JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1996, p. 342–344 Vol. 34, No. 2
0095-1137/96/$04.00+0


# Comparison of a Modified DNA Hybridization Assay with Standard Culture Enrichment for Detecting Group B Streptococci in Obstetric Patients

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Received 20 July 1995/Returned for modification 22 September 1995/Accepted 22 November 1995

**Infection with group B streptococcus (GBS) results in 12,000 to 15,000 cases of neonatal sepsis annually in the United States. GBS is transmitted vertically in up to 70% of infants born to colonized women. Early-onset GBS disease (septicemia, pneumonia, or meningitis occurring within 7 days of life) has a mortality rate of up to 50%, with permanent neurologic sequelae occurring in 15 to 50% of infants surviving meningeal infection. Because of the fulminant nature of neonatal infection, it would be useful to have a rapid assay for determining the GBS status of laboring women. This study illustrated how a commercially available DNA probe-based test was modified to achieve this goal. Modifications included the use of mixed cultures rather than pure isolates for detecting GBS, along with a shorter culture enrichment time and a sample concentration step. To this end, vaginal and rectal swabs from 402 pregnant women during their third trimester were cocultured and tested for GBS rRNA. The 8-h enrichment protocol resulted in an assay with a sensitivity of 95% and specificity of 98%, while the 3-h enrichment protocol revealed a sensitivity of 73% and specificity of 99%. In summary, GBS was detected in the majority of colonized women in less than 4 h. This study illustrated the usefulness of the approach in identifying the most heavily colonized women, who are at the highest risk of transmitting GBS to their neonates. The modified test would have a significant impact on both the medical management and antibiotic therapy for these women and their newborns.**

Group B streptococcus (GBS) is the leading cause of neonatal pneumonia, sepsis, and meningitis in the United States (2, 3, 5, 7, 10, 11, 13, 15, 16, 18, 19) and a common cause of maternal infection (11). Neonatal infection usually results from vertical transmission during delivery or acquisition in utero just prior to delivery (1–5, 9–11, 15, 17, 19). Early-onset disease in the neonate is defined as the clinical presentation of disease within the first 5 to 7 days of birth (10, 11, 19), with approximately half of these infants becoming symptomatic within 1 to 12 h of life (11). In the premature infant, early-onset disease may have a mortality rate as high as 50% (1, 2, 5, 11, 18).

In 5 to 35% of pregnant women, the vagina and rectum are colonized with GBS (1, 5, 10–12, 16, 18, 19). Vaginal carriage may be intermittent because of spontaneous clearing and recolonization in approximately half of all women (2, 15, 18, 19). Identification of carriers at the time of labor to prevent neonatal infection requires a rapid test with the sensitivity of the current "gold standard" culture enrichment procedure. Intrapartum chemoprophylactic treatment of GBS-positive women after the onset of labor or membrane rupture, but before delivery, has been shown to decrease neonatal colonization and early-onset invasive disease.

The established protocol in our laboratory for GBS detection employs selective enrichment in Todd-Hewitt broth which contains yeast extract, colistin, and nalidixic acid (LIM broth) (14), followed by culture on Columbia blood agar base with colistin and nalidixic acid (CNA), enriched with 5% sheep blood and confirmation by latex agglutination. Although quite sensitive, this method has an average turnaround time of 36 to 72 h. Consequently, patients who are at high risk of infection (those with preterm labor, prolonged rupture of membranes, or maternal intrapartum fever) are treated prophylactically with antibiotics (1, 3, 8, 9, 11, 13, 17, 18). Rapid determination of a woman's GBS colonization status, whether high or low, at the time of delivery would ensure proper antibiotic treatment and/or alleviate unnecessary prophylaxis. Prompt and accurate treatment of all GBS-positive, pregnant women during labor could help to prevent neonatal sepsis, meningitis, pneumonia, and possible death.

