Comparison of a Simple Latex Agglutination Test with Hemolysis-in-Gel, Hemagglutination Inhibition, and Radioimmunoassay for Detection of Rubella Virus Antibodies

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Rubella virus antibodies were measured in 300 sera from pregnant women visiting a maternity center by using a new, simple latex test, Rubalex. The results were compared with those obtained by using hemolysis in gel, hemagglutination inhibition, and radioimmunoassay. The sensitivity of the latex test was 100.0, and 99.6% when compared with hemolysis in gel, hemagglutination inhibition, and radioimmunoassay, respectively. Its comparative specificity was 96.2, 95.7, and 90.7%, and the predictive value of a positive result was 99.2, 99.2, and 98%, respectively. When assayed with the British standard anti-rubella serum its sensitivity was 11 IU/ml. The latex test gave a positive result within 2 min, and 87% of the positive samples had already reacted after 1 min. The negative results remained as such for at least 8 min. No prozone effect was observed for sera with hemagglutination inhibition titers from 256 to 2,048. We concluded that the latex test, Rubalex, was readily applicable for measuring rubella immunity with a reaction time of 2 min in undiluted samples.

Serum antibodies against rubella are usually determined for three purposes: (i) to establish the immune status, (ii) to diagnose a rubella infection, and (iii) to diagnose congenital rubella. The commonly used standard technique for this purpose has been the hemagglutination inhibition (HI) test (2, 5, 10). This is, however, time consuming and sensitive to nonspecific inhibitors. The hemolysis-in-gel (HIG) test (16, 17) was developed to overcome the problems of the HI test. The HIG test, however, requires overnight incubation and therefore does not give rapid results.

Latex tests have been in wide use in clinical laboratories for many years. Results of the first latex test for rubella antibody determinations (Rubiscan; Hyson, Westcott & Dunning, Baltimore, Md.) was recently reported to correlate well with those of the conventional techniques (1, 6, 12, 14, 15). In this study we compared another rubella latex test (Rubalex; Orion Diagnostica, Espoo, Finland) with three conventional techniques: HIG, HI, and radioimmunoassay (RIA).

MATERIALS AND METHODS

Sera. A total of 300 serum samples were obtained from a maternity center and sent to the National Public Health Institute, Kuopio, Finland. Sera were coded and then tested by the HIG technique. Of the 300 sera, 250 were unselected, whereas 50 sera were selected because they were negative in the HIG test. The sera were stored at -20°C until subsequent testing with latex agglutination (LA), HI, and RIA.

As a rubella reference serum the British standard for anti-rubella serum (69/60) was used. It had a potency of 720 IU/ml (4).

HIG test. The HIG test was performed by using a commercial Orivir Rubella kit (Orion Diagnostica, Espoo, Finland). The smallest measurable zone of hemolysis was 3 mm in diameter and was regarded as positive.

HI test. The HI test was performed by the standard rubella HI test of the Centers for Disease Control, Atlanta, Ga. (2).

The sera were treated with heparin-MnCl₂ to remove nonspecific inhibitors, and 1-day-old chicken erythrocytes were used as indicator cells. A titer of 1:8 was considered the lowest positive result.

LA test. The Rubalex test was used for the LA test. A volume of 25 µl of undiluted serum was pipetted onto a test card. One drop (25 µl) of a solution containing latex particles coated with rubella virus antigen was added and mixed carefully with the serum, and the card was tilted for 2 min. The test was regarded as positive if agglutination of the latex particles was observed within this time. If the agglutination was completed earlier, the time was recorded.

RIA. The solid-phase RIA for rubella immunoglobulin G (IgG) and IgA antibodies was carried out as described previously (9, 13). The antibody concentrations were expressed in arbitrary units by means of a standard curve (9). Values of 5 U or more were considered positive.

Calculations. The calculations for sensitivity, specificity, and predictive values were done with binary tables and the data in Table 1 as previously described (8).

RESULTS

The 300 sera described above were tested by the Rubalex test, and the results were compared with those obtained by using conventional tests for rubella antibody titrations, such as HIG, HI, and RIA. Since the result was considered positive in the latex test if there was any visible agglutination, the limit of positive response in the other techniques was also set close to the detection limit, being the lowest measurable HIG titer (3 mm), the lowest RIA titer above the background (5 U), and the reciprocal of the lowest dilution giving positive results in the HI test (1:8).

The results of the comparison are summarized in Table 1, in which the results obtained with the latex test are compared with the corresponding results in the conventional tests. All the tests were in good agreement. The sensitivity of the LA test (LA positive/reference test positive) was calculated to be 98, 99.6, and 100% in comparison with those of the HI test, the RIA, and the HIG test, respectively. The

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sensitivity of the LA test was also determined by using serially diluted British standard anti-rubella serum. The LA test gave a positive result with a dilution of 1:64 of the standard serum, corresponding to a sensitivity of 11 IU/ml. When the standard serum was tested in the HIG test, the detection limit of 3 mm was obtained with a dilution of 1:96, corresponding to 7.5 IU/ml. The relative specificity of the LA test (LA negative/reference test negative) was calculated from the values in Table 1 to be 95.7, 90.7, and 96.2% in comparison with those of the HI test, the RIA, and the HIG test, respectively.

