Rapid Slide Coagglutination Test for Identifying and Typing Group B Streptococci

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Received for publication 25 February 1977

A Cowan I strain of Staphylococcus aureus was labeled with either group B streptococcal grouping or typing antiserum. These antibody-labeled reagent cells (ARC) were used in a slide coagglutination test to identify and type group B streptococci from blood agar plates. All streptococci were also identified by the standard Lancefield capillary precipitin test. In a blind study, all 141 group B streptococci were correctly identified by the coagglutination grouping test. None of the 148 non-group B streptococci caused agglutination of ARC. The coagglutination grouping test required an acid extract prepared from only four colonies and could be completed less than 30 min after colonies were removed from plates. The coagglutination typing test correctly identified 98.6% of the types of the 141 group B streptococcal strains tested. At least 88.6% of these streptococci could be typed directly from blood agar plates within 5 min by the coagglutination typing test. The remaining 11.4% of the group B streptococci were acid extracted (less than a 30-min procedure), and the extract was used for coagglutination typing. Coagglutination typing can be performed with only four colonies. The coagglutination grouping and typing tests are inexpensive, rapid, reliable, and easy to perform.

The group B streptococcus Streptococcus agalactiae has been implicated as a causative agent in a wide variety of human infections. Included are meningitis, pneumonia, endocarditis, arthritis, empyema, abscesses, osteomyelitis, septicemia, and genitourinary tract infections (16). Neonatal infections are of particular concern because of their high incidence and mortality rate (1, 2, 7). Early diagnosis is imperative in neonatal infections to ensure prompt and appropriate therapy.

S. agalactiae has traditionally been identified by the Lancefield capillary precipitin (CP) reaction (15). This technique requires an 18- to 24-h culture period and a time-consuming extraction procedure and is, therefore, of limited value in neonatal infections. A variety of methods have been developed and used to simplify and speed the identification of group B streptococci. The 2-h hippurate hydrolysis test (10) and CAMP reaction as described by Darling (4) have been used for rapid presumptive identification. Fluorescent-antibody techniques (14) and counterimmunoelectrophoresis (8) have also been used for definitive identification, but the equipment and reagents necessary for these tests are not available in many laboratories.

Christensen et al. reported a method for the rapid and specific identification of streptococci (3) using antibody-labeled Staphylococcus aureus cells (ARC). We have modified the procedure and used it to identify and type group B streptococci.

MATERIALS AND METHODS

Reagent cells. S. aureus reagent cells were prepared with modifications of the methods of Minor and Marth (12). Dialysis tubing (3.25 inches [approximately 8.3 cm] wide; S.S.D.C., Union Carbide, Chicago, Ill.) was cut into flat 90-mm disks. Filter paper was inserted between each disk, and the disks were autoclaved in a moist chamber at 121°C for 15 min. A sterile disk was placed on the surface of brain heart infusion (BHI; Difco) agar to cover the surface. A Cowan I strain of S. aureus was grown in BHI broth (Difco) at 35°C and transferred three or four times in BHI broth at 9- to 14-h intervals. Cells were washed three times in sterile phosphate-buffered saline (PBS; 1.096 g of Na2HPO4, 0.315 g of NaH2PO4, H2O, and 8.5 g of NaCl per liter, pH 7.3) and resuspended in PBS to a 10% (vol/vol) concentration. A 0.1-ml quantity of cells was inoculated onto the disk on the BHI plate and spread with a sterile glass rod. Plates were incubated in an upright position at 35°C for 18 h.

The luxuriant growth of S. aureus cells was harvested by adding 1 ml of PBS to the plate, removing the cells with a sterile glass rod and pipette, and repeating the process. The cells were washed three times in PBS and resuspended in PBS with 0.5%
formaldehyde to a 10% concentration. After standing for 3 h at room temperature, the treated cells were washed three times and resuspended in PBS to a 10% concentration. This suspension was heated for 20 min in an 80°C water bath and resuspended in PBS with 0.002% Merthiolate to a final 10% concentration. Reagent cells could be stored for several months at 4°C.

ARC. The ARC were prepared with modifications of the methods of Edwards and Larson for the identification of group A streptococcus (5). Group B streptococcus grouping and typing antiserum (types Ia, Ib, II, and III) were obtained from the Center for Disease Control, Atlanta, Ga. Reagent cells were labeled with individual antiserum by adding 0.035 ml of antiserum to 0.25 ml of cells and allowing the mixture to stand at room temperature for 2.5 h. The mixture was centrifuged, and the supernatant was discarded. These ARC were reuspended in 2 ml of PBS with 0.002% Merthiolate and 0.02 ml of 2% Procion brilliant blue, M-RS (Colab Laboratories, Chicago Heights, Ill.). The ARC were stable for at least 4 months when stored at 4°C.