A commercially available kit, ACCUPROBE (AP) (GEN-PROBE, Inc., San Diego, Calif.), utilizes a nucleic acid hybridization methodology for the identification of GBS 16S rRNA from pure isolates of GBS. The present investigation illustrates the success of this commercially available kit in identifying GBS from mixed cultures rather than pure isolates to produce a more rapid assay.

## MATERIALS AND METHODS

The present investigation was approved prior to its initiation by the Research Review and Human Experimentation Committee at Magee-Women's Hospital and Research Institute. In phase I of the study, separate vaginal and rectal swabs (Culturette II Swab; Becton Dickinson Microbiology Systems, Cockeysville, Md.) were collected from 123 women who were in their third trimester of pregnancy. The vaginal and rectal swabs were cocultured in 3 ml of LIM broth and incubated overnight at 37°C in 5% $CO_2$. Following incubation, a 50-μl sample was tested directly with the AP system for GBS 16S rRNA according to the manufacturer's instructions. Briefly, 50 μl of the LIM broth specimen was incubated in the probe-containing reagent tube at 37°C for 10 min to lyse the bacterial cell walls to liberate rRNA. This tube contains an acridium-labeled single-stranded DNA probe, which is complementary to GBS 16S rRNA. Hybridization of the target RNA and the probe DNA was permitted to occur at 60°C for 10 min. The resulting formation of an RNA-DNA hybrid acts to protect the acridium ester from hydrolysis, thus allowing differentiation of a hybridized probe from a non-hybridized probe. Hydrogen peroxide was then added to the mixture, in which it

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TABLE 1. Phase I: comparison of standard culture with ACCUPROBE with overnight broth cultures[a]

| Given result by ACCUPROBE | No. of samples with given result by standard culture | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 26 | 3 | 29 |
| Negative | 0 | 94 | 94 |
| Total | 26 | 97 | 123 |

[a] Values for ACCUPROBE compared with standard cultures following 12 to 18 h of enrichment were as follows: sensitivity, 100%; specificity, 96.9%; positive predictive value, 89.7%; negative predictive value, 100%; accuracy, 97.6%. Prevalence for ACCUPROBE was 23.6%.

TABLE 2. Phase II: comparison of standard overnight culture with ACCUPROBE performed following 8-h enrichment[a]

| Given result by ACCUPROBE | No. of samples with given result by standard culture | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 110 | 5 | 115 |
| Negative | 5 | 283 | 288 |
| Total | 115 | 288 | 403 |

[a] Values calculated for ACCUPROBE compared with overnight standard cultures following an 8-h enrichment are as follows: sensitivity, 95.7%; specificity, 98.3%; positive predictive value, 95.7%; negative predictive value, 98.3%; accuracy, 97.5%. Prevalence for ACCUPROBE was 29.5%.

reacted with the protected acridium ester, resulting in the emission of light, which was measured in a luminometer (6). A cutoff value of 50,000 relative light units, set by the manufacturer, was used to determine whether a sample was positive or negative. For comparison, a Columbia CNA plate was inoculated with the same overnight LIM broth culture. Following overnight incubation, this plate was examined for the presence of beta-hemolytic colonies, which were confirmed as GBS by the Patho Dx Latex Grouping Kit (Diagnostic Products Corp., Los Angeles, Calif.). Quantitative growth of GBS is described as being either 1, 2, 3, or 4+. These numbers reflect GBS colony growth on 25, 50, 75, or 100% of the area of a CNA plate, respectively.

In phase II of this investigation, an additional 403 vaginal and rectal swabs were collected from third-trimester obstetrical patients and cocultured as described previously with the exception of LIM broth enrichment times. In this study, 3- and 8-h LIM broth enrichment time points were used. At the end of 3 h of enrichment, 2.5 ml of the 3.0-ml sample was concentrated at 2,000 $\times$ *g* for 10 min. The pellet was resuspended in 50 μl of the manufacturer's lysis reagent and tested for GBS 16S rRNA as described earlier. To the remaining 0.5-ml sample, 2.5 ml of fresh LIM broth was added, and the specimen was reincubated at 37°C in 5% $CO_2$ for an additional 5 h. At the end of 8 h of total enrichment, 2.5 ml of broth was again concentrated and tested as described previously. For comparison, a sample of broth was also plated onto Columbia CNA to look for beta-hemolytic colonies.

