Method for Rapid Detection of Group B Streptococci by Coagglutination

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A quick and reliable technique for the identification of group B streptococci has been developed. The method requires no elaborate equipment or expensive reagents and can be used to detect the group B organisms in mixed broth cultures or to identify suspect colonies selected from agar plates. The method is a coagglutination technique in which 1 drop of specifically sensitized protein A-containing *Staphylococcus aureus* is mixed with 1 drop of supernatant of an actively growing culture. The soluble group-specific carbohydrate substance of the group B streptococci reacts with the staph particles to produce agglutination that is macroscopically readable. One colony of group B streptococci taken from an agar plate and inoculated into Todd-Hewitt broth will give a positive reaction within 6 h of incubation; with a larger inoculum, the positive reaction occurs within a shorter period. The method was applied for detection of group B streptococci in mixed broth cultures. In laboratory studies involving random mixtures of organisms, 59.3% of positive cultures were detected within the first 8 h of incubation, and 71.7% were found within 24 h. In clinical studies with mixed broth cultures grown directly from vaginal swabs, 78.6% of the positive cultures were detected within the first 8 h of incubation, and 92.9% were found within 24 h.

Within the last decade, the importance of group B streptococci in human infections has been realized (2, 9). Since the disease may manifest itself as a sudden, unexpected, and often fatal infection, a rapid and reliable method is needed to identify the group B streptococci. Standard methods (beta-hemolysis, hippurate, Lancefield grouping, CAMP) require 24 to 48 h for identification. Edwards and Larson (7) have described a coagglutination method for identifying colonies of beta-hemolytic streptococci directly on blood agar plates. Others (3, 8, 11, 12) have also used coagglutination to identify streptococci.

Rosner (17) selected three to five colonies of beta-hemolytic streptococci from blood agar plates, incubated them in Todd-Hewitt broth (THB) for 4 h, and tested the broth suspension by coagglutination. He successfully grouped 92.6% of beta-hemolytic group A, B, C, and G streptococci after 4 h of incubation.

The present study describes a further modification of the coagglutination technique. The streptococci are grown in pure or mixed cultures in THB, and then the organism-free supernatant is tested. Macroscopic agglutination is observed from the reaction of the soluble group-specific carbohydrate substance of the group B streptococci and specifically sensitized staph particles.

MATERIALS AND METHODS

Bacterial strains. Beta-hemolytic streptococci of groups A, B, C, G, and F were obtained from the Medical Center Hospital of Vermont and the Vermont State Health Laboratory, Burlington; Katherine Merritt, Dartmouth Medical Center, Hanover, N.H.; and Hazel W. Wilkinson, Center for Disease Control, Atlanta, Ga. Nonhemolytic group B strains were supplied by Martha Roe of the Childrens Hospital, Denver, Colo. Other organisms used were isolated from cultures provided by the Medical Center Hospital of Vermont, Burlington. *Staphylococcus aureus* Cowan I, ATCC 12598, used in preparation of the group B coagglutination reagent, was supplied by Earl A. Edwards of the Navy Research Center, San Diego, Calif.

Antisera. Rabbit antisera against group B streptococci were purchased from Difco Laboratories, Detroit, Mich.

Preparation of group B coagglutination reagent. This reagent was prepared according to the method of Edwards and Larson (7). *S. aureus* Cowan I, ATCC 12598, were grown overnight in Trypticase soy broth. The organisms were washed five times (Sorvall GLC-1, 3,000 rpm for 15 min/spin, or refrigerated Sorvall RC-2 [4°C], 10,000 rpm for 5 min/spin) in phosphate-buffered saline (PBS) (9 g of NaCl, 1.37 g of Na₂HPO₄, 0.24 g of NaH₂PO₄·H₂O, and 1,000 ml of distilled water), and 1 ml of packed cells was suspended in 9 ml of 0.5% formaldehyde. The formaldehyde suspension was incubated for 3 h at room temperature and washed three times with PBS. The

packed cells were diluted 1:10 in PBS and heated at 80°C for 1 h over a hot plate with a magnetic stirrer. The organisms were washed three times with PBS and were diluted 1:10 in PBS. The formaldehyde-stabilized staphylococci suspension was stored at 4°C. A mixture of 1 ml of 10% staphylococci and 0.1 ml of reconstituted group B antiserum was prepared and incubated at room temperature for 3 h. The suspension was mixed for 30 s on a Vortex mixer at 0.5-h intervals during the 3-h incubation period. The suspension was washed once with PBS and diluted to the original volume. The suspension was diluted 1:3 in PBS and 0.1 ml of Gram crystal violet per 10 ml of suspension was added to aid in visualization of agglutination. The group B coagglutination reagent was stored at 4°C.