Organisms and media. Stock cultures and fresh clinical isolates of group A, B, C, D, F, and G streptococci were obtained from the Wisconsin State Laboratory of Hygiene and Madison General Hospital. These isolates were identified by growth in 5.5% NaCl broth, bile esculin hydrolysis, hippurate hydrolysis, and the Lancefield CP test for grouping and typing (6, 15). Streptococci were transferred to 7.5% sheep blood agar plates (vial infusion base; BBL), incubated under 10% CO₂ at 35°C for 18 h, and then tested by the coagglutination procedure.

Coagglutination grouping procedures. The majority of the group B streptococci could be grouped only if a hot-acid extract was prepared. The grouping procedure was always attempted directly with whole cells initially and then with the extract if necessary. Care was taken to pick pure cultures and to subculture when necessary. Two 15-mm paraffin rings were applied to a glass slide, and 0.5 drop of 0.85% saline was added to each ring from a Pasteur pipette. Two colonies were suspended in the saline, and 1 drop of the grouping ARC or unlabeled reagent cells was delivered to each ring from a Pasteur pipette. The slide was rocked continuously for up to 4 min, and macroscopic agglutination was observed.

The second method involved removing well-isolated colonies from the plate, preparing a hot-acid extract of the cells, and testing the extract with ARC. This extract was prepared by emulsifying at least four colonies in 3 to 4 drops of saline in a test tube (12 by 75 mm) and adding a drop of 0.04% metacresol purple and sufficient 0.2 N HCl to make the suspension a salmon pink color. The tube was placed in a boiling water bath for 10 min, neutralized with 0.2 N NaOH, and centrifuged until a clear supernatant was obtained. The test was performed by delivering 0.5 drop of the extract supernatant to two paraffin rings on a glass slide. A drop of the ARC was added to one ring, and a drop of unlabeled reagent cells was added to the second ring. The slide was rocked continuously for up to 4 min, and macroscopic agglutination was observed.

**Coagglutination typing procedures.** Most group B. streptococci could be typed directly from plates without preparing an extract. Five 15-mm paraffin rings were applied to a glass slide, and a drop of 0.85% saline was delivered to each ring. Four similar streptococcal colonies were simultaneously collected on a bacteriological loop and suspended to a uniform turbidity in each of the saline-containing rings. A drop of one of the four types of ARC was added to four rings, and a drop of unlabeled reagent cells was added to the fifth. The slide was rocked continuously for 3 min, although macroscopic agglutination was usually observed after 30 s. If the streptococci failed to agglutinate any of the ARC or a very weak agglutination reaction occurred, then a hot-acid extract (described above) was prepared and the coagglutination test was repeated with the extract. Agglutination developed more slowly with an extract and was of a finer texture than that observed with whole cells.

**RESULTS**

**Coagglutination grouping.** Initially the coagglutination grouping procedure was performed by adding ARC to streptococcal cells suspended in saline on slides. Only 14% of the 141 group B streptococci tested in this manner caused ARC agglutination. Non-group B streptococci frequently autogglutinated in the saline control.

Because of the poor results obtained with the above method, the procedure was modified. Streptococci were removed from blood agar plates, and acid extracted, and the extract was tested with reagent cells labeled with group B antiserum. Table 1 shows the coagglutination results obtained with ARC and streptococcal cell extracts derived from the modified hot-acid extraction method. All group B streptococcal

**Table 1. Specificity of the coagglutination test for identifying group B streptococci**

<table>
<thead>
<tr>
<th>Lancefield group</th>
<th>Stock strains No. positive by coagglutination</th>
<th>Clinical strains No. positive by coagglutination</th>
<th>No. positive by Lancefield CP test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>96</td>
<td>96</td>
<td>45</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

* Results were obtained with reagent cells labeled with group B antiserum and tested with whole streptococcal cells or by the modified hot-acid extraction technique.

* Results were obtained with standard Lancefield hot-acid extracts and standard group antisera.
isolates produced strong ARC agglutination. These ARC failed to agglutinate with extracts from any of the 148 non-group B streptococci tested. Neither the group B nor the non-group 
B streptococci agglutinated the unlabelled reagent 
cells. Cell extraction and coagglutination 
grouping could be completed in less than 30 
min. Thus, 100% of the group B streptococci 
were correctly identified if the coagglutination 
test was performed on cell extracts. The CP 
results were obtained with standard Lancefield 
hot-acid extracts and standard grouping anti-
sera.

