

## Rapid Latex Agglutination Test for Rubella Antibody

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The latex agglutination card test (Rubascan) for the detection of rubella antibody was compared with the standard hemagglutination inhibition and enzyme-linked immunosorbent assay tests. There was complete agreement with sera which had hemagglutination inhibition titers of  $\geq 16$ . Sera with low levels of antibody which were positive in the enzyme-linked immunosorbent assay, however, gave negative latex agglutination results approximately 25% of the time (false negatives), whereas sera which were negative in the enzyme-linked immunosorbent assay gave false-positive results in about 3% of the cases. The use of capillary "finger stick" plasma instead of venous sera resulted in additional false-negative latex agglutination tests among patients with very low antibody titers. Because of the simplicity of the method, it should be possible to use this test in physicians' offices and in large immunization campaigns. Care should be taken to become completely familiar with the procedures and reading of the agglutination patterns. Control sera should always be used. Interpretation of results should take into consideration the rates of false-negative and false-positive results noted above. These rates apply to sera with little or no antibody. In particular, negative tests should be confirmed with more specific methods in critical cases, such as pregnant women exposed to rubella or women of childbearing age who are being considered for immunization. There was no problem with the latex agglutination findings for sera with higher titers. Since results are available in 8 min, physicians should be able to counsel their patients rapidly and immunize, if necessary, while the patient is still present.

More than 20 kits are now available for the detection of rubella antibody (1, 3). Most of the kits use the hemagglutination inhibition (HI) method. Some, however, employ indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA) or immunofluorescence. Most of these tests require 1 to 2 days for completion and considerable technical ability and equipment for performing the test properly. Recently, a new, simple latex agglutination (LA) test (Rubascan) became available for the rapid detection of rubella antibody. This method was evaluated and compared with the HI and ELISA tests for rubella.

### MATERIALS AND METHODS

**Serum specimens.** The serum specimens used for this study were selected from a large panel of specimens which have been studied in our laboratory for rubella antibody. Because of the importance of distinguishing samples with no detectable rubella antibody from those with low levels of antibody, 76 of the 152 sera used for this study were known to have no detectable HI antibody to rubella at a 1:8 dilution.

Twenty-three sera were included which had only a titer of 1:8 by the HI method. The remaining sera had various levels of antibody. These sera were also tested by the ELISA method for rubella antibody detection (2).

**HI test.** All of the sera were retested with our standard HI test (4). Briefly, this test employed pretreatment of the sera with Kaolin and the use of 1-day-old chicken cells for hemagglutination.

**ELISA test.** All sera were tested by the ELISA method, and the level of antibody was quantitated as previously described (2).

**LA test.** The LA kit called Rubascan, marketed by Hynson, Westcott and Dunning, Baltimore, Md., was used for these tests. The tests involved the mixing of 1 drop of undiluted serum (25  $\mu$ l) with 1 drop of the prepared latex antigen (latex particles coated with rubella virus antigen), approximately 15  $\mu$ l. The mixing was done with a plastic spatula on a marked circle on a firm, dark card. The card was then rotated for 8 min on a mechanical platform with a plastic cover and wet sponge to minimize evaporation. At the end of 8 min, the test was read and reported as positive if there was evidence of agglutination of the latex (Fig. 1).

**Venous serum compared with capillary "finger stick" heparinized plasma.** Venous serum samples and simultaneous finger stick heparinized plasma samples were

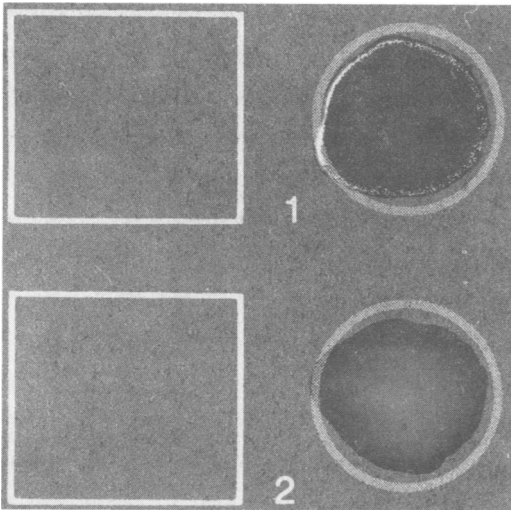


FIG. 1. LA test. (1) Positive LA test. (2) Negative LA test.

obtained from 10 volunteers. The capillary finger stick blood was obtained in a heparinized microhematocrit (Clay-Adams, Inc., Baltimore, Md.) tubes and centrifuged to obtain the plasma. The titers of the venous serum specimens were then determined by the HI method, and the capillary heparinized plasma samples were tested with the LA test.

Discrepant results were obtained with one volunteer, who had a HI titer of 8 but no detectable antibody when the capillary heparinized plasma was tested with the LA test. Further testing of this individual was conducted with additional samples of venous serum, venous plasma (heparinized microhematocrit tube and heparinized Vacutainer tube), and capillary plasma (heparinized microhematocrit tube and heparinized Brewer plasma collection card obtained from Hynson, Wescott and Dunning).