Testing procedure. Organisms or swabs to be tested were inoculated into THB and incubated in a 10% CO₂ incubator at 36°C. Broths were centrifuged for 5 min in a bench-top clinical centrifuge set at maximum speed. One drop of supernatant fluid was mixed with 1 drop of group B coagglutination reagent on an RPR card (Hynson, Westcott, and Dunning, Inc.). The drops were mixed with a wooden applicator stick, and the RPR card was rotated for 5 min on a serological rotator set at 90 rpm. The suspension was observed macroscopically for agglutination and interpreted according to the following criteria: negative—little or no observable agglutination of particles in the center of the circle, with diffuse blue coloration of entire circle area; positive—agglutinated particles clustered in the center and at the periphery of the circle, with little or no diffuse blue coloration of the circle area.

Identification of colonies selected from blood agar plates. Organisms grown overnight on blood agar plates were suspended in 1-ml aliquots of THB and incubated in a 10% CO₂ incubator at 36°C. At 1-h intervals, the tubes were centrifuged and the supernatants were tested with group B coagglutination reagent. Organisms tested included beta-hemolytic streptococci of groups A, B, C, G, and F. Among the group B strains were one each of types Ia, Ib, Ic, II, and III, and three nonhemolytic strains. Various organisms including *Streptococcus faecalis*, alpha- and nonhemolytic streptococci, and nonstreptococcal organisms (including *Escherichia coli*) were tested.

Identification of group B streptococci in random mixtures. A total of 198 mixtures were prepared which contained *Bacillus* species, *Candida* species, *Corynebacterium* species, *E. coli*, *Listeria monocytogenes*, *Proteus* species, *Pseudomonas* species, *Streptococcus faecalis*, *Staphylococcus* species, and alpha- and nonhemolytic streptococci as well as various beta-hemolytic streptococci of groups A, B, C, G, and F. Each organism was grown in Trypticase soy broth and diluted within the range of 10³ to 10⁹ colony-forming units per milliliter. Samples of various volumes were combined to make up test mixtures. A sample of each test mixture was inoculated into a 2.5-ml aliquot of THB, and after 2, 4, 6, 8, and 24 h of incubation at 36°C in a 10% CO₂ incubator, the broths were centrifuged and the supernatants were tested with group B coagglutination reagent. A reliability index was computed according to the following formula:

Reliability index =

$$\frac{(\text{no. of tubes containing group B streptococci giving positive results}) + (\text{no. of tubes without group B streptococci giving negative results})}{\text{total no. of tubes}}$$

Identification of group B streptococci in broth cultures grown directly from vaginal swabs. Eighty vaginal specimens were tested. Each swab was plated on a blood agar plate, submerged in a 2.5-ml aliquot of THB, and agitated vigorously. The swab was discarded, and the broth culture was incubated in a 10% CO₂ incubator at 36°C. At 2, 4, 6, 8, and 24 h, the cultures were tested with the group B coagglutination reagent. After the 24-h reading, the THB cultures were plated on blood agar plates.

RESULTS

The testing of group B streptococci selected from blood agar plates (Table 1) showed that when one colony of group B streptococci was used, a positive result could be observed within 6 h of incubation. Testing using more than one colony as inoculum showed an inverse relationship between the number of colonies used and the time required to produce a positive reaction. All group B streptococci tested, including non-hemolytic strains, gave positive results, and all non-group B organisms tested produced negative reactions. The method showed excellent specificity.

In studies of 198 random mixtures, 71.7% of the 145 mixtures containing group B streptococci were detected as positive within 24 h of incubation. Of these, 59.3% were detected within 8 h of incubation, with a very small number (10.4%) requiring only 2 to 4 h and an additional small number (12.4%) requiring 24 h. The overall reliability index was 0.79. No false-positive results were encountered.

