Evaluation of Commercial Rubella IgG Avidity Assays

JCM01243-06

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Running Title: Commercial Rubella IgG Avidity Assays

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October 6, 2006, version 2
Abstract

We compared the performance of five commercial rubella IgG avidity assays. The Adaltis (Kappa = 0.28) and Diesse (Kappa = 0.33) assays showed poor correlation, Behring (Kappa = 0.68) showed good correlation, and Euroimmun (Kappa = 0.95) and Radim (Kappa = 0.94) assays showed excellent correlation with a well-established in-house rubella 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 infection relies upon the detection of rubella-specific IgM, rubella virus isolation, or a greater than fourfold rise in rubella IgG titre (1). Consideration must be given, however, to the possibility of false-positive rubella 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 (CRS) in the newborn. There is therefore particular importance in the interpretation of a positive rubella IgM result during pregnancy, where the implications of a false-positive or misinterpreted result are significant (2). In addition to seeking 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 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 HIV-infected patients (10). In addition, the avidity induced by different rubella antigens has been characterized in patients with primary and remote infection, as well as vaccinees (8, 9). In this study, we compared the performance of five commercial rubella IgG avidity assays.

Two panels of sera were used for this 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 pre-natal rubella
screening.

An in-house rubella IgG avidity assay based on the method developed and described by
Thomas et al (11, 12) was used as the "gold-standard" avidity assay. Briefly, a 1:10
dilution of rubella antigen (BioGenesis, Poole, United Kingdom) was prepared in
phosphate-buffered saline (PBS) and sonicated in a bath style sonicator for 5 minutes.
The diluted antigen was added to coating buffer (0.015M Sodium carbonate, 0.035M
Sodium Bicarbonate, pH 9.6) for a final dilution of 1:1000. Fifty microlitres was added to
each well of a Nunc Polysorb microtiter plate (VWR, Mississauga, Canada) sealed, and
incubated overnight at 4°C. Control and patient serum were diluted 1:200 in 2% normal
goat serum (NGS) (Invitrogen, Burlington, Canada) in PBS plus 0.05% Tween (PBST).
The plates were washed 3 times with PBST before 50 µl of the diluted serum was
inoculated into duplicate wells of the microtiter plates. Plates were then incubated at
room temperature for one 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 35mM diethylamine (DEA), (Sigma-Aldrich, Oakville, Canada)
in 2% NGS/PBST (treated wells). Plates were incubated at room temperature for 5
minutes and then aspirated. This cycle was repeated for a total of three times, and then
followed by 6 washes with PBST. Fifty µl of a 1:2500 dilution of goat anti-human IgG
with horseradish peroxidase conjugate (Jackson, West Grove, Pennsylvania, USA) was
added per well. Plates were reincubated for 1 hour in a humidified chamber. After
washing plates 3 more times with PBST, 50 µl of o-phenylenediamine dihydrochloride (OPD) substrate and buffer (Sigma-Aldrich, Oakville, Canada) in deionized water was added to each well. Plates were incubated in the dark for 15 minutes before adding 50 µl 2M sulphuric acid. The optical densities (OD) were read on a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale California, USA) at 490nm using SoftMAX Pro 2.6.1 software (Molecular Devices, Sunnyvale California, USA). Avidity indices were calculated using the following formula:

\[ \text{Avidity Index} = \left( \frac{\text{OD of DEA-washed well}}{\text{OD of buffer-washed well}} \right) \times 100 \% \]

Figure 1 shows the results of the in-house rubella IgG avidity assay. The low panel clearly yields low avidity results (< 50%) and the high panel clearly yields 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 EIAgen (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); Radim EIA Well (Radim SpA, Rome, Italy). The commercial assays were carried out according to manufacturers’ instructions.

The high and low avidity serum panels were run on each of the commercial rubella avidity assays (figure 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 greater than 0.8 is considered excellent, 0.6 to 0.8 good, 0.4 to 0.6 fair, and less than 0.4 poor.

The % avidity result for each kit was determined according to the manufacturer's criteria, and as such, not all sera were able to have % avidity results calculated due to indeterminate or incalculable results. Indeterminate or incalculable results reflect kit differences to a significant degree since such results for a particular sample were not the same for all kits. In addition, low titer sera (in the low avidity acute serum panel) also contributed to a significant number of incalculable results. All but the Euroimmun kit required a minimum OD for results to be calculable. However, for the Euroimmun method, only samples testing IgG positive by Euroimmun would be tested for avidity. The threshold was higher for the Adaltis and Diesse kits, which required an OD threshold of ≥0.40 in the untreated well in order for results to be interpretable. This may in part account for the greater number of incalculable results for the Adaltis kit (reflected in the n=66 in the complete data set in table 1). Assays producing measurable results were included in the calculation of the weighted Kappa. Results of the comparison of each kit to the gold-standard are presented in table 1 and figure 2. The weighted kappa statistic 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 is based on a different sample size. To determine whether or not the results were affected by these slightly different sample sizes, the entire analysis was repeated on a reduced common dataset 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 from the other assays using the z-test (p<0.01) for both the full and reduced datasets.

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

Acknowledgements

We thank Dr. Dan Château for his expertise in the statistical analysis. Cadham Provincial Laboratory (Winnipeg, Canada) is gratefully acknowledged for provision of some of the sera.
References


Figure Legends

Figure 1: Performance of the in-house “gold-standard” rubella IgG avidity assay using the low (diamonds) and high avidity panels (squares). The high (>60%) and low (<50%) avidity cut-off values are indicated by the dashed horizontal lines.

Figure 2: Scatterplots of commercial rubella IgG avidity assays (a) Adaltis, (b) Behring, (c) Diesse, (d) Euroimmun, and (e) Radim with respect to the in-house gold-standard assay. High and low avidity cut-off 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 value for commercial rubella 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>n</td>
<td>Weighted Kappa</td>
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<tr>
<td>Adaltis</td>
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</tr>
<tr>
<td>Behring</td>
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<td>Diesse</td>
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<td>Euroimmun</td>
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<tr>
<td>Radim</td>
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<td>0.94</td>
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</tbody>
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\[a\] The complete data set consists of the entire low and high avidity panels.  
\[b\] The reduced common data set consists of only the samples which gave readable results for all commercial assays used.
Figure 1
Figure 2

2a. Adaltis

2b. Behring

2c. Diesse

2d. Euroimmun

2e. Radim