Comparison of Three Rapid Methods for Detection of Antibodies to Streptolysin O and DNase B

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Group A streptococci produce many infections, but the two most common are pharyngitis and impetigo. During infection, the host may produce antibodies to one or more extracellular products of group A streptococci, and these antibodies are useful markers of recent streptococcal infection (9-11). Immune responses to streptococcal antigens vary and are related to the site of infection, age, etc. About 80% of patients show a response to a single streptococcal antigen. However, if multiple responses are measured, the percentage approaches 95%. Thus, the performance of more than one antibody test is desirable in patients with negative cultures and a normal or borderline titer for one streptococcal antibody (9-11).

Several commercial tests are available that are designed to detect antibodies to multiple streptococcal antigens of clinical importance. We hypothesized that these tests could be useful as screening methods to detect antibodies to either streptolysin O (ASO) or DNase B (ADB) or both, and if the results are positive, conventional ASO or ADB titer determinations could be performed on only those samples. We chose three systems for evaluation. The Streptozyme test has been evaluated by a number of investigators (3, 5-8); however, there is limited information on the LeapStrep test (4). Published information on the Check-Spectra test is not available.

Sera were obtained from patients at the Hospital of the University of Pennsylvania and from Smith Kline Laboratories (King of Prussia, Pa.). Sera were divided into aliquots and stored at −20°C until testing was to be performed. ASO were measured by the microtiter method of Klein and Jones (7). Reagents were obtained from Difco Laboratories (Detroit, Mich.). ADB were measured by neutralization of the enzyme activity of DNase B upon a calf thymus DNA substrate with a color indicator (methyl green) (1) and a commercial tube test (Streptozyme B; Wampole Laboratories, Cranbury, N.J.) performed as indicated by the manufacturer. The endpoint titers of all sera used in this study were determined.

Three commercial systems that are claimed to detect both ASO and ADB were evaluated. LeapStrep (Organon Teknika, Malvern, Pa.) is a liposome-latex agglutination test and was performed on sera diluted 1:100. Streptozyme (Wampole Laboratories) is a hemagglutination procedure performed on sera diluted 1:100. Check-Spectra (Diagnostic Technology, Hauppauge, N.Y.) tests had low sensitivities for detecting ASO (35.3 and 21.4%, respectively) and ADB (22.2 and 33.3%, respectively).

We evaluated three commercial systems for ability to detect antibodies to streptolysin O (ASO) and DNase B (ADB). Streptozyme (Wampole Laboratories, Cranbury, N.J.) exhibited high sensitivity (100%) for detecting ASO but low sensitivity for ADB (22.2%). The LeapStrep (Organon Teknika, Malvern, Pa.) and Check-Spectra (Diagnostic Technology, Hauppauge, N.Y.) tests had low sensitivities for detecting ASO (35.3 and 21.4%, respectively) and ADB (22.2 and 33.3%, respectively).

TABLE 1. Performance characteristics of rapid antibody tests compared with those of conventional methods for detecting ASO and ADB

<table>
<thead>
<tr>
<th>Antibody profile</th>
<th>No. tested</th>
<th>% Sensitivity of LeapStrep</th>
<th>% Sensitivity of Streptozyme</th>
<th>% Sensitivity of Check-Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASO+/ADB+</td>
<td>17</td>
<td>35.3</td>
<td>100</td>
<td>21.4</td>
</tr>
<tr>
<td>ASO+/ADB−</td>
<td>9</td>
<td>22.2</td>
<td>22.2</td>
<td>33.3</td>
</tr>
<tr>
<td>ASO−/ADB+</td>
<td>17</td>
<td>70.6</td>
<td>100</td>
<td>64.7</td>
</tr>
<tr>
<td>ASO−/ADB−</td>
<td>28</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* The ASO or ADB test was positive if the titer was ≥1:170.

* The overall sensitivities of LeapStrep, Streptozyme, and Check-Spectra were 46.5, 83.7, and 44.2%, respectively, and the specificities were 96.4, 85.7, and 92.9%, respectively. ND, Not determined.
ASO and ADB titers were tested \((n = 17)\), Streptozyme exhibited 100% sensitivity compared with LeapStrep (70.6%) and Check-Spectra (64.7%). There was good correlation between Streptozyme and ASO titers (Fig. 1) but less correlation with LeapStrep and Check-Spectra. There was poor correlation between the three commercial systems and ADB titers (Fig. 2).

