Comparison of Meritec-Strep with Streptex for Direct Colony Grouping of Beta-Hemolytic Streptococci from Primary Isolation and Subculture Plates

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Meritec-Strep (Meridian Diagnostics, Inc., Cincinnati, Ohio), a coagglutination method, was compared with Streptex (Wellcome Diagnostics, Research Triangle Park, N.C.), a latex agglutination method, for the identification of beta-hemolytic streptococcal groups A, B, C, F, and G by the direct colony method. A total of 124 beta-hemolytic streptococcal isolates were tested, which included 77 from group A, 15 from group B, 10 from group C, 1 from group F, and 21 from group G. All were tested from subculture, and 74 (60%) were also tested from primary isolation plates. For Meritec-Strep, usually one colony was directly applied to the reaction card for testing each grouping reagent, while for Streptex, five colonies were tested after a 1-h extraction process. Complete agreement was obtained for all isolates tested from subculture with the kits. From primary isolation plates, Meritec-Strep correctly identified 97.3% of the isolates compared with 94.6% correctly identified for Streptex. Meritec-Strep produced a false-negative for one group A isolate and positive reactions for group A and F reagents with another group A isolate. A diphtheroid contaminant caused the positive group F reaction. Streptex produced false-negative results for one group A and three group C isolates. Most positive reactions were strong and rapid (less than 30 s) for both kits. The negative test control provided in the individual group A and B kits was nonreactive for all isolates. Meritec-Strep accurately identified isolated colonies of beta-hemolytic streptococci on primary isolation and subculture plates. It provided faster results than Streptex by eliminating the time and manipulation of antigen extraction and needed fewer colonies when individual group A or B reagents were used.

Commercially available latex agglutination and coagglutination kits are routinely used to accurately identify the clinically common beta-hemolytic streptococcal groups A, B, C, F, and G. Although it is common and acceptable practice to identify streptococcal groups directly from colonies on agar plates, as opposed to testing fluids from 2-, 4-, or 24-h broth cultures of beta-hemolytic colonies which have been extensively studied (4, 8), few studies have evaluated the accuracy of direct colony methods. Evaluations from primary isolation and subculture plates have been reported for Streptex (Wellcome Diagnostics, Research Triangle Park, N.C.), SeroSTAT (Scott Laboratories, Inc., Fiskeville, R.I.), and Phadebact (Pharmacia Diagnostics, Piscataway, N.J.). The studies have reported accuracies of 83 to 99% with Streptex (1–4, 5, 8) and SeroSTAT (2, 5, 8) and 98 to 100% for Phadebact (6–8). Interestingly, few of these studies (1, 3, 6–8) have reported on identification of streptococci from primary isolation plates, an application that provides faster results with less manipulation. In general, this testing produced the lower accuracies defined above. This was partially due to false-positive reactions that occurred when normal flora colonies contaminated the streptococcal colonies tested (1, 7, 8). In addition, false-negative reactions that occurred were from testing an inadequate number of colonies. Regardless of the grouping kit used, an adequate number of colonies and isolated colonies is needed for testing.

Each of the three kits varies in its grouping capability and testing requirements for the direct colony method. Streptex has been evaluated for grouping streptococcal groups A, B, C, D, F, and G. This test requires at least five isolated colonies, but more frequently, higher concentrations of organisms were used (1, 4, 5, 8). All studies used a 1-h enzyme extraction of carbohydrate antigen. SeroSTAT and Phadebact have been evaluated for grouping A, B, C, and G streptococci. Testing requires at least five isolated colonies, which are suspended in a drop of buffer (or grouping reagent for Phadebact) on a slide for each group. With SeroSTAT, higher inoculum concentrations were usually used to enhance reactivity (2, 8). Trypsin extraction was used routinely to resolve negative and equivocal reactions, which involved 15% of the isolates tested in one study (2, 5). With Phadebact, suspending the organism in buffer was found to enhance the agglutination reaction, but the use of fewer than five colonies did not produce reliable agglutination (6, 7).

The testing requirements and procedures necessary with these kits prompted the evaluation of Meritec-Strep (Meridian Diagnostics, Inc., Cincinnati, Ohio) and the direct colony test method. It is a coagglutination kit not previously reported for the grouping of beta-hemolytic streptococcal groups A, B, C, F, and G. It requires only one colony to test each grouping reagent and has a unique method of applying organisms to the reaction oval that eliminates the time and handling needed for antigen extraction and organism suspension processes. Individual grouping kits for groups A and B are available. The kit was compared with the Streptex direct colony method, which is currently used in this laboratory.

MATERIALS AND METHODS

Organisms. All cultures of beta-hemolytic streptococci obtained from clinical specimens submitted to the clinical microbiology laboratory from 8 January to 14 February 1986 were included in this study.