Coagglutination typing. When the typing 
procedure was performed, 88.6% of the group B 
strains tested could be typed directly with 
whole cells without acid extraction. The ARC 
agglutination patterns observed with different 
types of group B streptococci are shown in Ta-
ble 2. Streptococcal types Ib and II produced 
strong agglutination reactions only with ho-
alogous reagent cells. Type Ic streptococci 
gave strong agglutination reactions with both 
type Ia and Ib reagent cells, and type II(Ib) 
streptococci gave strong agglutination reac-
tions with both type Ib and II reagent cells (16).

Streptococcal types Ia and III strongly aggluti-
nated homologous reagent cells and frequently 
weakly agglutinated type III and Ia reagent 
cells, respectively. To correctly establish types 
by the coagglutination procedure, these weak 
agglutination reactions should be disregarded 
when strong agglutination reactions are simul-
taneously observed\(^\text{a}\) with type Ia or III reagent 
cells.

When nontypable (NT) strains (13) were 
tested by the whole-cell coagglutination pro-
cedure, ARC did not agglutinate or, rarely, weak 
ARC agglutination occurred (Table 2). Sixteen 
strains of group B streptococci were NT by 
coagglutination when using whole cells (Table 
3). These strains were treated with the modified 
hot-acid extract method, and the coagglutina-
tion test was repeated. Interpretation of aggluti-
nation patterns was as described in Table 2. 
After extraction and testing with ARC, nine 
strains remained NT, one strain was type II, 
and six strains were type III.

Table 4 shows the agreement of typing re-
results obtained by the coagglutination procedure 
using whole streptococcal cells and the modified 
hot-acid extraction procedure with typing by 
the standard Lancefield hot-acid extraction 
test. Overall 98.6% of the types established by 
the coagglutination procedure agreed with 
types established by the CP test. When clinical 
isolates were typed, 100% agreement was ob-
tained between the two methods. There was 
100% agreement between the two methods for 
type Ia, Ic, II(Ib), II, and NT streptococci. Disa-
greement between the two methods occurred 
with two stock cultures. Strain 216 was type Ib 
by the CP test, but Ib and II antigens were 
detected by the coagglutination procedure. 
Strain 437 was type III by the CP test, but type 
III and II antigens were detected by the coag-
glutination procedure. These discrepancies 
could be due to cross-reacting antibodies in the 
antiser, since the antiserum were prepared for

| Table 2. Coagglutination patterns obtained with different types of group B streptococci |
|---|---|---|---|---|
| Agglutination type | Patterns\(^\text{a}\) with ARC | Anti-Ia | Anti-Ib | Anti-II | Anti-III |
| Ia | + | - | - | - | ± |
| Ib | - | + | - | - | - |
| Ic | + | + | - | - | - |
| II(Ib) | - | + | + | - | - |
| II | - | - | + | - | - |
| III | ± | - | - | + | - |
| NT\(^\text{b}\) | - | - | - | - | - |

\(^\text{a}\) Strong agglutination of ARC; \(-\), no agglutination of ARC; \(\pm\), weak agglutination of ARC frequently occurred and no agglutination rarely 
occurred.

\(^\text{b}\) Nontypable (NT) strains do not agglutinate 
ARC either before or after acid extraction.

Table 3. Number of group B streptococci that were 
typable by the coagglutination technique with whole 
cells or only after extraction

| Lancefield type | No. typable by given method |
|---|---|---|
| From whole cells | From extract |
| Ia | 34 | - |
| Ib | 16 | - |
| Ic | 2 | - |
| II(Ib) | 3 | - |
| II | 24 | 1 |
| III | 46 | 6 |
| NT | 16\(^\text{c}\) | 9 |
| Total | 125 | 16 |

\(^\text{a}\) Number of strains giving a positive coagglutina-
tion reaction when tested with whole cells from 
four colonies.

\(^\text{b}\) Number of strains that gave a negative or 
weakly positive coagglutination reaction when 
tested with whole cells from four colonies and had to 
be retested with the modified hot-acid extraction 
technique.

\(^\text{c}\) Sixteen strains were found to be nontypable 
(NT) when tested with whole cells from four colo-
nies; when these same 16 strains were retested after 
acid extraction, 6 were found to be type III and 1 was 
found to be type II. The remaining 9 were NT.
Edwards and Larson (5) identified group A streptococci by adding ARC to paraffin-ringed colonies on blood agar plates and observing agglutination on the media. We attempted to perform the coagglutination procedure in this manner, but found the results more difficult to interpret than if the test was performed on a glass slide. Apparently the type antigens of group B streptococci can mask or cover the group antigen (9, 11) and thereby interfere with coagglutination grouping of whole cells on media or slides. Thus it was generally necessary to prepare a hot-acid extract of the group B streptococci, which apparently removed the type antigens and allowed the coagglutination grouping reaction to occur. The coagglutination grouping of whole streptococcal cells can be performed on a slide, but if no agglutination occurs, the cells must be extracted and the coagglutination procedure repeated. Care must be taken to pick only pure cultures from plates and subculture when necessary.