## RESULTS

The evaluation of the LA test for detection of rubella antibody compared with the HI test for presence or absence of antibody is shown in Table 1. For the 152 sera tested, there were 10 (7%) for which there was disagreement between the two tests. There was complete agreement between the two tests for all sera with HI titers of  $\geq 16$ . These sera were also positive with the ELISA method. The area of disagreement between the two tests for the low-HI-titer sera (8) and sera with no detectable HI antibody ( $< 8$ ) was further analyzed by the use of the more sensitive ELISA test data (Table 2). The HI test was positive for 23 of the 99 sera, whereas the ELISA test was positive for 28 of these sera. This was a 22% increase in positive results with the ELISA method. When the ELISA results were then compared with the LA tests for these sera, there were two false-positive LA tests for

the 71 negative sera and seven false-negative LA results for the 28 positive sera.

The results with venous serum for the HI test compared with capillary heparinized plasma for the LA test are shown in Table 3. One of the 10 volunteers tested showed an HI titer of 8 but a negative LA test. Further testing of this volunteer showed that the LA tests with venous serum or plasma were positive, but the capillary plasma samples were consistently negative with the same test.

## DISCUSSION

The LA (Rubascan) test has several advantages over other methods for detecting rubella antibody. These include (i) no pretreatment of the serum specimens, (ii) only 1 drop of serum and 1 drop of prepared sensitized latex needed, (iii) results available 8 min after mixing the test reagents, and (iv) agglutination read visually. The simplicity and speed of the test cannot be matched by any other method.

The results obtained with the LA test correlated reasonably well with the findings with the HI and ELISA methods. All sera with HI titers of  $\geq 16$  were positive with both the ELISA and LA tests. The ELISA test is more sensitive than the HI, and this was helpful in analyzing the data for sera with HI titers of  $< 8$  and 8 in relation to the LA tests. The ELISA test detected antibody in 5 of 76 sera which had no detectable HI antibody. This resulted in an increase from 22 to 28 (22%) in the number of sera with antibody. When the ELISA results for these negative and low-titer sera were used as the "standard," there were two false-positive LA tests among the 71 negative sera and seven (25%) false-negative LA results for the 28 low-titer positive sera.

Accuracy with negative and low-titer sera is always the most difficult to achieve with any method and constitutes the real "test" of any new procedure (1). Based on these studies, the LA method gave a low rate of false-positive results (ca. 3%) but a high rate of false-negative results (25%) among low-titer sera. Clinically,

TABLE 1. Rubella antibody titers by HI compared with qualitative LA tests

Titer	HI		LA	
	No. of samples	No. positive	No. positive	No. negative
<8	76	5	5	71
8	23	18	18	5
16	16	16	16	
32	12	12	12	
64	9	9	9	
128	11	11	11	
256	5	5	5	

TABLE 2. Comparison of HI and ELISA tests with LA test for 99 sera with HI titers of  $\leq 8$ 

Test	LA	
	Positive	Negative
HI		
Negative	5	71
Positive	18	5
ELISA		
Negative	2	69
Positive	21	7

the rate of false-positive tests is most important, since false-positive results imply immunity in nonimmune individuals or a seroconversion in an individual who has not been infected. Fortunately, the frequency of false positives was quite low with the LA test. The physician, however, will have to keep in mind the possibility that with the LA method, as with other methods, some false results may occur. The higher rate of false-negative tests among patients with low antibody might result in unnecessary concern for the patient regarding susceptibility and possibly unnecessary immunization. For this reason, negative tests should be confirmed with more specific methods in clinically important cases, such as pregnant women exposed to rubella or women of childbearing age who are being considered for immunization. The manufacturer and the laboratory will have to maintain careful control tests to ensure the highest specificity and sensitivity possible. Hopefully, the manufacturer will improve the specificity of the test for low-titer sera.

The use of capillary plasma for the LA test appeared attractive, since only 25  $\mu$ l of serum is required for the test and a finger stick sample could be substituted for a venipuncture. With this approach, however, we found an additional source of false-negative LA tests when testing a volunteer with an HI antibody titer of 8. With this individual, capillary plasma constantly gave a negative reaction, whereas venous serum or plasma gave positive results. The use of capillary blood apparently resulted in sufficient dilution of the low level of antibody in the sera of this patient in "tissue juices" to give a false-negative LA. If finger stick capillary plasma were used with this method, additional false-negative reactions could be expected.

Because of the simplicity of the LA test, it should be particularly helpful in clinical practice

TABLE 3. Rubella antibody detection: venous serum compared with capillary finger stick heparinized plasma

Venous serum HI titer	Capillary heparinized plasma LA test (heparinized microhematocrit tube)
<8	-
<8	-
8	- <sup>a</sup>
8	+
8	+
64	+
64	+
64	+
250	+
512	+

<sup>a</sup> Further testing of this volunteer venous serum: HI, 8; LA, positive; venous plasma (heparinized microhematocrit tube): HI, 8; LA, positive; venous plasma (heparinized Vacutainer tube): HI, 8; LA, positive; capillary plasma (heparinized microhematocrit tube): LA, negative; capillary plasma (Brewer card): LA, negative.

and large-scale immunization programs. Control positive and negative sera are provided and should be used with each test. Good lighting is needed to ensure proper reading of the agglutination. The card must be read immediately at the end of the 8 min of shaking. The technician should practice reading the agglutination pattern before beginning actual testing. Venous serum should be used to avoid unnecessary false-negative results or to confirm negative tests obtained with capillary plasma.

The speed of the LA test (8 min) is particularly attractive. The physician can now determine the need for immunization while the patient is present for the initial visit and can counsel and immunize immediately. This should eliminate the need for recalling the patient for a second visit to accomplish immunization.

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