Heath-Fracica and Estevez (4) recently compared the LeapStrep test with conventional ASO and ADB methods by using an approach similar to that used in our study. They found the sensitivities and specificities of the LeapStrep test to be 77 and 69%, respectively, for detecting ASO and 76 and 75%, respectively, for detecting ADB. They used a reference cutoff of \(\geq 1:240\), however, which is slightly higher than our cutoff of \(\geq 1:170\). To determine whether the performance of the LeapStrep test in our study improved with a higher cutoff of \(\geq 1:240\), we reanalyzed our data and used this cutoff value. For the group of sera that exhibited positive ASO and negative ADB results by this criterion \((n = 11)\), LeapStrep had a sensitivity of 45.5%, Check-Spectra had a sensitivity of 45.5%, and Streptozyme detected 100%. With sera that showed positive ASO and ADB titers \((n = 11)\), LeapStrep and Check-Spectra detected 82%, whereas Streptozyme detected 100%. For sera with negative ASO and positive ADB titers \((n = 10)\), LeapStrep and Check-Spectra had 30% sensitivity, and Streptozyme detected 70%. Thus, even when the cutoff value was increased, Streptozyme had higher sensitivity. The reason for the increased sensitivity of these tests when sera positive for both ASO and ADB were examined may be related to the observation that the mean

**FIG. 1.** Correlation of screening test titers with ASO titers. Regression lines are shown for Streptozyme (---; \(r = 0.929\)), LeapStrep (-----; \(r = 0.861\)), and Check-Spectra (-----; \(r = 0.723\)). Titers are represented as reciprocal titers. Strep AB, Streptococcal antibody.

**FIG. 2.** Correlation of screening test titers with ADB titers. Regression lines are shown for Streptozyme (---; \(r = 0.271\)), LeapStrep (-----; \(r = 0.325\)), and Check-Spectra (-----; \(r = 0.440\)). Titers are represented as reciprocal titers. Strep AB, Streptococcal antibody.
titers of sera positive for both ASO and ADB were higher (1:348 and 1:459, respectively) than those of sera positive for ASO alone (1:254) or ADB alone (1:288), or positive reactions may be more evident when both antibodies are detected. Another possibility may be that sera that exhibit high ASO and ADB titers also have increased amounts of antibodies to other streptococcal exoantigens. Because we measured only ASO and ADB titers, we do not know what proportion of sera contained significant titers of antibodies to other streptococcal antigens, such as hyaluronidase.

Several studies have compared Streptozyme with conventional antibody detection methods. Golubjatnikov et al. (3) found that this test had a high sensitivity (97%) compared with those of conventional ASO tests when a cutoff of ≥256 Todd units was used as a positivity standard and a sensitivity of 75% for ADB with a positivity cutoff of ≥1:480; the specificity was 57%, however. The low specificity and high sensitivity were related to the high cutoff values used in their study. Klein and Jones (7) tested Streptozyme by using a cutoff of ≥1:170 and found 98.5% sensitivity and 68% specificity; however, the sensitivity for detecting ADB was 85%, with 59% specificity. Gerber et al. (2) recently evaluated Streptozyme and found that this reagent was limited in detecting antibody rises in paired sera from infected patients and also exhibited lot-to-lot variation. We did not evaluate lot-to-lot variation in our study, since only a single lot was used for all testing. Earlier studies on Streptozyme also showed good performance for detecting ASO and high ADB titers (6, 8).

In summary, we found the Streptozyme test to have the highest sensitivity of all three methods studied for detecting sera with ASO titers of ≥1:170. None of the commercial systems were particularly good at detecting ADB titers at this same level. We would recommend that neither Leap-Strep nor Check-Spectra be used as a screening test for ASO or ADB because of the low test sensitivity. Streptozyme, in contrast, may be useful for screening sera only if ASO are of interest. However, like the other two systems, Streptozyme does not appear to be useful for screening sera to detect ADB.

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LITERATURE CITED