Some false-negative results were found. Growth curve studies conducted before this investigation showed that 6×10^8 colony-forming units of group B streptococci per ml must be present before enough diffusible antigen is produced for detection with the group B coagglutination reagent. Quantitative platings of the false-negative specimens in this study confirmed these findings. If, for any reason, the group B streptococci failed to reach this critical concentration, a false-negative reaction resulted.

Of 80 vaginal specimens, 14 contained group B streptococci. All 14 of the cultures were correctly identified by coagglutination. Of the 14, 35.7% were found at 6 h, 42.9% were found at 8 h, and 14.3% were found at 24 h. Therefore, 78.6% were detected within 8 h, and 92.9% were

TABLE 1. Colonies of group B streptococci selected from blood agar plates

No. of strains tested	No. of colonies used	% Initially positive at each reading ^a (cumulative % positive)			
		3 h	4 h	5 h	6 h
34	1	11.7 (11.7)	44.1 (55.8)	32.5 (88.3)	11.7 (100)
45	3-5	28.9 (28.9)	62.2 (91.1)	8.9 (100)	0 (100)

^a All tubes were negative when tested at 1 and 2 h.

found within 24 h. One specimen was identified as positive at 32 h. No positive readings were found at 2 or 4 h. One false-positive result was found which was due to a cross-reaction with a strain of *E. coli*.

DISCUSSION

The coagglutination method has many advantages over other methods now used to identify group B streptococci. The vast majority of current methods, including hippurate hydrolysis (1), pigment production (14), Lancefield grouping (13), and the CAMP reaction (6, 15), require at least 18 to 24 h of incubation after the suspect beta-hemolytic colony has been located on blood agar. The fluorescent-antibody technique (16) and counterimmunoelectrophoresis (5, 10) require only 2 to 4 h to perform but call for special equipment.

The coagglutination method has been used to identify suspect beta-hemolytic colonies directly on blood agar plates (7). Rosner (17) selected colonies from blood agar plates and suspended them in THB; after 4 h of incubation the broth suspensions were tested by coagglutination, and positive readings were obtained. However, some isolates gave troublesome multiple agglutinations or gave no reaction at all within the 4-h incubation period.

In the coagglutination method used in this study, colonies were selected from blood agar plates, suspended in THB, and, after incubation, the organism-free culture supernatant was tested. Positive reactions were detected in all cases (even with a single-colony inoculum) within a 6-h period. No cross-reactions were observed with streptococci of any other group or with nonstreptococcal organisms. Cross-reactions which have been noted between group B and G streptococci (4) were not encountered. The technique is simple to perform, no special equipment is required, and the results are accurate and easy to read.

The coagglutination method has also been shown to be of value in detecting group B streptococci in mixed broth cultures. Preliminary studies using broth mixtures prepared in the laboratory to simulate vaginal specimens

showed that 59.3% of all positive cultures could be detected within 8 h of incubation, and 71.7% could be found within 24 h. Studies using mixed broth cultures grown directly from actual vaginal specimens gave even more promising results, with 78.6% of the positive cultures being detected within 8 h of incubation and 92.9% being found within 24 h of incubation.

One false-positive result was encountered during this study. The specimen contained a strain of *E. coli* that was found to cross-react with some lots of group B antiserum. Since the false-positive reactions appeared to be related to the quality of the antiserum rather than to the coagglutination method itself, the occurrence of false-positive reactions could be eliminated by careful quality control of all antisera used.

It was also found that grossly bloody swab specimens gave a weak reaction immediately upon inoculation of the THB. The reaction did not intensify as the incubation progressed. No positive reactions were obtained as the result of blood on the swab. A 0-h reading is recommended for use with bloody swabs to serve as a base reading. A 0-h positive reading would indicate that the specimen may be unsuitable for testing by this method.

This coagglutination technique provides presumptive evidence of the presence of group B streptococci in many instances within 8 h after receipt of the specimen in the laboratory. By centrifuging the cultures to eliminate the bacterial cells and testing only the cell-free supernatant, troublesome autoagglutinations have been eliminated. The group-specific substance of the group B streptococci diffuses into the broth medium and can be easily detected in the culture supernatant. The detection of group B streptococci in mixed broth cultures by this method may also lead to the identification of nonhemolytic group B streptococci that may be overlooked in traditional methods requiring beta-hemolysis to locate suspect colonies.

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