Evaluation of Rapid, Commercial Latex Techniques for Serogrouping Beta-Hemolytic Streptococci

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The clinical need to rapidly and correctly differentiate beta-hemolytic streptococci into Lancefield groups has prompted the development of commercially available rapid agglutination techniques. A modified Streptex (Wellcome Diagnostics, Research Triangle Park, N.C.) technique and the PathoDx latex Strept Grouping Kit (Diagnostic Products Corporation, Los Angeles, Calif.) technique were applied to 220 strains of beta-hemolytic streptococci that were serologically grouped by standard techniques. Agreements between standard and modified Streptex and PathoDx techniques were 99.1 and 100%, respectively. Modified Streptex produced a false-negative for one group G isolate and a weak positive reaction for a group G reagent with a nongroupable isolate. Sixty-five strains were evaluated in concentrations high enough to cause potential discrepancies with antigen detection reagents were tested with Streptex and PathoDx reagents. No cross-reactions were observed with any reagent tested when challenged with these 65 strains. When combined with colonial morphology and hemolytic reaction, both modified Streptex and PathoDx were rapid, specific tests for identifying streptococci, with PathoDx being slightly faster.

Beta-hemolytic streptococci have traditionally been grouped by a capillary precipitin reaction between antibodies extracted from the streptococcal cell envelope and group-specific antisera. The standard procedure is to prepare extracts (i.e., acetone, formaldehyde, and autoclave) from the growth of strains in broth (6). Although this Lancefield precipitation technique still represents the standard technology, the expense, expense, and time involved have restricted its use in most laboratories. However, with the development of immunofluorescence, coagglutination, and latex agglutination techniques serological grouping of streptococci has become technologically simple (6, 9). Kits for serogrouping various beta-hemolytic streptococci by coagglutination (Meritec-Strep, Meridian Diagnostics, Inc., Cincinnati, Ohio; and Phadebact, Pharmacia Diagnostics, Piscataway, N.J.) and latex agglutination (SeroSTAT, Scott Laboratories, Inc., Fiskeville, R.I.; and Streptex Grouping Kit, Wellcome Diagnostics, Research Triangle Park, N.C.) are commercially available and have been evaluated with regard to a number of parameters (1–5, 7, 8, 10–12). Recently, the Streptex test has been modified, reducing the antigen extraction incubation time from 60 to 10 min. In addition, the PathoDx latex Strept Grouping Kit (Diagnostic Products Corporation, Los Angeles, Calif.) has been released, with an immediate room temperature nitric acid extraction procedure that requires no incubation time. The purpose of this evaluation was to compare the accuracies of these two commercially available rapid techniques for serogrouping streptococci.

Bacterial strains. Strains used in this study were obtained from recent clinical specimens submitted to the clinical microbiology laboratory of Primary Children’s Medical Center. If stored, specimens were frozen in skim milk or 20% glycerol and Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at −70°C. Subcultures of stock isolates were passaged three times on agar plates (BBL), with each passage followed by incubation at 37°C in ambient air or in a 5% CO₂ atmosphere for 24 h. Reference strains of group A, B, C, F, and G beta-hemolytic streptococci obtained from the American Type Culture Collection and from Harry Hill, University of Utah School of Medicine, were used to check the grouping results. Streptococcal strains were extracted by the Lancefield procedure (hot HCl) and serologically grouped by capillary precipitin tests with Wellcome Streptococcus Grouping Seria (Wellcome Diagnostics); they were identified to the species level according to the physiological characteristics described by Faeklman and Carey (6). Other isolates were identified by conventional methods (9).

Streptex. Streptex reagents A, B, C, F, and G were used to test each isolate. The Streptex Grouping Kit was used according to instructions defined by the manufacturer. The procedure was that described above for isolated beta-hemolytic colonies, except that the enzyme extraction suspension of five colonies was incubated for only 10 min in a 37°C water bath (1). Agglutination was interpreted as 0 (the milky appearance of the suspension remained essentially unchanged) or 1+ to 4+ (specifying agglutination intensity).

PathoDx. The PathoDx latex Strept Grouping Kit includes dropper bottles with grouping latex reagents for antigens A, B, C, F, and G; control antigens ABC and FG; and extraction reagents (reagents 1, 2, and 3). The test was performed according to instructions defined by the manufacturer. One colony was tested with each reagent unless the colony was small. The colonies, picked with a disposable applicator stick, were mixed with extraction reagents 1 and 2, which previously had been dropped into labeled test tubes (12 by 75 mm). Control antigens ABC and FG were dropped into the extraction tubes prepared earlier as explained above; extraction reagent 3 was then immediately added. Extract (50 μl) was then dropped onto the labeled test control or on a determination card, followed by specific latex, one per test or control. Latex and extract were subsequently mixed with a stir stick and rocked for 60 s or less. Agglutination was graded as 0 (no agglutination) or 1+ to 4+ (relating to agglutination intensity).

