New Latex Agglutination Test for Rapid Determination of Rubella Immune Status

ALICE S. WEISSFELD†* AND ALEX C. SONNENWIRTH

Department of Pathology and Department of Microbiology and Immunology, Washington University School of Medicine, and Department of Laboratory Medicine, The Jewish Hospital of St. Louis, St. Louis, Missouri 63110

Received 14 May 1982/Accepted 16 July 1982

A prototype rubella latex agglutination card assay (Hynson, Westcott and Dunning, Baltimore, Md.) was compared with a standard hemagglutination inhibition test for the detection of rubella antibodies in 500 sera. The sensitivity and specificity of the latex agglutination assay were 100% and 94%, respectively. This assay did not require pretreatment of serum, and the entire assay could be performed in 10 min.

Several different test methods are currently available for detecting serum antibodies to rubella virus. These include neutralization, hemagglutination inhibition (HAI), complement fixation, passive hemagglutination, enzyme-linked immunosorbent assay, and indirect immunofluorescence tests. Diagnostic test kits are commercially available for most of these procedures. Recently, a new test was developed for the determination of rubella immunity. This assay, marketed as the Rubiscan card test by Hynson, Westcott and Dunning (Baltimore, Md.), is a passive latex agglutination test. We evaluated a prototype kit by comparing it with the standard HAI test recommended by the National Committee for Clinical Laboratory Standards and the Center for Disease Control (3, 4). This report is a summary of our findings.

A total of 200 encoded sera received from the manufacturer and 300 random sera submitted to the Serology Laboratory at The Jewish Hospital of St. Louis for rubella immune status determination were screened. Samples of each serum were stored at −70°C to avoid unnecessary freeze-thaw cycles.

The standard HAI test was performed with 1-to 3-day-old chick erythrocytes and heparin-manganese chloride extraction. All reagents other than erythrocytes were purchased from Flow Laboratories (McLean, Va.); chick and chicken erythrocytes were purchased from Truslow Farms (Chestertown, Md.). Results were evaluated as either antibody present (titer, \( \geq 1:8 \)) or antibody not present (titer, \( <1:8 \)).

The rubella latex agglutination (RLA) card assay was performed according to the manufacturer's instructions. Although the current commercial kit provides protocols for testing specimens either undiluted or diluted 1:10, all sera used in this study were diluted 1:10 to approximate the sensitivity level obtained with the reference HAI method. Serum dilutions were carried out directly on the test card; an initial 1:5 dilution was prepared in each square, and the final 1:10 dilution was prepared by making a single serial dilution into the adjacent circle. The test specimen was then spread to fill the entire circle, one drop of latex antigen was dispensed onto each circle, and the card was rotated under a moistened humidifying cover for 8 min at 100 rpm; the rotator was the same one used with the Macro-vue RPR card test for syphilis (Hynson, Westcott and Dunning, Baltimore, Md.). At the end of 8 min the card was rotated and tilted briefly by hand and then observed under a high-intensity incandescent lamp. Results were evaluated as either positive (agglutination present) or negative (no agglutination) for rubella antibodies.

A comparison of HAI and RLA results with the panel of 200 sera received from Hynson, Westcott and Dunning is shown in Table 1. The RLA assay gave no false-negative results. The 3 of 44 sera that were antibody-negative by HAI and positive by RLA were quantitated with the RLA card assay, and each had a titer of 1:40. Thus, although these samples were considered to be false positives when compared with HAI results, the specimens may, in fact, have contained rubella antibody.

A comparison of HAI and RLA results for 300 clinical specimens submitted to The Jewish Hospital of St. Louis serology laboratory is shown in Table 2. Of 119 sera that were antibody negative by HAI, 7 were positive by RLA. However, six of these false-positive sera were rubella antibody-positive by an enzyme-linked immunosor-
TABLE 1. Comparison of standard HAI method with RLA card assay in a panel of 200 sera received from the manufacturera

<table>
<thead>
<tr>
<th>HAI</th>
<th>RLA</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>41</td>
</tr>
</tbody>
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a Overall results: sensitivity, 156/156 = 100%; specificity, 41/44 = 93%; false positives, 3/44 = 7%; false negatives, 0/156 = 0%.

bent assay (BioEnzaBead, Litton Bionetics, Kensington, Md.), two had previously been shown to contain neutralizing antibody to rubella virus, and five gave titers of at least 1:20 when quantitated by the RLA card assay.

The overall sensitivity and specificity of the RLA card assay for the detection of rubella antibodies in all 500 sera tested was 100% and 94%, respectively. Our findings were in agreement with those of Castellano et al. (1), who briefly described their experience with the RLA card assay in a recent paper comparing several commercially available diagnostic test kits for rubella.

The most desirable assay for rubella antibodies will be both highly specific and highly sensitive, since false-positive tests could result in failure to vaccinate susceptible women and false-negative tests could result in unnecessary abortions. For many years, HAI has been viewed as the standard against which other, newer tests for rubella have been judged. No serological test is 100% accurate, however. Even in the HAI test the incomplete removal of nonspecific inhibitors (especially in hyperbilirubinemic sera) can lead to false-positive results, and the removal of rubella-specific antibodies during absorption can lead to false-negative results. In the present study, the results obtained by HAI and RLA showed complete agreement for 490 of the 500 sera (98%). The discrepant sera were all falsely positive when compared with the HAI results. However, it is becoming increasingly well documented that sera which are determined to be rubella antibody negative by HAI may be positive when newer, more sensitive methods, such as indirect immunofluorescence and enzyme-linked immunosorbent assay, are employed (1, 2, 5, 6); this may also be the case with the RLA card assay, which appears to be a highly sensitive and specific new test kit for the determination of an individual’s immune status for rubella virus. Sera need not be pretreated, and the entire procedure can be performed in less than 10 min with equipment which most clinical laboratories already use for syphilis testing.

The technical expertise of Jeanne Ruperto is greatly appreciated.

LITERATURE CITED