The predictive value of the LA-positive result (true positive/LA test positive) was calculated to be 99.2, 98, and 99.2% with the HI test, the RIA, and the HIG test, respectively. Some of the sera reacted very rapidly in the LA test, suggesting a possible correlation between the speed of the reaction and the antibody titer. However, the samples reacting fastest in the LA test (complete agglutination in 30 s to 1 min) represented the whole range of HIG values as well as HI or RIA titers. The speed of the agglutination reaction was therefore not dependent solely on the antibody titer of the sample.

Characteristics of the agglutination reaction. As a rule, the agglutination reaction was found to be very easy to interpret. Although the latex test involved incubation for 2 min, in most cases (87%) the reaction was already complete after 1 min. A few sera reacted weakly, and the interpretation of these results was difficult. One of these sera (no. 43426; see Table 2) was regarded as weakly positive when the agglutination was tested by using a transparent microscope slide instead of the cardboard test cards of the LA test kit.

To determine the effect of prolonged reaction time on the results, 22 negative and 15 weakly positive sera were tested in the LA test, and the stage of agglutination was recorded after 2, 4, and 8 min. None of the negative sera became positive during the prolonged reaction time. Of the weakly positive sera, 10 out of 15 agglutinated more intensively after a longer reaction time. This experiment showed that the testing time of 2 min is enough for the differentiation of negative from positive samples. A longer incubation time, e.g. 3 min, would, however, make the test easier to interpret for weakly reacting sera.

Discrepant samples. Thirteen of the sera gave discrepant results when tested by different techniques. A closer examination of these samples can be seen in Table 2. The five LA-negative, HI-positive samples (including four samples with an HI titer of 1:128) were apparently false positives in the HI technique as a result of incomplete removal of the nonspecific inhibitors (11), because all the other tests gave negative results with these samples. The two LA-positive, HI-negative samples were also negative in the RIA-IgG but had a weak positive reaction in the HIG test. The next five LA-positive sera were also positive in the HI test, two of them were positive in the RIA-IgG but negative in the HIG test, and three were positive in the HIG test but negative in the RIA-IgG. The one RIA-IgG-positive, LA-negative sample (no. 43426) was also negative in the HI and HIG tests. When tested several times by the LA test, this serum gave variable results (see above). When tested with a commercial rubella IgG-enzyme immunoassay (Rubenzyme; Abbott Laboratories), it was positive. This serum obviously only gave the false-negative result with the LA test.

To determine the possible effects of other immunoglobulin classes than IgG on the discrepancy of the results, all the discrepant samples were tested for the presence of rubella-specific IgM or IgA. However, none of the sera had IgM antibodies against rubella when measured with a commercial rubella IgM-enzyme immunoassay kit (Ruben M; Northumbria Biologicals) and only one (no. 43495 in Table 2) had a low-titer IgA reaction.

Prozone phenomenon. The prozone phenomenon, often seen in immunological reactions with high-titer antibodies, has also been reported to occur sometimes in the rubella latex test (7). Therefore, 10 sera with HI titers ranging from 256 to 2,048 were measured in the LA test both undiluted and diluted 10-fold. We did not observe any inhibitory effect of the high-titer antibodies on the LA test. The sera caused a strong agglutination in the LA test both before and after dilution, although there was a slight increase in the time that elapsed before the appearance of the complete agglutination in some of the sera after dilution.

DISCUSSION

The Rubalex LA test has several advantages over conventional serological methods: (i) no predilution series of the samples are needed, (ii) the test can be carried out from a drop of sample and reagents, (iii) the performance of the test does not require any special equipment, and (iv) the result can be obtained within a few minutes. The rubella LA test described showed good correlation with the conventional reference methods, i.e., HI titration by the Centers for Disease Control and Prevention.

<table>
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* - Negative result in LA test; +, weak positive result in LA test; ++, intermediate positive result; ++++, strong positive result.
Disease Control method and the RIA-IgG, with a relative sensitivity of 98.0 and 99.6%, respectively, in the determination of immunity. The specificity as compared with the HI test was 95.7% but was only 90.7% in comparison with the RIA-IgG, apparently as a result of the fact that the LA test is slightly more sensitive than the specific IgG test.

When compared with the HIG test, which is also accepted by the Centers for Disease Control as a rubella antibody screening assay (3), the LA test attained a sensitivity of 100% and a specificity of 96.2%. The HIG test is not sensitive to the nonspecific inhibitors of hemagglutination or rheumatoid factor (17), making it a good reference method for the latex test. The sensitivity of the LA test was shown to be 11 IU/ml by the use of the British standard anti-rubella serum.

All the results suggested that the LA test is as good for the determination of rubella immunity as the reference methods are. The LA test has a much shorter reaction time than that of any of the reference methods. Of the 52 HIG-negative sera, two were found to be positive in the LA test—confirmed by the HI test—but none of the HIG-positive sera were negative in the latex test. In a recent study concerning another rubella LA test, 22 to 25% of low-antibody-titer sera were determined as false negatives (14) when compared with the HI test and enzyme immunoassay. We found only one false-negative result with the latex test described. The prozone phenomenon with high-titer sera was not demonstrable with our LA test. No correlation was found between the speed of agglutination and the HI, HIG, or RIA titers of the samples. This may be due to the short reaction time in the latex test. In the other tests, longer incubation times also provide the lower-affinity antibodies with the possibility of reacting with the antigen. All sera that were negative after 2 min were also negative after 8 min. None of these negative sera were found to be positive with the other techniques.

These results confirm that the present rubella latex test, Rubalex, which can be performed in 2 min without any special equipment, is a sensitive, specific, and simple test for the determination of rubella antibodies.

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LITERATURE CITED