Grouping discrepancies. All discrepancies with the hot
TABLE 1. Comparison of two rapid agglutination techniques for serogrouping beta-hemolytic streptococci from pure cultures

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lancefield extract-capillary precipitin</th>
<th>Modified Streptex grouping (10-min extraction)</th>
<th>No. correct by PathoDx grouping (0-min extraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. % of total isolates</td>
<td>No. correct</td>
<td>No. negative errors</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>220</td>
<td>77.2</td>
<td>218</td>
</tr>
<tr>
<td>Group A</td>
<td>44</td>
<td>15.4</td>
<td>44</td>
</tr>
<tr>
<td>Group B</td>
<td>43</td>
<td>15.1</td>
<td>43</td>
</tr>
<tr>
<td>Group C</td>
<td>42</td>
<td>14.7</td>
<td>42</td>
</tr>
<tr>
<td>Group F</td>
<td>40</td>
<td>14.0</td>
<td>40</td>
</tr>
<tr>
<td>Group G</td>
<td>43</td>
<td>15.1</td>
<td>42</td>
</tr>
<tr>
<td>Nongroupable</td>
<td>8</td>
<td>2.9</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>65</td>
<td>22.8</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td>285</td>
<td></td>
<td>283</td>
</tr>
</tbody>
</table>

<sup>a</sup> There were no negatives or errors.
<sup>b</sup> Repeat testing resulted in the same conclusion as initial testing.
<sup>c</sup> Negative, Negative reactions with all latex reagents.
<sup>d</sup> F, False-positive reaction with group G reagent.
<sup>e</sup> Representative of bacteria commonly found in the posterior pharynxes of humans in concentrations high enough to cause potential reactions with Streptococcal antigen grouping reagents. Strains tested included Klebsiella pneumoniae (n = 10), Staphylococcus aureus (n = 10), S. pneumoniae (n = 10), Citrobacter diversus (n = 5), Enterobacter cloacae (n = 5), Escherichia coli (n = 5), P. aeruginosa (n = 5), Strep group G, and viridans group streptococci (n = 10).

HCl-extraction-capillary precipitin testing were repeated once. One technologist performed all the tests.

Overall agreement between the Lancefield extract-capillary precipitin and the two rapid agglutination techniques is summarized in Table 1. Among the beta-hemolytic streptococcal strains, results of the evaluation of strains obtained from 5% sheep blood plates were 99.1% for modified Streptex and 100% for PathoDx when compared with the "gold standard" grouping procedure. Reactions for both modified Streptex and PathoDx were rapid (<1 min) and strong (3+), with few exceptions. Repeat testing for one group G isolate and one nongroupable isolate with modified Streptex reagents resulted in the same results. The group G isolate resulted in no reaction with any of the Streptex reagents. The nongroupable isolate resulted in a 2+ reaction with group G modified Streptex reagent.

Among the non-beta-hemolytic streptococcal strains and other organisms representative of bacteria commonly found in the posterior pharynxes of humans in concentrations high enough to cause potential reactions with streptococcal antigen grouping reagents, testing each organism with each commercial reagent resulted in no cross-reactions (Table 1). No multiple-group reactions were observed with modified Streptex or PathoDx.

Several evaluations have shown that agglutination testing affords the clinical microbiologist a rapid and reliable technique for serogrouping beta-hemolytic streptococci (1–5, 7, 8, 10–12). This evaluation confirmed the diagnostic potential of the 10-min extraction modification of the Streptex system and showed the excellent capabilities of the relatively new PathoDx latex Strept Grouping Kit, one that requires no extraction time.

Of the 220 beta-hemolytic streptococcal isolates grouped by the modified Streptex procedure, only two discrepancies were encountered; while the PathoDx latex Strept Grouping Kit was shown to agree 100% with Lancefield extract-capillary precipitin testing. Because our main interest was to evaluate the extremely short extraction procedures (modified Streptex, 10 min; PathoDx, 0 min) by using the recommendations of the manufacturer, this evaluation was performed with isolated colonies obtained from 5% sheep blood agar plates. Interfering cross-reactions caused by a mixed population of organisms were reported previously (8, 11, 12). Each commercial reagent was tested with colonies of organisms potentially cross-reactive and available on primary isolation plates from throat and other cultures (i.e., viridans group streptococci, Streptococcus pneumoniae, several strains of the family Enterobacteriaceae, and Pseudomonas aeruginosa). In this evaluation, neither modified Streptex nor the PathoDx latex Strept Grouping Kit resulted in erroneous reactions with these potentially problematic organisms. However, results from previous investigations emphasize the requirement for careful selection and testing of isolated colonies.

One false-negative reaction (no reaction with a group G isolate) and one false-positive reaction (a nongroupable isolate reacting with group G reagent) occurred with the modified Streptex test. False-negative reactions have been reported with broth techniques, and false-positive reactions have been noted with latex and coagglutination techniques (1, 8). Shared protein antigens and medium composition have been implicated as the reasons for these discrepancies.

We did not find substantial differences in rapidity and ease of execution among the commercial kits examined. However, with a 10-min extraction time, the modified Streptex represents a major time improvement over the previous 1-h extraction time. In addition, the PathoDx latex Strept Grouping Kit was routinely effective with one large colony, lessening the chance of a cross-reaction when more than one colony is picked on a primary plate. Moreover, the immediate room temperature nitrous acid extraction procedure requiring no incubation time was very convenient. Finally, the blue latex particles were extremely easy to read. Overall, these commercially available latex agglutination tests represent a simple, rapid, reliable alternative to Lancefield technology.

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LITERATURE CITED