All specimens for isolation of beta-hemolytic streptococci
TABLE 1. Specimen sources for 124 beta-hemolytic streptococcal isolates tested from primary isolation and subculture plates

<table>
<thead>
<tr>
<th>Source</th>
<th>A (n = 77)</th>
<th>B (n = 15)</th>
<th>C (n = 10)</th>
<th>F (n = 1)</th>
<th>G (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P Sub</td>
<td>P Sub</td>
<td>P Sub</td>
<td>Sub</td>
<td>P Sub</td>
</tr>
<tr>
<td>Throat</td>
<td>36 28</td>
<td>1 4</td>
<td>5 2</td>
<td>0</td>
<td>11 5</td>
</tr>
<tr>
<td>Wound</td>
<td>9 2</td>
<td>3 0</td>
<td>2 1</td>
<td>1</td>
<td>3 0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>1* 1*</td>
<td>3* 4*</td>
<td>0 0</td>
<td>0</td>
<td>0 2*</td>
</tr>
</tbody>
</table>

*a* P, Tested from primary plate and subculture.  
*b* Sub, Tested from subculture only.  
*c* Eye.  
*d* Blood.  
*e* Cervix and urine (two isolates).  
*f* Cervix, intrauterine device, blood, and urine.  
*g* Blood and sputum.

were inoculated onto 5% sheep blood agar plates containing Trypticase soy agar II (BBL Microbiology Systems, Cockeysville, Md.) and streaked. Primary cultures from throats were incubated for 15 to 24 h at 35°C in a room air incubator; cultures from all other sites were incubated in 4% CO2 and 78% humidity for 15 to 24 h before testing. Subcultures of beta-hemolytic streptococci were inoculated onto 5% sheep blood agar, streaked for isolated colonies, and incubated at 35°C in room air for 18 to 24 h before testing.

Beta-hemolytic streptococci on primary isolation plates that were well isolated as single colonies, or single colonies that were touching other colony types but could be selected with minimal contamination, were tested with Meritec-Strep and Streptex reagents. No repeat testing was performed on suspected false-negative or weakly positive reactions. All isolates were subcultured and tested with both kits. Beta-hemolytic streptococci on primary culture that were surrounded or overgrown with confluent different colony types, and single colonies too few in number for testing all groups of streptococci by both kits, were subcultured and tested by both kits. One technologist performed all the tests.

**Streptex.** The Streptex (Wellcome Diagnostics) A, B, C, F, and G reagents were used to test each isolate. Reconstitution of reagents, their use, selection of colonies, and interpretation of reactions were performed according to procedures defined by the manufacturer. The procedure has been reported previously (1). Five colonies of each isolate were tested after enzyme extraction for 1 h. Reactions were interpreted after 1 min as no agglutination or 1 to 4+ agglutination. Polyvalent antigen control produced 3 to 4+ reactions for all reagents each day the test was performed.

**Meritec-Strep.** The Meritec-Strep kit (Meridian Diagnostics) reagents included grouping reagents and positive and negative test controls. Killed, red-stained staphylococcal cells coupled with specific rabbit antibody to streptococcal groups A, B, C, F, and G and a negative control which contained killed, red-stained staphylococcal cells coupled with nonimmune rabbit serum were used to test each isolate. The negative control was included in this study because the individual test kits of groups A and B require its use. No reconstitution of reagents is needed. Use of reagents, selection of colonies, and interpretation of reactions were done according to procedures defined by the manufacturer. One colony was tested for each reagent except for very small colonies, which occurred infrequently. These small colonies were picked to cover the flat end of a wooden applicator stick (approximately 2-mm diameter). The inoculated flat end of the stick was rubbed over the entire surface of the reaction oval on a determination card. One drop of the grouping reagent was mixed with an applicator stick and then rocked for 3 min unless reaction occurred sooner, at which time the test was stopped. Reactions were graded no agglutination or 1 to 4+ agglutination. Polyvalent antigen control produced a 3 to 4+ reaction for all reagents each day tests were performed. All isolates tested were nonreactive with the negative control reagent.

**Confirmation of identification.** Testing of isolates from subculture with all grouping reagents by both kits was used as confirmation of identification. If both kits agreed in group reactivity, identification was accepted. Isolates were resubcultured and retested with both kits if discrepancies occurred. Since this resolved all problems, no other testing was used.

