Detection of Group B Streptococci by Agglutination Testing from Selective Broth Cultures*

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ABSTRACT

A rapid technique for the immunological identification of group B streptococci in vaginal swabs is reported. Vaginal swabs obtained from 567 pregnant women at term or during labor were incubated for eight hrs in Todd Hewitt broth containing 15 μg per ml nalidixic acid, one μg per ml polymixin, and 0.1 μg per ml crystal violet (NPC broth). After streaking the swabs on blood agar plates, both plain broth cultures and their nitrous acid extracted pellets were tested with a commercial latex agglutination reagent. Beta-hemolytic colonies grown on the blood agar plates after overnight incubation were grouped with commercial latex agglutination and coagglutination reagents for reference identification. Sensitivities for the broth culture and nitrous acid techniques were 86.8 percent and 94.7 percent, respectively; specificities were 97.4 percent and 98.7 percent respectively. Nitrous acid extraction of vaginal broth cultures followed by latex agglutination testing can significantly shorten the time needed to detect group B streptococci, resulting in the intrapartum detection of these organisms.

Introduction

The role of group B streptococcus (GBS) as a human pathogen has been clearly documented.²,⁴,⁸,¹¹ Systemic infections occurring during the first week of life are termed early-onset sepsis (EOS) and show a high incidence and mortality rate,⁴,⁷,¹⁴ with the source of infection generally being the maternal genital tract.² Because of the rapid course of the disease⁵ and because of difficulties in predicting, at the time of
prenatal examinations, maternal colonization at delivery, therapy and prophylaxis could be initiated sooner if detection and identification time were reduced.

Boyer et al have reported that GBS can be identified in about half of the colonized parturient women when examining six hr selective broth cultures by fluorescent antibody (FA) techniques. The organism could be identified in 93 percent of the colonized women by increasing the incubation time to 18 hrs. Although their technique appeared accurate and sensitive, the authors did not consider it a practical approach to large scale intrapartum antibiotic prophylaxis because of the logistic complexities associated with FA techniques. In a recent study, FA examination of uncultured vaginal samples could identify a subgroup of women heavily colonized with GBS.

Counterimmunoelectrophoresis, latex agglutination, and coagglutination testing of selective broth cultures have been described as an approach to rapid detection of GBS. Coagglutination shows a sensitivity similar to FA and superior to counterimmunoelectrophoresis. In this study, it was hoped to reduce further the time required for immunological detection of GBS in selective broth cultures of pregnant women by using a sensitive latex agglutination reagent and extracting the antigenic material by the nitrous acid technique.

Materials and Methods

Latex Agglutination

The Wellcogen Strep B (WSB) reagent consists of latex particles coated with rabbit antibodies directed against the group-specific GBS antigen. A control latex suspension consisting of particles coated with normal globulins and a positive control antigen are included in each kit. The WSB test has been successfully used for the detection of GBS antigen in body fluids from neonates with systemic infections. In the present study the authors sought to take advantage of the sensitivity of the WSB reagent by extending its use to the detection of GBS in vaginal broth cultures. The WSB test was performed by mixing 20 μl of each latex reagent with 40 μl of antigenic material on a glass slide. The latter was represented by pure GBS suspensions, broth cultures, or by nitrous acid extracts. The slide was rocked for three minutes and observed for agglutination. Test results of the WSB were graded as 4+ (large clumps appearing within one min), 3+ (large clumps appearing within three min), 2+ (small clumps appearing within one min) and 1+ (small clumps appearing within three min). For the purpose of the present study, only 3+ and 4+ results were considered as indicating the presence of group B antigen. When both test and control latex suspension showed agglutination, the test was repeated after heating the sample at 100°C for five min as per the manufacturer's instructions.

