (n=10), and SARS-CoV-2 total Ab against the nucleocapsid protein (n=10), and commercial glass sides for an indirect fluorescent Ab assay (Zeus Scientific Inc., Branchburg, NJ, USA) for detection of *Treponema pallidum* (Td) IgM (n=10).

All serum samples were used to evaluate the following seven assay reagents for the detection of infectious Abs in duplicate: Architect rubella IgM and CMV IgM (Abbott Ireland Diagnostics Ltd., Sligo, Ireland), Architect Toxo IgM, HIV Ag/Ab, HAV Ab IgM, and anti-HCV (Abbott GmbH, Wiesbaden, Germany) with the Alinity *i* system (Abbott Laboratories, Abbott Park, IL, USA), and Elecsys Anti-HBc IgM (Roche Diagnostics GmbH) with the Roche Cobas e801 system (Roche Diagnostics GmbH). The details of the assay reagents are listed in Table 1. All results were interpreted using the manufacturer's cutoff values, and gray zone results were considered positive for interpretation.

For samples that were repeatedly reactive or had unclear (gray zone) results with each assay reagent as well as re-testing of the initial results of the collected serum samples, the following supplemental tests were performed to investigate the cross-reactivity of test reagents: Elecsys Toxo IgM (Roche Diagnostics AG, Basel, Switzerland), VIDAS rubella IgM (bioMérieux SA, Marcy-l'Étoile, France), VIDAS CMV IgM (bioMérieux SA), LIAISON XL CMV IgM (DiaSorin S.p.A.), Elecsys HIV Duo (Roche Diagnostics AG), Elecsys anti-HAV IgM (Roche Diagnostics AG), and HCV blot 3.0 (MP Diagnostics Inc., Mandaluyong, Philippines), which is a nitrocellulose strip containing four recombinant HCV proteins, that is, the capsid and non-structural proteins 3, 4, and 5 regions of the HCV genome.

**Table 1.** Basic characteristics of Ab assays provided by manufacturers

| Assay | Cutoff value | | | Unit | Principle | Capture Ag/Ab (solid phase) | Label (reporter) | Interference or specificity for other condition | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NR | GZ | R | | | | | Tested no. | Reactive no. | Detail (no.) |
| Toxo IgM* | <0.50 | 0.50–0.60 | ≥0.60 | Index | CMIA | Anti-human IgM (mouse, monoclonal) Ab | Acridinium-labeled anti-Toxo p30 Ag mouse monoclonal F(ab')2 fragment and native *Toxoplasma gondii* lysate | 167 (Unspiked); 165 (spiked) | 2 (Unspiked); 10 (spiked) | R/GZ (1/1): ANA; false R (8): polyclonal IgM; false GZ (2): poly- & mono-clonal IgM |
| Rubella IgM† | <1.20 | 1.20–1.60 | ≥1.60 | Index | CMIA | Rubella whole virus (strain HPV 77) | Anti-human IgM (murine) acridinium-labeled conjugate | NA | NA | NA |
| CMV IgM† | <0.85 | 0.85–1.00 | ≥1.00 | Index | CMIA | CMV virus lysate (strain AD169) and recombinant CMV Ag | Murine anti-human IgM acridinium-labeled conjugate | NA | NA | NA |
| HIV Ag/Ab* | <1.00 | | ≥1.00 | S/CO | CMIA | HIV-1/HIV-2 Ag (recombinant) and HIV p24 Ab (mouse, monoclonal) | Acridinium-labeled HIV-1 Ag (recombinant), HIV-1/HIV-2 synthetic peptides, and HIV p24 Ab (mouse, monoclonal) conjugate | 322 | 13 | True R (12); false R (1) |
| HAV IgM* | <0.80 | 0.80–1.20 | >1.20 | S/CO | CMIA | HAV (human) | Anti-human IgM (mouse, monoclonal) acridinium-labeled conjugate | 83 | 0 | 0 |
| HBc IgM‡ | <1.00 | | ≥1.00 | COI | ECLIA | HBcAg labeled with a ruthenium complex and streptavidin-coated microparticles | Biotinylated anti-human IgM (mouse, monoclonal) | 131 | 0 | 0 |
| Anti-HCV* | <1.00 | | ≥1.00 | S/CO | CMIA | Recombinant HCV Ags (HCr43 and c100-3) | Murine anti-human IgM/IgG acridinium-labeled conjugate | 104 | 3 | True R (3) |

