Evaluation of the Directigen 1,2,3 Group A Strep Test for Diagnosis of Streptococcal Pharyngitis

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The Directigen 1,2,3 Group A Strep Test (DGAST; BBL Microbiology Systems, Cockeysville, Md.) was compared with conventional culture methods for the detection of group A streptococci. Among 327 children, the DGAST had a sensitivity of 75.0%, a specificity of 99.1%, and positive and negative predictive values of 97.5 and 89.3%, respectively, as compared with 48-h culture results. The lower sensitivity (60.0%) in 322 adults was related to the low incidence of group A streptococcal pharyngitis in that population (7.8%). The positive and negative predictive values for adults were 93.8 and 96.7%, respectively. Only 3 of 327 (0.9%) pediatric and 2 of 322 (0.6%) adult specimens yielded uninterpretable results in the DGAST.

The diagnosis of streptococcal pharyngitis has traditionally relied upon microbiological culturing performed in the laboratory. Recently, antigen detection methods have become sufficiently sensitive to detect group A streptococcal (GAS) antigens extracted directly from throat swabs (1-4, 7, 8). As commercial products for the direct rapid detection of GAS have become available, the diagnosis of streptococcal pharyngitis has shifted from the microbiology laboratory to the physician's office (6, 10, 11).

Rapid antigen detection systems for GAS have shown a wide range of sensitivities (62 to 95%), but the specificities are generally high (85 to 100%) (5, 12-14). Most of the available systems are based upon latex agglutination or an enzyme immunoassay; however, one test incorporates lipo- somal technology into a membrane-bound immunoassay procedure.

The Directigen 1,2,3 Group A Strep Test (DGAST; BBL Microbiology Systems, Cockeysville, Md.) (K. Uithoven, E. McFarland, R. Rosenstein, and T. Bloomster, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C341, p. 388) was compared with culturing for the diagnosis of GAS pharyngitis in acute-care clinics in Utah by Huck et al. (9). On the basis of reference culture methods incorporating both selective and nonselective media incubated for 48 h, these investigators reported that the DGAST produced an unacceptable number of false-positive and false-negative tests and concluded that culturing was still required to prevent missed diagnoses.

The present study was undertaken to determine the utility of the DGAST for the diagnosis of acute streptococcal pharyngitis in patients from a high-incidence pediatric population as compared with a low-incidence adult population in the same community.

Two patient populations in Iowa City, Iowa, were evaluated for GAS pharyngitis between 20 March and 12 April 1989. The first, a predominantly pediatric population, included 319 children aged 7 months to 16 years and 8 adults. An adult population of 322 patients ranging in age from 18 to 38 years was also evaluated. Asymptomatic patients and patients who had received antibiotics for any reason during the 2 weeks prior to the onset of pharyngitis were excluded from the study.

Swabs were obtained by a physician or nurse using the BBL Aerobic Transport System; however, the plugs were not depressed to release the transport medium. Dry swabs were transported at ambient temperature, cultured, and then tested with the DGAST as soon as possible but no longer than 24 h after specimen collection. All swabs were inoculated onto BBL Trypticase soy agar containing 5% sheep blood, and the plates were cross-streaked with a microbiological loop. Swabs were then extracted for GAS detection with the DGAST. Plates were incubated aerobically at 35°C and examined at 24 and 48 h. A quantitative estimation of beta-hemolytic streptococci on a scale of 1+ to 4+ growth (1+, 1 to 10 colonies; 2+, 11 to 50 colonies; 3+, >50 but <300 colonies; 4+, >300 colonies) was recorded. Colonies exhibiting typical morphology were further characterized by direct immunofluorescence staining for GAS with a BBL conjugate, and all beta-hemolytic streptococci were identified to group by latex agglutination with the BBL Strep Grouping Kit.

We were trained in the performance and interpretation of the DGAST by a Becton Dickinson and Co. technical representative. A blind panel of antigen extracts of various reactivities served as a proficiency check prior to initiation of the study. Tests were performed in accordance with the printed instructions supplied by the kit manufacturer. Each step of the extraction and test procedure included internal controls to ensure proper test performance. The time between inoculation of the culture media and extraction for DGAST testing was recorded and never exceeded 15 min. A positive test was indicated by the appearance of a distinct pink triangle in the center of the white membrane of the test devices. A negative test was indicated by the presence of a pink dot in the center of the white membrane. An uninterpretable test was one in which the membrane failed to clear and remained pink after the addition of the wash reagent. The completed tests were read immediately, and results were recorded as weakly to strongly positive (1+, 2+, 3+, or 4+), negative (− or +), or uninterpretable (absence of a pink dot or triangle).

Throat swabs for the diagnosis of acute GAS pharyngitis were obtained from 649 patients during a comparison of DGAST and culturing. Usable test results were obtained from 324 of 327 patients examined in a private community-based pediatric practice and 320 of 322 adults examined at the student health clinic of a major midwestern university. Five specimens produced uninterpretable results and were excluded from the study. The incidences of GAS pharyngitis

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TABLE 1. Performance summary of the DGAST as compared with culturing