Nitrous Acid Extraction

Nitrous acid (NA) extraction was performed by a microtechnique on pelleted material from GBS suspensions or from vaginal broth cultures. In either case, one ml aliquots were centrifuged at 1500 g for 10 min. The supernatant was decanted and excess fluid was removed by draining through filter paper. Forty μl of 4 N sodium nitrite and six μl of glacial acetic acid were sequentially added to the pellet. The tubes were then vortexed, capped, and left at room temperature for 10 min before neutralization with 25 μl of 5 M sodium hydroxide. With vaginal cultures only, the tubes...
were centrifuged again to remove debris before agglutination testing. Centrifuga-

**Laboratory Evaluation**

The sensitivity of various procedures for the detection of the group-specific antigen was evaluated by testing serially diluted GBS suspensions or undiluted broth cultures incubated for different periods of time. The strains employed in these experiments were fresh isolates from vaginal specimens. One colony was inoculated into one ml of Todd Hewitt broth† and incubated aerobically overnight at 35°C. After photometrically adjusting the suspension to a concentration of approximately 1.5 × 10⁷ colony forming units (CFU) per ml, serial dilutions were prepared in normal saline. These dilutions were processed for colony counts by standard methods and tested by WSB agglutinations with and without prior NA extraction.

The early kinetics of GBS growth were analyzed by noting the incubation period intervening before antigen was detectable. Todd Hewitt broth† supplemented with 15 μl per ml of nalidixic acid, one μl per ml of polymixin and 0.1 μl per ml of crystal violet (NPC broth), as described by Gray et al,¹⁵ was used for this purpose. One ml aliquots of NPC broth were inoculated with known numbers of CFU from stationary cultures and tested by latex agglutination 4, 6, 8, and 10 hrs after incubation at 35°C.

In preliminary experiments, selective broths containing erythrocytes, such as Baker's et al,⁶ proved unsuitable for treatment with NA. Removal of blood from Baker's medium resulted in marked inhibition of early GBS growth, even after the addition of rabbit serum (not shown).

**Field Evaluation**

Vaginal swabs were obtained from pregnant women at term (38 weeks gestation) and from parturient women admitted to the University Policlinic of Messina, Italy, over an 18 month period. Immediately after collection, the swabs were placed in tubes containing one ml of NPC broth. The swabs were either immediately transported to the laboratory or refrigerated until transported. Upon receipt, the tubes were incubated aerobically at 35°C for eight hr. The swabs were then carefully expressed against the walls of the tube, and each was inoculated onto Columbia and Columbia CNA agar plates‡ containing five percent sheep blood for reference identification. The remaining broth culture was used for latex agglutination with and without NA extraction. Blood agar plates were incubated for 18 hrs at 35°C, and beta-hemolytic streptococci were identified by the Streptex latex system§ and by the Phadebact Streptococcus test|| as previously described.¹⁰ Alpha or non-hemolytic colonies were grouped with the Streptex or Phadebact group B and D reagents only when the corresponding broth cultures agglutinated the WSB reagent.

**Results**

**Laboratory Evaluation**

The minimum number of CFU per ml required to agglutinate the WSB reagent was determined using untreated and NA extracted GBS suspensions. Since the NA extraction procedure requires treatment of centrifuged pellets, unextracted and resuspended pellets were also tested for comparison. Results with four strains

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‡ BBL, Cockeysville, MD.

§ Wellcome Diagnostics, Dartford, England.

|| Pharmacia Diagnostics, Piscataway, NJ.
RAPID DETECTION OF GROUP B. STREPTOCOCCI

Number of CFU/ml of Group B Streptococci Necessary to Agglutinate the Wellcogen Strep B Reagent With and Without Extraction With Nitrous Acid

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment of the Bacterial Suspension</th>
<th>Centrifugation* &amp; Extraction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS 1</td>
<td>None</td>
<td>1.22 x 10^7</td>
</tr>
<tr>
<td>KS 2</td>
<td>None</td>
<td>1.5 x 10^7</td>
</tr>
<tr>
<td>KS 3</td>
<td>None</td>
<td>9.4 x 10^6</td>
</tr>
<tr>
<td>KS 4</td>
<td>None</td>
<td>1.35 x 10^7</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
<td>1.23 x 10^7</td>
</tr>
</tbody>
</table>

*Results refer to colony forming units per ml (CFU/ml) present in the suspensions before centrifugation. Tests were performed on pellets. ND = not done.

are shown in table I. Mean numbers of CFU per ml required to agglutinate the WSB reagent were 1.23 x 10^7, 5.49 x 10^6, and 5.90 x 10^4 when using untreated suspensions, unextracted pellets, and NA extracted pellets, respectively. Thus, NA extraction resulted in a 93 fold increase in antigen availability.