Abbreviations: Ab, antibody; NR, nonreactive; GZ, gray zone; R, reactive; Ag, antigen; Toxo, *Toxoplasma gondii*; CMIA, chemiluminescent microparticle immunoassay; IgM, immunoglobulin M; ANA, antinuclear antibodies; HPV, human papillomavirus; NA, not available; CMV, cytomegalovirus; HIV, human immunodeficiency virus; S/CO, signal-to-noise ratio; HAV, hepatitis A virus; HBc, hepatitis B core; COI, cutoff index; ECLIA, electrochemiluminescence assay; HBcAg, hepatitis B core antigen; HCV, hepatitis C virus; IgG, immunoglobulin G.

The instruments used were from the following companies: *Abbott GmbH (Wiesbaden, Germany), †Abbott Ireland Diagnostics Ltd. (Sligo, Ireland), and ‡Roche Diagnostics GmbH (Manheim, Germany).

The study protocol was approved by the Institutional Review Board of Seoul Clinical Laboratories (Yongin, Korea) (approval no., IRB–21–006).

Statistical analyses were performed using GraphPad Prism software ver. 5.0 (GraphPad Software, San Diego, CA, USA) and R ver. 4.0.2 software (The R Foundation for Statistical Computing, Vienna, Austria). Performance data were presented as proportions or ratios with 95% confidence intervals. Agreements between assays were evaluated using Cohen's kappa (κ) statistic with the categories as poor (below 0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.81–1.00).

## RESULTS

Seven assay reagents detecting infectious Abs showed identical results for the serum samples reactive to the same Ab (each n=10, except for Toxo IgM, n=7). The overall results of the reactive sera against other infectious diseases (n=157–160) for the seven assay reagents are presented in Table 2. Repeatedly reactive or gray zone responses were observed in six serum samples by Architect Toxo IgM (five Toxo IgG and one HCV Ab), two by rubella IgM (two HSV IgM), 19 by Architect CMV IgM (one rubella IgM, one rubella IgG, two Toxo IgG, eight EBV VCA IgM, one HSV IgG, one HAV IgM, one HCV Ab, two HBc IgM, and two Td IgM), one by Architect HIV Ag/Ab (one Td IgM), one by Architect HAV IgM (one HBc IgM), and four by Architect anti-HCV (one Toxo IgG, one HIV Ag/Ab, one HAV IgG, and one HSV IgG). Elecsys anti-HBc IgM showed that all serum samples were nonreactive with other potentially interfering Abs.

All re-test results were consistent with the initial results of the collected serum samples, that is, the reactive response for each assay reagent. Supplemental testing results showed suspicious cross-reactivity of Architect rubella IgM to two reactive serum samples for HSV IgM (index, >3.50; cutoff, 1.10) (100% of repeatedly reactive). Architect CMV IgM showed the possibility of cross-reactivity of 15 reactive serum samples (1 rubella IgM [index, 2.23; cutoff, 1.60], 1 rubella IgG [372 IU/mL; cutoff, 10.0], 8 EBV VCA IgM [all >160 U/mL; cutoff, 40], 1 HAV IgM [12.24 signal-to-cutoff (S/CO) ratio; cutoff, 1.20], 1 HCV Ab [15.13 S/CO; cutoff, 1.00], 2 HBc IgM [8.79 and 5.86 cutoff index; cutoff, 1.00], and 1 Td IgM [reactive]) contrary to VIDAS CMV IgM (bioMérieux SA) results (78.9% of repeated reactivity) and eight identical reactive serum samples (1 rubella IgM, 1 rubella IgG, 2 EBV VCA IgM, 1 HAV IgM, 2 HBc IgM, and 1 Td IgM) contrary to LIAISON XL CMV IgM (DiaSorin S.p.A.) results (42.1% of repeated reactivity). Finally, Architect anti-HCV showed the possibility of cross-reactivity of one reactive serum sample with HAV IgG (11.21 S/CO; cutoff, 1.00) (repeated reactivity, 5.0%).