**RESULTS**

A total of 124 beta-hemolytic streptococcal isolates were tested from primary isolation and from subculture plates from sites listed in Table 1. These included 77 group A, 15 group B, 10 group C, 1 group F, and 21 group G isolates. A total of 92 isolates (74%) were from the throat, 21 (17%) were from wounds, and 11 (9%) were from other sources. Seventy-four isolates (60%) were tested directly from primary isolation plates. This included 46 (60%) of 77 group A isolates from all sites and 36 (56.3%) of 64 from the throat. Of the 50 isolates (40%) tested from subculture only, 6 (12%) of 50 were present in too few colonies to be tested by both methods for all groups. Four isolates were group A (two from wounds and two from throats), one isolate was group B (from urine), and one isolate was group C (from a wound).

Isolates tested from subculture with both kits produced 100% agreement (Table 2). Repeat testing for one group C isolate with Streptex and two group A isolates with Meritec-Strep was performed because it was felt that too few colonies were selected. No multiple-group reactions were observed with either kit. Reactions were strong (3 to 4+) and rapid (<30 s) for both kits, with few exceptions. Two group A isolates tested with Meritec-Strep produced 1 to 2+ reactions. These isolates were recovered from the throat and tested from subculture only. One group C isolate tested with Streptex produced a 2+ reaction, which was the same reaction obtained on a primary plate. The group F isolate was tested only from a subculture.

Of the 74 isolates tested from primary isolation plates, Meritec-Strep identified 72 (97.3%) and Streptex identified 70 (94.6%) (Table 2). Meritec-Strep identified 44 (95.6%) and Streptex identified 45 (97.8%) group A isolates. Three isolates tested by Meritec-Strep produced 2+ reactions: two...
TABLE 2. Results of Meritec-Strep and Streptex grouping of beta-hemolytic streptococci from primary isolation and subculture plates

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>% of isolates correctly identified</th>
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<tbody>
<tr>
<td></td>
<td>Subculture</td>
</tr>
<tr>
<td></td>
<td>Meritec-Strepb</td>
</tr>
<tr>
<td>A (77)</td>
<td>100f</td>
</tr>
<tr>
<td>B (15)</td>
<td>100</td>
</tr>
<tr>
<td>C (10)</td>
<td>100</td>
</tr>
<tr>
<td>F (1)</td>
<td>100</td>
</tr>
<tr>
<td>G (21)</td>
<td>100</td>
</tr>
<tr>
<td>Accuracy</td>
<td>100</td>
</tr>
</tbody>
</table>

a Isolated beta-hemolytic colonies were tested. NT, Not tested.
b All isolates were nonreactive in negative control.
c Two group A isolates and one group C isolate did not react on initial testing. Correct identification was obtained on repeat testing with larger inocula.
d One isolate was negative for all groups, and one isolate was positive for groups A and F (a diphtheroid contaminant caused the group F reaction).
e One isolate was negative for all groups.
f Agreement in identification by both kits when isolate was tested from subculture.

were from 11- to 16-h primary plates (wound and throat), and the other isolate was from an 18- to 24-h throat culture. Both kits misidentified the same group A isolate from an 18- to 24-h wound culture that grew colonies of normal flora organisms touching the group A colonies. Streptex produced no reaction for the groups tested. Meritec-Strep produced 3+ reactions for groups A and F and was nonreactive for the negative control. On subculture, beta-hemolytic colonies and non-beta-hemolytic diphtheroids were isolated. Both kits produced 4+ reactions with group A reagent only with the beta-hemolytic colonies. Meritec-Strep produced a 4+ reaction for group F only with the diphtheroid, and Streptex was negative for all groups. For another group A isolate, Meritec-Strep produced no reaction in all groups. The isolate was from an 18- to 24-h throat culture that grew colonies of normal flora touching the beta-hemolytic streptococcal colony. Both kits identified all group B isolates. Meritec-Strep identified all group C isolates, and Streptex identified four isolates (57.1%). The three isolates not identified were from throat cultures incubated for 18 to 24 h; two isolates were selected from colonies touching normal flora colonies, and the other isolate was well isolated. Both kits identified all group G isolates.

DISCUSSION

The present study is the first report on the coagglutination kit Meritec-Strep and the direct colony testing procedure. This kit was shown to agree 100% with Streptex for the 124 beta-hemolytic streptococcal isolates tested from subculture. Three isolates (a group C isolate for Streptex and two group A isolates for Meritec-Strep) required repeat testing. No multiple-group or false-positive single-group reactions occurred with either Streptex or Meritec-Strep. These results with Streptex are in agreement with those of previous studies in which the direct colony method was evaluated on beta-hemolytic isolates from subculture (1-5). Only one isolate each from groups A, C, and G gave a false-negative reaction. In only one of these studies was the sensitivity less than 100% for group F. In that study it was 59% (5). Even though the group F isolate recovered in the present study produced a strong reaction in both kits, more isolates must be tested to determine accuracy for this group. Only a few false-positive single-group reactions have been reported; one group F isolate was identified as group C, and a nongroupable isolate was identified as group F (1, 5). Two group D isolates also gave a false-positive reaction for group G only, but the hemolytic reaction was not defined (1). These results are in contrast to those in the many studies that evaluated broth methods, in which cross-reactions with group A, C, and G isolates and false-negative reactions occurred, especially with Phadebact. This discrepancy was related in part to shared protein antigens and the composition of the broth used. The extraction process of the latex agglutination system and, to a limited degree, trypsin resolved these problems (4, 6, 8). Phadebact has not been evaluated by the direct colony method from subculture.

