Evaluation of Commercial Rubella Immunoglobulin G Avidity Assays*†

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We compared the performances of five commercial rubella virus immunoglobulin G (IgG) avidity assays. The Adaltis (kappa = 0.28) and Diesse (kappa = 0.33) assays showed poor correlation, the Behring assay (kappa = 0.68) showed good correlation, and the Euroimmun (kappa = 0.95) and Radim (kappa = 0.94) assays showed excellent correlation with a well-established in-house rubella virus IgG avidity assay. The Euroimmun and Radim assays were statistically significantly better than the other commercial assays (P < 0.01).

Laboratory confirmation of acute rubella virus infection relies upon the detection of rubella virus-specific immunoglobulin M (IgM), rubella virus isolation, or a greater-than-fourfold rise in the rubella virus IgG titer (1). Consideration must be given, however, to the possibility of false-positive rubella virus IgM results where the prevalence of rubella is low (2, 15). Also, IgM may persist beyond the expected period of 4 weeks (14). Acute rubella infection during the first trimester of pregnancy carries a high risk of congenital rubella syndrome in the newborn. The interpretation of a positive rubella virus IgM result during pregnancy, where the implications of a false-positive or misinterpreted result are significant, is therefore particularly important (2). In addition to seeking patient history pertaining to vaccination status, recent exposure, and signs or symptoms of clinical infection, additional laboratory testing provides clinicians and patients with more information upon which to base clinical-management decisions. Immunoglobulin G avidity testing has been shown to be useful for differentiating recent from past rubella infection or reinfection and is particularly useful for investigating suspected rubella in pregnant women. Rubella virus IgG avidity has been examined in patients with recent and remote rubella infections, (3, 6, 7), reinfection (5), in congenital rubella syndrome (4, 13), and in human immunodeficiency virus-infected patients (10). In addition, the avidities induced by different rubella virus antigens have been characterized in patients with primary and remote infection, as well as vaccinees (8, 9). In this study, we compared the performances of five commercial rubella virus IgG avidity assays.

Two panels of sera were used for the analysis (n = 94). The low-avidity panel consisted of 49 sera collected from acute rubella cases as previously described (15). The high-avidity panel consisted of 53 sera from pregnant women undergoing routine prenatal rubella screening.

An in-house rubella virus IgG avidity assay based on the method developed and described by Thomas and Morgan-Capner (11, 12) was used as the gold standard avidity assay. Briefly, a 1:10 dilution of rubella virus antigen (BioGenesis, Poole, United Kingdom) was prepared in phosphate-buffered saline and sonicated in a bath style sonicator for 5 min. The diluted antigen was added to coating buffer (0.015 M sodium carbonate, 0.035 M sodium bicarbonate, pH 9.6) to a final dilution of 1:1,000. Fifty microliters was added to each well of a Nunc Polysorb microtiter plate (VWR, Mississauga, Canada), which was sealed and incubated overnight at 4°C. Control and patient sera were diluted 1:200 in 2% normal goat serum (NGS) (Invitrogen, Burlington, Canada) in phosphate-buffered saline plus 0.05% Tween (PBST). The plates were washed three times with PBST before 50 μl of the diluted serum was inoculated into duplicate wells of the microtiter plates. The plates were then incubated at room temperature for 1 hour in a humidified chamber. After aspiration of the dilute serum from the plate, 100 μl of 2% NGS-PBST was added to the wells (untreated wells) in parallel with 100 μl of 35 mM diethylenetriamine dihydrochloride substrate and buffer (Sigma-Aldrich, Oakville, Canada) in 2% NGS-PBST (treated wells). The plates were incubated at room temperature for 5 min and then aspirated. This cycle was repeated a total of three times and then followed by six washes with PBST. Fifty microliters of a 1:2,500 dilution of goat anti-human IgG with horseradish peroxidase conjugate (Jackson, West Grove, PA) was added per well. The plates were reincubated for 1 h in a humidified chamber. After the plates were washed three more times with PBST, 50 μl of o-phenylenediamine dihydrochloride substrate and buffer (Sigma-Aldrich, Oakville, Canada) in deionized water was added to each well. The plates were incubated in the dark for 15 min before 50 μl 2 M sulfuric acid was added. The optical densities (OD) were read on a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA) at 490 nm using SoftMx Pro 2.6.1 software (Molecular Devices, Sunnyvale, CA). Avidity indices were calculated using the following formula: avidity index = (OD of diethylamine-washed well/OD of buffer-washed well) × 100 (%).