Compared with the results of supplemental tests, four (Architect Toxo IgM, HIV Ag/Ab, HAV IgM, and Elecsys anti-HBc IgM) showed negative, positive, and total agreement of 100%, and almost perfect agreement (κ=1.000). The two reagents (Architect rubella IgM and anti-HCV) showed a total agreement of 98.8% and 99.4% and almost perfect agreement (κ=0.903 and 0.960), respectively. The Architect CMV IgM reagent showed total agreement of 91.0% (κ=0.607, substantial) against with VIDAS CMV IgM (bioMérieux SA), and total agreement of 95.2% (κ=0. 814, almost perfect) against the LIAISON XL CMV IgM (DiaSorin S.p.A.) (Table 3).

## DISCUSSION

Immunoassays are particularly sensitive and specific for the detection of various analytes. Although the frequency of interference of current immunoassay reagents is estimated to be less than 2% owing to the efforts of manufacturers, such as the addition of blocking agents, immunoassays are still subject to interference by endogenous Abs [5]. However, in monoclonal Ab-based immunoassays (widely used murine anti-human Ig), heterophile Abs as common interfering Ab can cause false-positive or false-negative interference by binding to capture and labeled Ag or Ab [6,7]. Heterophile Abs can naturally arise in the body due to Ag diversity and can also be produced in patients with autoimmune or inflammatory conditions. These endogenous heterophile Abs can be present in more than 10% of patients and up

**Table 2.** Tested results for seven Ab assays against reactive samples of infectious Abs

| Assay | Sample no. | Initial R/GZ | | Repeatedly R/GZ | | Suspicious cross-reactivity | |
|---|---|---|---|---|---|---|---|
| | | No. (% of total) | Corresponding samples | No. (% of total) | Corresponding samples | No.* (% of total) | Corresponding samples |
| Toxo IgM | 160 | 6 (3.8) | 5 Toxo IgG, 1 HCV Ab | 6 (3.8) | 5 Toxo IgG, 1 HCV Ab | 0 | None |
| Rubella IgM | 157 | 2 (1.3) | 2 HSV IgM | 2 (1.3) | 2 HSV IgM | 2 (1.3) | 2 HSV IgM |
| CMV IgM | 157 | 21 (13.4) | 1 Rubella IgM, 1 rubella IgG, 2 Toxo IgG, 9 EBV VCA IgM, 1 HSV IgG, 1 HAV IgM, 1 HCV Ab, 2 HBc IgM, 2 Td IgM, 1 HBeAg | 19 (12.1) | 1 Rubella IgM, 1 rubella IgG, 2 Toxo IgG, 8 EBV VCA IgM, 1 HSV IgG, 1 HAV IgM, 1 HCV Ab, 2 HBc IgM, 2 Td IgM | 15 (9.6) † | 1 Rubella IgM, 1 rubella IgG, 8 EBV VCA IgM, 1 HAV IgM, 1 HCV Ab, 2 HBc IgM, 1 Td IgM† |
| | | | | | | 8 (5.1) ‡ | 1 Rubella IgM, 1 rubella IgG, 2 EBV VCA IgM, 1 HAV IgM, 2 HBc IgM, 1 Td IgM‡ |
| HIV Ag/Ab | 157 | 1 (0.6) | 1 Td IgM | 1 (0.6) | 1 Td IgM | 0 | None |
| HAV IgM | 157 | 1 (0.6) | 1 HBc IgM | 1 (0.6) | 1 HBc IgM | 0 | None |
| HBc IgM | 157 | 0 | None | 0 | None | 0 | None |
| Anti-HCV | 157 | 4 (2.5) | 1 Toxo IgG, 1 HIV Ag/Ab, 1 HAV IgG, 1 HSV IgG | 4 (2.5) | 1 Toxo IgG, 1 HIV Ag/Ab, 1 HAV IgG, 1 HSV IgG | 1 (0.6) | 1 HAV IgG |

