Evaluation of Screening Tests for the Detection of Antistreptolysin O Antibodies

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The accuracy of two screening tests, one utilizing serum and the other utilizing whole blood, was compared with the accuracy of the conventional macrotitration method for the detection of antistreptolysin O antibodies. Of the 569 specimens tested with the serum screening procedure and the macrotitration method, 235 and 282, respectively, were positive for antistreptolysin O antibodies. Comparative testing of 200 specimens with the peripheral blood screening test and the macrotitration method gave 60.5% by the macrotitration procedure. Discrepant results with both procedures were obtained with specimens having 166 Todd units. The results suggest that these screening procedures can be used to screen specimens for the presence of significant antistreptolysin O antibodies.

Todd standardized a neutralization method for measuring the levels of antistreptolysin O (ASO) antibodies in patient sera (7). This procedure is based on the ability of free reduced streptolysin O, not neutralized by ASO antibodies, to lyse erythrocytes, which serve as an indicator. The endpoint is the highest dilution of serum having no hemolysis and is expressed in Todd units. This technique was subsequently modified by Rantz and Randall (5), and later a microtitration procedure of Klein et al. was introduced (3). Agglutination tests utilizing latex and erythrocytes as carriers of streptolysin O have been used for the detection of ASO antibodies (1, 2, 4, 8). These procedures are also capable of detecting antibodies to other streptococcal extracellular enzymes.

More recently Ricci et al. (6) proposed a new method that utilized whole blood and eliminated the need for adding indicator erythrocytes. This procedure is based on the capacity of the sulfhydryl groups of streptolysin O to lose their ability to hemolyze erythrocytes when oxidized while retaining their binding capacity for specific antibodies. After allowing ASO antibodies, if present, to combine with this oxidized antigen, a reducing agent is added to restore the original sulfhydryl groups. The unbound streptolysin O now has regained its ability to hemolyze erythrocytes. This principle has been adapted to two single-tube screening procedures, one with serum and the other with peripheral whole blood, for the identification of specimens with significant ASO antibody levels. The purpose of this investigation was to evaluate the accuracy of these commercial screening procedures in detecting specimens with significant ASO antibody levels when compared with the results obtained by the Rantz and Randall macrotitration method.

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MATERIALS AND METHODS

Rantz-Randall multiple-tube test (macrotitration method). The reagents and procedures for the macrotitration test were obtained from Difco Laboratories, Detroit, Mich. Specimens having a titer of 166 or greater were interpreted as being positive.

One-tube serum screening test. The reagents and procedures for performing the one-tube serum screening test were obtained from Difco. Serum was diluted 1:23 with supplied diluent, and 0.25 ml was added to a predispensed standardized amount of streptolysin O. A 0.1-ml sample of a 10% solution of rabbit erythrocytes was added, and the mixture was incubated in a water bath at 37°C for 5 min. A small amount of an enzyme reactivating reagent was added, and the tube was incubated at 37°C for an additional 5 min. Incubation was continued for 15 to 20 min for specimens that did not hemolyze after the first 5 min. Serum with an ASO antibody titer of 1:100 or less hemolyzed the erythrocytes within 5 min. Serum with an ASO antibody titer between 1:100 and 1:170 displayed partial to no hemolysis after 5 min, but full to partial hemolysis after 25 min. Serum having a titer above 1:170 displayed no hemolysis. Specimens displaying no hemolysis were interpreted as being positive.

One-tube peripheral blood screening test. The materials and methodology for performing the one-tube peripheral blood screening test were supplied by
Difco. Peripheral blood was diluted with supplied diluent, and 0.4 ml was used to rehydrate a predissolved, standardized amount of streptolysin O. The mixture was incubated in a water bath for 5 min at 37°C. A small amount of the enzyme-reactivating reagent was added, and the mixture was incubated for 5 min. Incubation was continued for 15 to 20 min for specimens that did not demonstrate hemolysis after 5 min. Specimens with an ASO antibody titer of 1:100 or less demonstrated hemolysis in 5 min. Serum having an ASO antibody titer between 1:100 and 1:170 displayed partial to no hemolysis after 5 min, but full to partial hemolysis was visible after 25 min. Serum having a titer above 1:170 displayed no hemolysis. Positive specimens were those that showed no hemolysis.