The coagglutination typing test was even more rapid than the coagglutination grouping test, since no extraction step was generally required. In fact, if a hot-acid extract is routinely used for the coagglutination typing test, erroneous typing results may be obtained. Twenty-three strains that did type correctly with whole cells as compared with the standard Lancefield hot-acid extraction procedure were also extracted with the modified hot-acid extraction technique, and the coagglutination typing test was repeated. When the test was repeated using modified hot-acid extracts, it was found that 44% of the strains were incorrectly typed as compared with the standard Lancefield typing results. A cell extract should only be used for typing if whole streptococcal cells fail to agglutinate the typing ARC. The coagglutination typing test was very reliable, since 98.5% of the coagglutination typing results agreed with types established by the CP test.

Reagent cells must be prepared from a strain of _S. aureus_ that produces sufficient protein A to effectively absorb antiserum. We tested several strains of _S. aureus_ that apparently produced small amounts of this protein and consequently gave unsatisfactory agglutination reactions. The Cowan I strain of _S. aureus_ was finally selected because it produced large amounts of protein A (3). The method of preparing reagent cells can also influence the amount of protein A produced. When the Cowan I strain of _S. aureus_ was grown in a CO₂ atmosphere, these cells failed to absorb antisera. Inferior reagent cells may result if formaldehyde is not immediately removed from treated cells by

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**Table 4. Agreement of group B streptococcal types established by the coagglutination test with types established by the Lancefield CP test**

<table>
<thead>
<tr>
<th>Lancefield type</th>
<th>Stock strains</th>
<th>Clinical strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive by coagglutination*</td>
<td>No. positive by Lancefield CP test</td>
</tr>
<tr>
<td>Ib</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Ic</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>II(1b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>NT</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>96</td>
</tr>
</tbody>
</table>

* Number of strains that agglutinated reagent cells labeled with group B typing antiserum when tested with whole streptococcal cells or the modified hot-acid extraction technique.

# Number of strains of the various Lancefield types that precipitated in a standard Lancefield hot-acid extraction procedure with standard typing antisera.

# One strain was type Ib by the CP test, but Ib and II antigens were detected by the coagglutination test.

# One strain was type III by the CP test, but III and II antigens were detected by the coagglutination test.

use in the Lancefield CP tests. These discrepancies could not be attributed to multiple strains or different types within the same culture.

**DISCUSSION**

On the basis of these results, the coagglutination grouping procedure appears to be a reliable, rapid, and easy test for the identification of group B streptococci. The coagglutination grouping procedure was specific for group B streptococci when 141 group B streptococci and 148 non-group B streptococci were tested. Once isolated beta-hemolytic streptococci appear on media, group B streptococci can be accurately identified by the coagglutination test in less than 30 min, whereas grouping by the Lancefield CP technique takes at least 18 h. With limited experience, a microbiologist can interpret the coagglutination reactions more easily than CP reactions. All antisera used in these tests were from the Center for Disease Control (Atlanta), but we also used commercially available group B antiserum (Burroughs Wellcome Co.) to prepare ARC, and these ARC gave satisfactory grouping reactions when a limited number of streptococci were tested (results not shown).
washing. Excessive heat treatment of reagent cells will cause them to spontaneously agglutinate. Reagent cells are not difficult to prepare, but must be prepared exactly as described for optimal results.

The coagglutination test must include a negative control consisting of unlabeled reagent cells and the unknown streptococci. In our initial experiments with various types of blood agar plates, nonspecific agglutination of ARC and unlabeled reagent cells was sometimes observed. If the procedure was carried out properly and vein infusion blood agar plates were used, nonspecific agglutination was not observed. If the extract supernatant is contaminated with the aggregated cell sediment, the latter can mimic ARC agglutination. When coagglutination test reactions are observed for more than 4 min, nonspecific agglutination is frequently observed. The above problems are rare in the coagglutination tests, but microbiologists must be aware that they can occur.

This simple coagglutination grouping test should be most useful in busy clinical laboratories, which may not have time to serologically group these streptococci by other methods. The coagglutination typing test should be useful for extensive epidemiological and pathogenicity studies on the group B streptococci.

ACKNOWLEDGMENTS

We wish to thank A. Helstad for his assistance in the preparation of this paper. D. Drow for supplying many streptococcal cultures. E. Edwards and T. Minor for their suggestions, and the personnel in the Bacteriology Unit at the Wisconsin State Laboratory of Hygiene for their suggestions, assistance, and streptococcal cultures.

LITERATURE CITED