Evaluation of a Latex Agglutination Test for Detection of Antibodies to Rubella Virus in Selected Sera

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A new latex agglutination test for rubella virus was used to test sera with a hemagglutination inhibition titer of =8 (97 specimens) or $\geq$256 (158 specimens). Maximum latex agglutination test sensitivity was achieved when low-titer sera were tested undiluted and high-titer sera were diluted (1:10). A modification of the protocol of the manufacturer resulted in a latex agglutination-hemagglutination inhibition agreement of 96.7%.

The detection of humoral antibodies is the only feasible means available to clinical laboratories for assessing immune status to rubella virus. Radioimmunoassay, enzyme-linked immunosorbent assay, passive hemagglutination, and indirect immunofluorescence are among newer techniques developed for the detection of these antibodies. However, these are relatively complex, time-consuming techniques, and several require special equipment. Reports indicate acceptable agreement of these testing systems with the hemagglutination inhibition (HAI) test (1, 3, 5, 6, 8), still considered the standard against which all other procedures are judged.

Rubascan, a passive latex particle agglutination (LA) test recently marketed by Hynson, Westcott & Dunning, Inc. (Baltimore, Md.), is a simple, rapid (8-min) card test requiring minimal technical training. All reagents are included in the test kit, and the only equipment required is a rotator. The manufacturer suggests two different qualitative procedures, i.e., testing undiluted sera for maximum sensitivity or sera at a 1:10 dilution to approximate HAI sensitivity levels. Evaluations of Rubascan show excellent agreement with the HAI test in the assessment of immune status (1, 4, 7).

This report describes the relative sensitivity of the procedures for testing undiluted and diluted sera and illustrates the effect of prozones on LA test reactivity.

All sera tested had been submitted to the serology laboratory for a determination of immune status. Both HAI (Rubindex, Ortho Diagnostics, Raritan, N.J.) and LA procedures were performed according to the directions of the manufacturer. Results of LA testing were interpreted as positive (agglutination) or negative (no agglutination). Sera were selected for LA testing based on HAI test results: <8 (76 specimens), 8 (21 specimens), and $\geq$256 (158 specimens).

A comparison of results is shown in Table 1. Of the 76 sera with no detectable antibody (titer, <8), agglutination of latex particles occurred in 5 instances when sera were tested undiluted (1:1) and in 1 instance when sera were tested diluted (1:10). All 21 sera containing low levels of antibody (titer, 8) were positive by the undiluted procedure, although 6 of the 21 diluted sera were negative. LA test results were negative in 6 of the 158 high-titer, undiluted sera ($\geq$256), but all 158 specimens were positive when diluted.

In addition to the six negative results obtained with undiluted sera, seven other sera exhibited weak agglutination (Table 2). These weak reactions were observed in LA testing of five sera with a titer of 256, one serum with a titer of 512, and one serum with a titer of 1,024. No reactivity was seen with four sera (titer, 256) or two sera (titer, 512). The manufacturer states that any agglutination constitutes a positive test. However, careful scrutiny was necessary to make an observation of agglutination with the seven high-titer sera. Meegan et al. (4) noted weak agglutination in six instances when high-titer sera were tested undiluted, but upon retesting these samples at a 1:2 dilution, the agglutination pattern was strong. Similar results were observed in our study. All 13 sera, which were nonreactive (6 specimens) or weakly reactive (7 specimens) undiluted, were strongly reactive when diluted (1:10). This indicates that a prozone phenomenon needs to be considered when using the undiluted procedure. Although a direct correlation between this phenomenon and the HAI titer at high levels was not demonstrated.
TABLE 1. Comparison of HAI and LA test results

<table>
<thead>
<tr>
<th>LA result</th>
<th>Dilution</th>
<th>&lt;8&lt;sup&gt;a&lt;/sup&gt;</th>
<th>8</th>
<th>≥256</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1:1</td>
<td>5</td>
<td>21</td>
<td>152</td>
</tr>
<tr>
<td>Negative</td>
<td>1:1</td>
<td>71</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Positive</td>
<td>1:10</td>
<td>1</td>
<td>15</td>
<td>158</td>
</tr>
<tr>
<td>Negative</td>
<td>1:10</td>
<td>75</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> HAI titer.

(Table 2), only 14.6% of the high-titer sera tested had titers of >512, and a more extensive study may more clearly illustrate a relationship between antibody concentration and frequency of prozones.

Several investigators report a positive test by LA when there is no antibody detectable by HAI testing (2, 4, 7). The greater sensitivity is most pronounced when LA testing is performed on undiluted sera. Meegan et al. (4) found neutralizing antibody (titer, 8 to 32) in seven of eight LA test-reactive, HAI test-nonreactive sera. We were able to demonstrate a positive LA reaction in 5 of 76 undiluted sera which had an HAI titer of <8.

The Immunization Practices Advisory Committee (Morbid. Mortal. Weekly Rep. 30:37–47, 1981) indicates that antibody to rubella virus may be demonstrated by methods more sensitive than HAI testing, and any detectable antibody is presumptive indication of immunity and protection against subsequent viremic infection. The LA undiluted test procedure may very well be a more sensitive method than the HAI test for detecting low levels of viral antibodies.

The greatest LA reactivity was obtained with the undiluted protocol when sera had a low HAI titer (<8) and with the diluted protocol when sera had a high HAI titer (≥256). If LA and HAI tests are considered equally specific, a latex testing protocol which requires that all sera be initially tested undiluted, followed by repeat testing of all nonreactive sera at a 1:10 dilution, would give maximum sensitivity. In our study, this protocol would result in LA-HAI test agreement with 321 of 332 sera (96.7%).

Therefore, we recommend a change in the protocol of the manufacturer for testing undiluted sera. Sera should be initially tested undiluted, and nonreactive sera should be further tested at a 1:10 dilution. Any agglutination in either method should be interpreted as positive. This change will provide maximum sensitivity and eliminate the reporting of false-negative results due to a prozone reaction.

LITERATURE CITED


TABLE 2. Results of LA tests on undiluted sera with HAI titers of ≥256

<table>
<thead>
<tr>
<th>LA test</th>
<th>No. of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>256&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>76</td>
</tr>
<tr>
<td>Weak</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> HAI titer.