The sensitivity of the NA extraction procedure was also assessed in terms of the minimum incubation period needed before GBS could be detected in NPC broth using different inocula. Untreated and NA extracted cultures were tested by WSB agglutination after 4, 6, 8, and 10 hrs of incubation at 35°C. Results of representative experiments are shown in table II. The earliest time at which agglutination was detectable depended on the number of CFU forming the initial inoculum. Extraction by nitrous acid always resulted in shortening of the time required before antigen was detectable and/or in stronger reactions. With an inoculum consisting of approximately 66 CFU, the group specific antigen could be detected in NA extracts after an incubation period of four hrs. Testing unextracted cultures by WSB agglutination, however, also showed good sensitivity, detecting GBS growth after 10 hrs of incubation with a very low initial inoculum (three CFU). These results prompted us to evaluate WSB testing of vaginal cultures after an eight hr incubation period.

FIELD EVALUATION

By reference techniques, GBS were found in 38 of 567 (6.7 percent) vaginal cultures (table III). This percentage is similar to vaginal colonization percentages reported for parturient women in other parts of Italy and Europe. Of the 567 cultures directly tested with the

<table>
<thead>
<tr>
<th>TABLE II</th>
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| Incubation Period Necessary to Detect the Presence of Group B Streptococci in a Selective Broth With the Wellcogen Strep B Latex Agglutination Test Using Various Inocula |

<table>
<thead>
<tr>
<th>Inoculum (CFU)</th>
<th>4 hrs U</th>
<th>6 hrs U</th>
<th>8 hrs U</th>
<th>10 hrs U</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>66</td>
<td>3+</td>
<td>3+</td>
<td>4+</td>
<td>ND</td>
</tr>
</tbody>
</table>

U = Test performed directly on uncentrifuged broth culture.
NA = Test performed on nitrous acid extracts from the culture pellet.
CFU = Colony forming units.
ND = Not done.

TABLE III

Comparison of Group B Streptococcus Detection in Vaginal Broth Cultures Using the Wellcogen Strep B Latex Agglutination Test With Reference Cultural Methods

<table>
<thead>
<tr>
<th>Agglutination/ Culture Result*</th>
<th>Percent</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>Broth culture†</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>NA extracts‡</td>
<td>36</td>
<td>2</td>
</tr>
</tbody>
</table>

*Number of specimens with indicated agglutination and reference culture results.
†Agglutination test performed directly on uncentrifuged broth culture.
‡Agglutination test performed on nitrous acid extracts from the culture pellet.
WSB reagent, there were 47 (8.3 percent) positive reactions. Of these, 33 were positive by reference methods and 14 were not, resulting in a sensitivity of 86.8 percent and a specificity of 97.4 percent (table III). Testing the NA extract from the same cultures resulted in better sensitivity (94.7 percent) and specificity (98.7 percent). Latex agglutination was positive using both the direct and NA extraction procedures with a culture containing alpha-hemolytic GBS.

Agglutination of WSB non-specific agglutination latex control was never judged stronger than $2^+$ and occurred with 103 of 567 unextracted cultures (18.2 percent) and 66 of 567 (11.6 percent) NA extracts. Heating the samples at 100°C for five min reduced the occurrence of these non-specific reactions to 9.9 percent and 6.2 percent with unextracted cultures and NA extracts respectively.

