Comparison of Antistreptolysin O Latex Screening Test with the Antistreptolysin O Hemolytic Test

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This report describes a comparison of the Behringwerke antistreptolysin O (ASO) latex screening test with the ASO hemolytic test. Agreement between the two tests was poor when Difco streptolysin O (SLO) reagent was employed in the hemolytic test; approximately 34% of the sera with ASO titers in the normal range of the hemolytic test gave false-positive latex test reactions. However, the percentage of false-positive latex test reactions was only 5% when Behringwerke SLO reagent was used in the hemolytic test. An assay of the Difco and Behringwerke SLO reagents against an ASO standard indicated that the Difco SLO reagent was more potent than the Behringwerke SLO reagent. The lack of agreement between the Behringwerke latex test and the hemolytic test using Difco SLO reagent is attributed to the potency of the SLO reagents.

The antistreptolysin O (ASO) test is used as a laboratory aid in the detection of group A streptococcal infections. The most frequently used ASO test is a hemolytic test (3) which employs red blood cells (RBC) as an indicator system. An ASO latex test designed to screen sera with increased ASO levels from sera with normal ASO levels has been described recently (2). The latex test is a rapid slide test using a single dilution of the patient's serum and does not require RBC as an indicator system. Streptolysin O (SLO) is added to the patient's serum in a quantity sufficient to bind 200 international units of ASO per ml. If more than 200 international units of ASO per ml is present in the serum, it will cause agglutination of the subsequently added SLO-coated latex particles. The latex test reagents are produced by Behringwerke AG and distributed in the United States by Certified Blood Donor Service, Inc. The purpose of the present investigation was to determine how the latex screening test compared with the ASO hemolytic test.

MATERIALS AND METHODS

One hundred and seven human sera were tested by both the ASO latex screening test and the National Communicable Disease Center (NCDC) ASO microhemolytic test (1). Hemolytic tests were carried out by using Difco SLO reagent and Behringwerke SLO reagent (not to be confused with the Behringwerke latex test SLO reagent). The ASO titers of the sera ranged from less than 60 to 2,720; however, 94% had titers in the normal range (≤ 240) with Difco SLO reagent.

The ASO latex screening test was carried out according to the directions supplied with the commercial kit. Briefly, this consisted of adding 0.3 ml of the SLO reagent to 0.1 ml of the patient's serum in a test tube, shaking the tubes to mix the contents, and incubating at room temperature for 15 min. One drop of this SLO-serum mixture was transferred to a test plate and mixed with one drop of the latex reagent. The plate was rotated by hand for 4 to 6 min, and the test was read for agglutination or no agglutination.

Details of the hemolytic micro ASO test have been described previously (1). Serum dilutions of 1:60, 1:85, 1:120, 1:170, 1:240, 1:340, 1:480, 1:680, 1:960, 1:1,360, 1:1,920, and 1:2,720 were prepared, and 0.025 ml of SLO reagent was added to 0.05 ml of each dilution of serum. After incubation of the serum-SLO mixture at 37 C for 15 min, 0.025 ml of a 2% suspension of sheep RBC was added to each well and mixed. The plates were incubated at 37 C for 45 min, centrifuged, and read. The ASO titer was recorded as the reciprocal of the highest dilution of serum showing no hemolysis.

RESULTS AND DISCUSSION

Data from the comparison between the latex screening test and the ASO hemolytic test are presented in Table 1. Although an ASO titer of 240 is usually considered to be the upper limit of normal, it has been lowered to 170 in the present investigation because the latex test is designed to detect ASO titers above 200. Of the 107 sera tested with the hemolytic test using Difco SLO reagent,
TABLE 1. Results of the ASO latex screening test and the ASO hemolytic test

| ASO hemolytic test titer | No. of sera tested by Difco SLO reagent | No. positive with latex test | No. of sera tested by Behringwerke SLO reagent | No. positive with latex test |
|-------------------------|----------------------------------------|----------------------------|--------------------------------|----------------------------|
| <60                     | 23                                     | 0                          | 9                                  | 0                          |
| 60                      | 16                                     | 0                          | 2                                  | 0                          |
| 85                      | 20                                     | 4                          | 15                                 | 0                          |
| 120                     | 20                                     | 14                         | 14                                 | 1                          |
| 170                     | 15                                     | 14                         | 16                                 | 2                          |
| 240                     | 7                                      | 7                          | 17                                 | 11                         |
| 340                     | 4                                      | 4                          | 19                                 | 16                         |
| 480                     | 0                                      | —                          | 10                                 | 10                         |
| 680                     | 0                                      | —                          | 2                                  | 2                          |
| 960                     | 0                                      | —                          | 1                                  | 1                          |
| 2,720 or >              | 2                                      | 2                          | 2                                  | 2                          |
| Total                   | 107                                    | 45                         | 107                                | 45                         |

TABLE 2. Joint distribution of hemolytic test titers

| Behringwerke SLO reagent | Difco SLO reagent |
|--------------------------|-------------------|
|                          |                   |
| <60                      |                   |
| 60                       |                   |
| 85                       |                   |
| 120                      |                   |
| 170                      |                   |
| 240                      |                   |
| 340                      |                   |
| 480                      |                   |
| 680                      |                   |
| 960                      |                   |
| 2,720 or >              |                   |

94 had titers in the normal range. Positive latex test reactions ("false positives") were obtained on 34% of these 94 normal sera. No false-negative latex test reactions were obtained.

Agreement between the latex test and the hemolytic test when using Behringwerke SLO reagent was much better. The percentage of false-positive latex test reactions was greatly reduced—only 5% of the 56 sera with hemolytic test titers in the normal range gave a false-positive latex reaction. However, the latex test was falsely negative on 9 (18%) of the 51 sera with elevated hemolytic test titers. Six of these false negatives had a hemolytic test titer of 240, and three had a titer of 340.

Hemolytic test ASO titers were higher when Behringwerke SLO reagent was used than when the Difco SLO reagent was used. Approximately 48% of the sera had elevated titers (≥240) with the Behringwerke reagent, whereas only 12% were in the elevated range with the Difco reagent. The joint distribution of the hemolytic test titers is shown in Table 2. The average difference in titer between Behringwerke and Difco SLO reagents is two dilutions. This difference is consistent at all titer levels. As an additional check on the potency of the two SLO reagents, an assay was performed with an ASO standard, prepared from human gamma globulin containing 10 antistreptolysin units (ASU) per ml. The potency of the ASO standard was adjusted against the international ASO standard (4) containing 10 international units per ml. The assay showed that the Difco SLO reagent was stronger, requiring 1.0 ASU per tube to completely inhibit hemolysis; whereas the Behringwerke SLO reagent required only 0.63 ASU per tube to completely inhibit hemolysis. Thus, the poor agreement between the latex test using Behringwerke SLO reagent and the hemolytic test using Difco SLO reagent appears to be due to the difference in potency of the SLO reagents.

The ASO latex screening test appears to be useful for screening a few sera at a time, e.g., in a physician's office. However, it is not suitable for screening large numbers of sera because of its relatively high cost, $87.50 per 100 tests compared to $3.30 per 100 tests for the ASO microhemolytic test. Furthermore, all sera positive by the latex test must also be tested with a hemolytic ASO test to determine their titers.

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