<table>
<thead>
<tr>
<th>Specimen source</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Children</td>
<td>78.6 (70/89)</td>
<td>75.0 (78/104)</td>
<td>95.7 (225/235)</td>
<td>99.1 (218/220)</td>
</tr>
<tr>
<td>Adults</td>
<td>68.2 (15/22)</td>
<td>60.0 (15/25)</td>
<td>99.7 (297/298)</td>
<td>99.7 (294/295)</td>
</tr>
<tr>
<td>All patients</td>
<td>76.6 (85/111)</td>
<td>72.1 (93/129)</td>
<td>97.9 (522/533)</td>
<td>99.4 (512/515)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Children</td>
<td>87.5 (70/80)</td>
<td>97.5 (78/80)</td>
<td>92.2 (225/244)</td>
<td>89.3 (218/244)</td>
</tr>
<tr>
<td>Adults</td>
<td>93.8 (15/16)</td>
<td>93.8 (15/16)</td>
<td>97.7 (297/304)</td>
<td>96.7 (294/304)</td>
</tr>
<tr>
<td>All patients</td>
<td>88.5 (85/96)</td>
<td>96.9 (93/96)</td>
<td>95.3 (522/548)</td>
<td>93.4 (512/548)</td>
</tr>
</tbody>
</table>

between 20 March and 12 April 1989 were 32.1% (104 of 324) for patients in the pediatric clinic and 7.8% (25 of 320) for adult patients.

Data from an evaluation of the DGAST in the pediatric patients are presented in Table 1. The DGAST sensitivity was slightly higher (78.6 versus 75.0%) when calculations were based upon 24-h culture results. The predictive values of a positive DGAST were 87.5% (70 of 80) at 24 h and 97.5% (78 of 80) at 48 h of culture incubation. The specificities of the DGAST were 95.7% (225 of 235) at 24 h and 99.7% (218 of 220) at 48 h of culture incubation. The predictive value of a negative DGAST was 92.2% (225 of 244) at 24 h of incubation but decreased to 89.3% (218 of 244) at 48 h of incubation.

The DGAST results for adult patients (Table 1) indicated sensitivities of 68.2% (15 of 22) and 60.0% (15/25) for 24 and 48 h of culture incubation, respectively. The specificity was 99.7% (297 of 298 or 294 of 295) at both 24 and 48 h, and the predictive value of a positive test was 93.8% (15 of 16) at either incubation time. The predictive values of a negative test were 97.7% (297 of 304) and 96.7% (294 of 304) at 24 and 48 h of incubation, respectively.

On the basis of 48-h culture results, the overall sensitivity of 72.1% (93 of 129), specificity of 99.4% (512 of 515), and positive and negative predictive values of 96.9% (93 of 96) and 93.4% (512 of 548), respectively, are typical of direct antigen detection systems (5).

Agreement between culturing and the DGAST was 98.4% for cultures with + growth from both patient populations. For both populations, the correlation between DGAST positivity and 3+ growth was 82.4%; the corresponding values for 2+ growth and 1+ growth were 58.8 and 26.5%, respectively.

Of the beta-hemolytic colonies examined, 37.7% (78 of 207) were not GAS (8 group B, 50 group C, 13 group F, and 7 group G). No cross-reactivity between the DGAST and non-group A beta-hemolytic streptococci occurred, as indicated by the high specificity observed. However, six cultures initially identified as GAS by the BBL direct immunofluorescence fluorescein isothiocyanate conjugate were subsequently shown to be group C streptococci by latex agglutination.

The sensitivity and specificity obtained in this evaluation of the DGAST were considerably better than those reported by Huck et al. (9). While our specificity based upon 48-h culture results was somewhat higher than that obtained in the studies cited in the product insert (99 versus 92%), our sensitivity was considerably lower (72 versus 90%) for combined specimens. Only 36.4% (8 of 22) of the 1+ cultures produced positive DGAST results after 24 h of incubation. These data are similar to those reported in the product insert, in which 44.4% (four of nine) of 1+ cultures were reported to produce positive DGAST results. This number declined to 27.6% (8 of 29) when 48-h culture results were used. The large number of cultures with few colonies probably resulted from loss of the viability of organisms during dry-swab transport of specimens and inefficient transfer of organisms from dry swabs to culture media. Of 80 culture-positive pediatric specimens, 41.3% (33 of 80) were 4+, 25.0% (20 of 80) were 3+, 10.0% (8 of 80) were 2+, and 23.8% (19 of 80) were 1+ in the DGAST. Eight equivocal (±) specimens recorded negative by the DGAST were culture positive. The large number of DGAST results with + (n = 19) and ± (n = 8) reactivities and producing positive cultures was a further indication that the amounts of antigen on these swabs were decreased to marginally detectable levels by culture inoculation prior to extraction of the swabs for the DGAST. The 16 equivocal (±) DGAST results which were interpreted negative for statistical analysis indicate some difficulty in reading the test cutoff. The DGAST would be markedly improved by a modification to enhance the present subtle distinction between a weakly positive result and a negative result. The high predictive value of both the positive and the negative tests in the pediatric practice indicates that the DGAST is suitable for screening acutely ill patients, in whom the incidence of GAS pharyngitis is high and in whom most specimens produce >1+ culture results.

In summary, the DGAST was evaluated with pharyngeal specimens from 649 patients equally divided between a high-incidence pediatric population and a low-incidence adult population. The cumulative sensitivity (72.1%), specificity (99.4%), positive predictive value (96.9%), and negative predictive value (93.4%) were higher than previously reported; however, these values may vary with the population tested and the reference methods used. The DGAST may be used most effectively in the screening of acutely ill symptomatic patients, in whom the incidence of GAS pharyngitis is high. Because of the low sensitivity as compared with that of the culture method used in our laboratory, we recommend that all negative DGAST results be followed up with culturing.

We thank the staff of Pediatric Associates, Iowa City, Iowa, and the Student Health Service of the University of Iowa for their cooperation during this study. We also express appreciation to Diana Danaher and BBL for providing test kits and training our staff in the performance of DGAST. Carolyn Wieland and staff prepared the manuscript.

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