Discussion

Therapy of EOS is often unsuccessful if initiated when the infant is overtly symptomatic.2,4 Thus, there is an obvious need for effective preventive measures and for earlier detection of the disease.1,23 Selective prophylaxis with antibiotics depends on the detection of maternal and/or infant GBS colonization as well as of obstetrical risk factors.18,25 Since genital tract colonization at delivery cannot be reliably predicted by prenatal cultures,3 intrapartum screening for the presence of GBS in the genital tract is recommended in selected populations.18,25 Moreover, intrapartum prophylaxis appears appropriate since there is sometimes evidence of intrauterine acquisition of GBS and/or maternal infection. Commonly employed methods for the identification of GBS take one to two days and are often incompatible with prophylaxis during labor or immediately after delivery. Indeed, as stressed by Boyer et al.9 the increasing indications for pharmacologic augmentation and for cesarean section have considerably shortened the mean duration of labor.

Detecting the group-specific carbohydrate in selective broth cultures by immunological methods has been shown to reduce the time required for definitive identification of GBS.9,13,20,22,24,26 Proposed techniques include counterimmunoelectrophoresis,13 coagglutination,20,22 latex agglutination,24 and FA examination.9,26 The latter method appears considerably more sensitive than counterimmunoelectrophoresis and highly specific.9 In the study of Boyer et al using an FA technique, sensitivity was 49, 81, 83, and 93 percent after 6, 12, 18, and greater than 18 hrs, respectively, of incubation in a selective broth.9 The main disadvantage of FA techniques is that experienced laboratory personnel should be available on a round-the-clock basis if this method is to be applied to the widespread screening of parturient women. On the contrary, latex agglutination and coagglutination are simple to perform and to read and do not require special equipment and highly trained personnel.

Using the supernatant of selective broth cultures, coagglutination shows a sensitivity comparable to that of FA, being able to detect 78.6 percent and 92.9 percent of the colonized women within eight and 24 hrs, respectively.22 In our experience, coagglutination testing of vaginal broth cultures often gives multiple, non-specific reactions. This problem has been recently reported to occur in 55 percent of intrapartum vaginal cultures and to be reduced by heating the samples at 90°C for 10 min.21

Since there is a higher risk of EOS for neonates born to mothers colonized with high GBS densities,1 rapid techniques have been proposed to detect only highly colonized (about $10^4$ to $10^5$ CFU per swab) parturient women by FA examination26 and coagglutination.20 Ryan
et al identified a subgroup (33 percent) of women colonized with GBS using direct FA examination of vaginal specimens, without culture. Specimens positive by this technique shared high (average of $10^5$) colony counts. Coagglutination with a commercial reagent* has been used in a recent study to identify, within five hrs, a subset of heavily colonized (average of $3 \times 10^4$ CFU per ml per swab) pregnant women.

Our results indicate that the time required for GBS detection in broth cultures can be significantly decreased by testing NA extracts of the culture sediment with the WBS reagent. With this technique, 94.7 percent of colonized women could be detected after eight hrs. Using the WBS reagent without NA extraction resulted in lower sensitivity (86.8 percent versus 94.7 percent) and slightly lower specificity (97.4 percent versus 98.7 percent). The increase in sensitivity after NA extraction can be explained by an increase in antigen availability secondary to disruption of the bacterial cell wall. The increase in specificity on the other hand, could be due to destruction of labile crossreacting antigens. In support of this explanation, it was noticed that heating the samples in order to reduce non-specific reactions was less often necessary with NA extracts. Non-specific reactions of the WSB test have been reported to occur with protein rich specimens but were not a major problem in the present study.

The methods described in the present study have been designed to detect GBS in the maternal genital tract independently from the magnitude of colonization. However, although vaginal cultures incubated for less than eight hrs were not tested, data on early kinetics of the GBS growth suggest that swabs containing 70 CFU or more can be detected after four hrs of incubation, using NA extracts. In addition, preliminary data from our laboratory suggest that GBS can be detected, without culture, in a high proportion of colonized women by directly testing NA extracts of vaginal specimens with the WSB reagent. In conclusion, it appears that NA extraction and the WSB test can contribute to the improvement of methods for the rapid intrapartum detection of GBS. It is hoped that these techniques will be useful in the prevention of EOS.

References


* Phadebact Streptococcus Test, Pharmacia, Piscataway, NJ.