Figure 1 shows the results of the in-house rubella virus IgG avidity assay. The low-avidity panel clearly yielded low-avidity results (<50%), and the high-avidity panel clearly yielded high-avidity results (>60%), thus defining the in-house assay as the gold standard for the purpose of this study.

The five commercial IgG avidity kits assessed were Adaltis EIAgens (Adaltis Italia, Casalecchio di Reno, Italy), Enzygnost anti-rubella virus IgG avidity (Dade Behring, Marburg, Germany), Diesse Enzywell (Diesse Diagnostica Senese, Siena,
Italy), Euroimmun (Lübeck, Germany), and Radim EIA Well (Radim SpA, Rome, Italy). The commercial assays were carried out according to the manufacturers’ instructions.

The high- and low-avidity serum panels were run on each of the commercial rubella virus avidity assays (Fig. 2). Replicate testing was performed on separate days. The mean of replicate runs was used for this analysis. The weighted kappa statistic was used to assess the level of agreement between the gold standard and each commercial assay (Table 1). A weighted kappa of greater than 0.8 was considered excellent, 0.6 to 0.8 good, 0.4 to 0.6 fair, and less than 0.4 poor.

The percent avidity result for each kit was determined according to the manufacturer’s criteria, and therefore, not all sera were able to have percent avidity results calculated due to indeterminate or incalculable results. Indeterminate or incalculable results reflect kit differences to a significant degree.

![Graph](image)

**FIG. 1.** Performance of the in-house gold standard rubella virus IgG avidity assay using the low-avidity (diamonds) and high-avidity (squares) panels. The high-avidity (>60%) and low-avidity (<50%) cutoff values are indicated by the dashed horizontal lines.

**FIG. 2.** Scatterplots of commercial rubella virus IgG avidity assays with respect to the in-house gold standard assay. (a) Adaltis. (b) Behring. (c) Diesse. (d) Euroimmun. (e) Radim. High- and low-avidity cutoff values are indicated for the in-house gold standard assay (vertical dashed lines) and for the commercial assays (horizontal dashed lines).

**TABLE 1.** Weighted kappa values for commercial rubella virus avidity assays compared to in-house gold standard

<table>
<thead>
<tr>
<th>Commercial assay</th>
<th>Complete data set</th>
<th>Reduced common data set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weighted kappa</td>
<td>Standard error</td>
</tr>
<tr>
<td>Adaltis</td>
<td>66</td>
<td>0.28</td>
</tr>
<tr>
<td>Behring</td>
<td>82</td>
<td>0.68</td>
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<tr>
<td>Diesse</td>
<td>83</td>
<td>0.33</td>
</tr>
<tr>
<td>Euroimmun</td>
<td>81</td>
<td>0.95</td>
</tr>
<tr>
<td>Radim</td>
<td>85</td>
<td>0.94</td>
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*The complete data set consists of the entire low- and high-avidity panels.

*The reduced common data set consists of only the samples which gave readable results for all commercial assays used.
commercial assays. Avidity assays performed well and can be considered reliable data sets. The number of incalculable results for the Adaltis kit (reflected in the n of 66 in the complete data set in Table 1). Assays producing measurable results were included in the calculation of the weighted kappa. The results of the comparison of each kit to the gold standard are presented in Table 1 and Fig. 2. The weighted kappa statistics for the Euroimmun and Radim assays were 0.95 and 0.94, respectively, demonstrating excellent correlation with the gold standard. The weighted kappa values for the Adaltis and Diesse kits were 0.28 and 0.33, respectively, indicated poor correlation with the gold standard. The kappa value for the Behring kit, 0.68, indicated good correlation with the gold standard. Since incalculable results were not included in this analysis, each weighted kappa was based on a different sample size. To determine whether the results were affected by these slightly different sample sizes, the entire analysis was repeated on a reduced common data set of 66 samples, for which the in-house gold standard assay and each commercial kit produced a measurable avidity result. The kappa values for the Euroimmun and Radim assays (0.95 and 0.94, respectively) were statistically significantly better than those of the other assays using the z test (P < 0.01) for both the full and reduced data sets.

In conclusion, the Euroimmun and Radim rubella virus IgG avidity assays performed well and can be considered reliable commercial assays.

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REFERENCES