A total of 569 specimens, submitted to the Serology Laboratory of Lincoln Medical and Mental Health Center for the detection of antibodies to streptococcal exoenzymes, were used to evaluate the accuracy of the serum one-tube screening test compared with the macrotitration method. For the comparison of the macrotitration test with the single-tube peripheral blood screening test, serum and peripheral blood samples in EDTA were taken from 200 patients with documented pharyngitis, due to group A beta-hemolytic streptococci. Beta-hemolytic streptococci were serogrouped by using phadebact reagent (Pharmacia Diagnostics, Piscataway, N.J.). Of these 200 specimens, 196 were evaluated by all three procedures.

**RESULTS**

The results of the comparative testing of the one-tube serum screening test and the macrotitration procedure are presented in Table 1. Of the 569 specimens tested, 49.7% were positive by the macrotitration method, and 41.3% were positive by the screening method. Forty specimens that were negative with the serum screening method had a titer of 166 Todd units with the macrotitration procedure. Seven specimens had a titer of 250 Todd units, but were negative by the serum screening tests. Two specimens were positive by the screening test, but gave negative results with the macrotitration.

Table 1 also lists the results of 200 specimens tested with the one tube peripheral blood screening test and the macrotitration test. By the screening method, 60.5% were positive; with the macrotitration method, 65% were positive. A titer of 166 Todd units was obtained with eight specimens that were negative with the screening test. Titers of 250 and 333 Todd units were obtained with two specimens that were negative by the peripheral blood test.

Of the 200 specimens from patients with documented pharyngitis due to group A beta-hemolytic streptococci, 196 were tested by all three procedures (Table 1). Positive results were obtained for 58.7% of the specimens evaluated with the peripheral blood screening method. The serum screening procedure was positive for 59.2% of these 196 specimens; with the Rantz-Randall multiple-tube test, 63.8% were positive. Two specimens that were positive by the peripheral blood screening test gave negative results with the serum screening test and had ASO titers of 12 and 50 Todd units. One specimen with an ASO titer of 166 Todd units was positive with the peripheral blood screening test, but negative with the serum screening test. Negative results were obtained with three specimens by the peripheral blood screening test, but gave positive results with the serum screening test. These specimens had titers of 166, 250, and 333 Todd units.

**DISCUSSION**

The most widely used tests for the diagnosis of poststreptococcal disease are the microtitration and the macrotitration procedures. These are very lengthy procedures requiring reagent preparation, several titration steps, and 45 min of total incubation time.

The two screening tests evaluated have several advantages over the conventional methods. The single-dilution scheme eliminates unnecessary titration of negative sera. The incubation time is shortened to 30 min for both procedures. The single-tube peripheral blood screening test has the additional advantage of not requiring the addition of erythrocytes because the patient's blood is used for the reaction. The use of whole blood and the small volume required for testing allows for its possible use in pediatric physicians' offices. If necessary, positive specimens can be forwarded to the laboratory for further testing.

The high rate of false-negative results by the screening test with specimens having an ASO antibody titer of 1:166, a titer that is not considered significant, could be due to the fact that these screening kits have been standardized to detect specimens with an antibody titer of 1:170 and above. The Rantz-Randall method, howev-

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**TABLE 1. Comparative testing of one-tube serum and peripheral blood screening tests and the macrotitration test**

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of specimens</th>
<th>No. positive</th>
<th>No. negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening Serum</td>
<td>569</td>
<td>235</td>
<td>334</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>116</td>
<td>80</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>200</td>
<td>121</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>115</td>
<td>81</td>
</tr>
<tr>
<td>Macrotitration</td>
<td>569</td>
<td>282</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>131</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>125</td>
<td>71</td>
</tr>
</tbody>
</table>
er, gives a titer within a myriad of ranges; that is, within experimental error, a specimen that is reported as having a titer of 1:166 may have a titer between 1:125 and 1:166. The specimens that were negative by the screening procedure were probably specimens having antibody titers within the range of 1:125 and 1:166. This would be below the 1:170 antibody level which these screening tests have been standardized to detect. This should not lead to misdiagnosis, since it is recommended by rheumatologists that testing should be performed at biweekly intervals after 4 to 6 weeks after the streptococcal infection. Testing at these intervals could not be performed with the patients in this study due to the low rate of compliance to medical advice of the population served by the hospital. However, since the discrepancies at the higher titers were minimal, it is safe to assume that an increase in antibody titer would be detected by these screening procedures.

The data presented suggests that the one-tube serum screening procedure and the one-tube peripheral blood test ASO titers could be used to screen specimens for ASO antibodies. Quantitative ASO titers can then be determined for positive specimens by using the macrotitration or the microtitration procedure.

LITERATURE CITED