Abbreviations: Ab, antibody; R, reactive; GZ, gray zone; Toxo, *Toxoplasma gondii*; IgM, immunoglobulin M; IgG, immunoglobulin G; HCV, hepatitis C virus; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; VCA, viral capsid antigen; HAV, hepatitis A virus; HBc, hepatitis B core; Td, *Treponema pallidum*; HBeAg, hepatitis B e-antigen; HIV, human immunodeficiency virus; Ag, antigen.

*Determined by supplemental tests following as Elecsys Toxo IgM (Roche Diagnostics AG, Basel, Switzerland), VIDAS rubella IgM (bioMérieux SA, Marcy-l'Étoile, France), VIDAS CMV IgM (bioMérieux SA), LIAISON XL CMV IgM (DiaSorin S.p.A., Saluggia, Italy), Elecsys HIV Duo (Roche Diagnostics AG), Elecsys anti-HAV IgM (Roche Diagnostics AG), and MP Diagnostics HCV blot 3.0 (MP Diagnostics Inc., Mandaluyong, Philippines); †Compared with VIDAS CMV IgM (bioM rieux SA); ‡Compared with LIAISON®XL CMV IgM (DiaSorin S.p.A.).

**Table 3.** Agreement rates between seven Ab assays and supplemental tests* with total reactive samples (n=167)

| Assay | Positive | | Negative | | Total | | Kappa value |
|---|---|---|---|---|---|---|---|
| | No./total no. | Agreement % (95% CI) | No./total no. | Agreement % (95% CI) | No./total no. | Agreement % (95% CI) | |
| Toxo IgM | 7/7 | 100.0 (77.2–100.0) | 160/160 | 100.0 (97.6–100.0) | 167/167 | 100.0 (97.8–100.0) | 1.000 (1.000–1.000) |
| Rubella IgM | 10/10 | 100.0 (72.3–100.0) | 155/157 | 98.7 (95.5–99.7) | 165/167 | 98.8 (95.7–99.7) | 0.903 (0.769–1.000) |
| CMV IgM† | 10/10 | 100.0 (78.5–100.0) | 142/157 | 90.4 (84.5–94.0) | 152/167 | 91.0 (85.7–94.5) | 0.607 (0.432–0.781) |
| | 10/10 | 100.0 (84.5–100.0) | 149/157 | 94.9 (89.6–97.2) | 159/167 | 95.2 (90.8–97.6) | 0.814 (0.690–0.938) |
| HIV Ag/Ab | 10/10 | 100.0 (74.1–100.0) | 157/157 | 100.0 (97.6–100.0) | 167/167 | 100.0 (97.8–100.0) | 1.000 (1.000–1.000) |
| HAV IgM | 10/10 | 100.0 (74.1–100.0) | 157/157 | 100.0 (97.6–100.0) | 167/167 | 100.0 (97.8–100.0) | 1.000 (1.000–1.000) |
| HBc IgM | 10/10 | 100.0 (72.3–100.0) | 157/157 | 100.0 (97.6–100.0) | 167/167 | 100.0 (97.8–100.0) | 1.000 (1.000–1.000) |
| Anti-HCV | 10/10 | 100.0 (77.2–100.0) | 156/157 | 99.4 (96.4–99.9) | 166/167 | 99.4 (96.7–99.9) | 0.960 (0.881–1.000) |

Abbreviations: Ab, antibody; CI, confidence interval; Toxo, *Toxoplasma gondii*; IgM, immunoglobulin M; CMV, cytomegalovirus; HIV, human immunodeficiency virus; Ag, antigen; HAV, hepatitis A virus; HBc, hepatitis B core; HCV, hepatitis C virus.
*Same as the supplemental tests in Table 2. †The data above were compared to VIDAS CMV IgM (bioMérieux SA, Marcy-l'Étoile, France) and the data below were compared to LIAISON XL CMV IgM (DiaSorin S.p.A., Saluggia, Italy).

to 40% of the general population, with affinity to animal Abs [8,9].