Colonies of beta-hemolytic streptococci from the primary isolation plates were carefully selected to avoid contaminating organisms in order to reduce false-positive reactions. Only well-isolated colonies and those with a few different colony types touching the streptococcal colony were tested. Previous studies have shown that false-positive reactions occur with Streptococcus pneumoniae, alpha-hemolytic streptococci, or Klebsiella pneumoniae and group A, C, or F when Streptex is used (1, 8) and with group C when Phadebact is used (8). In the present study, the only false-positive reaction occurred with a diphtheroid (nonhemolytic) colony that produced a strong reaction with the group F reagent of Meritec-Strep only. The diphtheroid colony was either touching or mixed with the group A colonies tested; both the group A and F reagents were positive, but the negative control reagent was nonreactive. Had the group A kit been used instead of the complete grouping kit, the isolate would have been correctly identified, producing a sensitivity of 97.8%. Even though Streptex produced no false-positive reactions in our study, the potential is well documented. Results with Meritec-Strep and those of previous studies demonstrate the need for careful selection and testing of isolated colonies.

From primary isolation plates, Meritec-Strep identified 97.3% of the beta-hemolytic streptococcal isolates compared with 94.6% by Streptex. Each kit had one false-negative reaction for group A, and Streptex produced false-negative reactions for three group C isolates. Even though the tests for each kit were not repeated, the rate of false-negative reactions is insufficient to determine if the insufficient inoculum may have caused the false-negative reactions. These results are in agreement with previous studies by Streptex in which group C accounted for the greatest number of false-negative reactions (3, 8). Results from one study showed an accuracy of 84.5% for Streptex, the false-negative reactions occurring primarily with groups C, F, and G (1). Cultures that grew almost pure beta-hemolytic streptococcal colonies had an increased accuracy of 92.7%. Phadebact, in comparison, produced accuracy comparable to that of Meritec-Strep; however, two false identifications occurred (6-8). One group F isolate and one group G isolate were identified as group A. In the present study, no beta-hemolytic streptococcal isolates were falsely identified by the Meritec-Strep kit.

The direct plate method of grouping streptococci was evaluated in order to generate rapid results when isolated colonies are available. For cultures with no isolated colonies, subculture and subsequent direct plate testing offer an easier method than testing from broth culture. In the present study, 59.8% of all primary isolation cultures yielded colonies that could be tested as others have reported (1, 9). Because of the design of the study, only six cultures had too
few isolated colonies to be tested from primary culture. In a normal-use setting, these cultures could have been tested, which would have increased the percentage of beta-hemolytic colonies tested to 65%; therefore, approximately 35% of beta-hemolytic streptococcal colonies would need to be subcultured and thus delayed by 18 to 24 h.

After the Meritec-Strep kit had been used for several weeks, the size of the colony relative to the end of the applicator stick was easier to approximate so that enough organisms could be obtained for testing when colonies were less than 1 to 2 mm wide. The design of the study was to pick one organism to avoid overinoculation, which never occurred. Inadequate inocula, however, did produce negative reactions or less than 3+ reactions for four isolates from subculture and five isolates from primary culture. Seven of these negative reactions occurred during the first several weeks of the study. Both negative reactions from subculture, which were repeated to obtain a positive reaction, occurred on the same day, and five of the 1 to 2+ reactions occurred on two separate days. These observations suggest that within a few weeks, technologists can learn effective procedures for selecting colonies that will avoid inadequate inocula and repeat testing.

Reagent cost per isolate, based on list prices, was lower for Meritec-Strep. Complete and individual groupings were $3.60 and $1.08, respectively, for Meritec-Strep compared with $4.10 and $2.69, respectively, for Streptex. These costs are based on the use of one other serogroup as a specificity control for the individual serogroup testing for Streptex.

Meritec-Strep provides accuracy equal to that of Streptex for identification of beta-hemolytic streptococci from subculture and, importantly, from primary isolation plates. It has the advantages of being able to test five colonies or less, does not require reconstitution of reagents, and provides fast results by eliminating the time and handling required for antigen extraction or organism suspension. As with the other kits, isolated colonies are needed to reduce false-positive reactions.

ACKNOWLEDGMENT

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LITERATURE CITED