When faced with a discordant clinical result, we can double-check the sample name and type and communicate with physicians to obtain clinical information. Generally, several actions can be performed during the workup. A dilution study could be performed to check for a possible high-dose hook effect or interfering substances. Heterophile Ab-blocking reagents are commercially available and are commonly used to neutralize or inhibit heterophile Ab interference. Lastly, we can consider retesting the same sample with a different assay because an alternative assay with different susceptibility or resistance to the heterophile Ab of patients can provide accurate results for physicians [5,9].

Antigenic cross-reactivity for CMV IgM may occur in reactive EBV VCA IgM specimens. Notably, one study reported that the Axsym CMV IgM assay (Abbott Ireland Diagnostics Ltd.) lacked specificity due to acute EBV infection [1]. Similarly, in our study, CMV IgM assays showed reactive responses in reactive samples for EBV VCA IgM, with reactive rates in the order of Architect, LIAISON XL, and VIDAS assays. In addition to EBV VCA IgM, Architect CMV IgM assay showed suspicious cross-reactivity for rubella IgM, rubella IgG, HAV IgM, HBc IgM, and Td IgM. According to Ohyama et al. [10], CMV IgM is a valuable diagnostic marker of congenital CMV infection (CMV IgM positivity rates of 84.4% and 0.7% for the disease and non-disease groups, respectively). However, serological cross-reactivity to CMV IgM may interfere with diagnosing other infectious diseases, and caution is warranted when interpreting the reactive results for CMV IgM [11].

In addition to the Architect CMV IgM in this study, Architect immunoassay reagents for rubella IgM and anti-HCV showed cross-reactivity with most highly reactive serum samples to other infectious Abs, which is, more than the upper limit of the analytical range or 10 times the cutoff value of each assay. Interestingly, only 32.7% (65/199) of the TORCH (*Toxo*, rubella virus, CMV, and HSV) IgM multi-positive results were consistent with those of the indirect immunofluorescence assays, indicating that

cross-reactivity commonly causes false positives when screening IgM Abs [12]. Likewise, Architect rubella IgM showed cross-reactivity for reactive samples with HSV IgM in our study. The signal of reactive Architect anti-HCV was weak (1.42/1.40 S/CO; cutoff, 1.00) for the reactive serum with HAV IgG. Several studies have reported high false-positive rates for the Architect assay (Abbott GmbH) for diagnosing HCV infection, especially in samples with low S/CO [13,14].

This study has two main limitations. First, approximately 160 reactive serum samples were tested for other infectious diseases, although the aim was to investigate only the false-positive rate owing to sample and assay availability. Therefore, the possibility of false negatives was not considered. Second, we could not collect clinical information from the tested serum samples due to anonymization, and we did not perform gold-standard tests (such as PCR, western blot, and mass spectrometry) due to insufficient and inappropriate types of samples. Thus, it was impossible to precisely differentiate among the false-positive results, cross-reactivity or false positivity of the tested results, and superinfection of various pathogens. We could only suspect cross-reactivity of the test assays by referring to the supplemental test results.

In conclusion, there was good agreement among infectious Ab assays for diagnosing current infections with low false-positive rates, although some assays showed substantial cross-reactivity with pre-existing Abs against other infectious diseases. Therefore, if the test results for infectious Abs differ from the clinical findings, or if there is high reactivity to other infectious Abs, the results should be carefully interpreted because of the possibility of cross-reactivity. Similarly, for infectious diseases that are clinically difficult to differentiate, extensive screening for relevant infectious Abs is necessary.

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