## RESULTS

In phase I, parallel testing was performed on overnight (12 to 24 h) mixed cultures by using standard culture enrichment and the AP DNA probe hybridization assay (Table 1). Of the total 123 samples tested, 26 samples were positive by both methods. The AP system detected three additional positives that were negative by culture. The presence of GBS was confirmed in these three samples after subculturing onto Columbia CNA and subsequent grouping by latex agglutination. There were no false-negative results generated by the AP method, in using mixed cultures, resulting in a sensitivity of 100% and a specificity of 97%.

On the basis of the success of the AP system using overnight mixed broth cultures, as opposed to purified isolates, both a shorter enrichment period and a sample concentration step were implemented in phase II. Following 8 h of selective enrichment in LIM broth, the sensitivity of the DNA probe hybridization assay was 95.7% and the specificity was 98.3% (Table 2). Of the five AP-positive, culture-negative samples, one contained gross amounts of blood, and in four, GBS was not identified by the technologist. In retrospect, this was due to the presence of very faint, atypical hemolysis in two of the cultures and to poor medium quality in the other two cultures. Subsequent subculturing of these four cultures revealed 1+ growth of beta-hemolytic colonies on CNA plates, and these colonies were identified as GBS by latex agglutination. In contrast, there were five samples which were positive for GBS by culture that were negative by the AP procedure after 8 h of enrichment. Four of these five false-negative samples were weakly positive by culture ($\leq$1+), with the fifth specimen being a relatively strong positive (3+). Not surprisingly, the five culture-positive samples were also negative by AP after 3 h of enrichment. Overall, following 8 h of broth enrichment, the modified AP procedure and culture enrichment correctly identified similar percentages of colonized patients, 95% (114 of 119) and 96.6% (115 of 119), respectively.

Concentration and analysis of the LIM broth enrichment culture after 3 h resulted in a test with a sensitivity of 73.9% and a specificity of 98.6% (Table 3). Four false-positive samples were identified by AP with culture as the gold standard. The first false-positive sample contained gross amounts of blood, while the second was due to a technical error in the AP procedure. The remaining two AP-positive, culture-negative samples were determined to be failures of culture on the basis of reexamination of the plates as described earlier.

Of the 30 samples containing GBS that were negative by AP at 3 h, 25 became positive following the 8-h incubation. Of the remaining five samples, four showed 1+ growth on the culture, while the last discrepant result showed 3+ growth.

Of the 115 culture-positive samples detected following the 3-h LIM incubation, quantitation results were available on 52 isolates, and ranged from 1+ to 4+ growth. Not surprisingly, after 3 h of incubation, GBS was detected by the AP assay in 50% (6 of 12) of samples which had 1+ to 2+ growth, and 88% (34 of 40) of samples containing 3+ to 4+ growth. In summary, the majority of women who are heavily colonized with GBS can be identified by the modified 4-h AP assay.

## DISCUSSION

Conventional GBS testing in the obstetrical patient consists of selective broth enrichment and culture, followed by latex agglutination. Unfortunately, this approach requires 36 to 72 h to complete, making it impossible to use the information for rapid intervention in GBS-positive women in labor. Incorporating the conventional selective enrichment approach with the newer DNA-based technology created a test with comparable sensitivity and specificity in less time. The modified AP proce-

TABLE 3. Phase II: comparison of overnight culture with ACCUPROBE assay following 3-h enrichment[a]

| Given result by ACCUPROBE | No. of samples with given result by standard culture | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 85 | 4 | 89 |
| Negative | 30 | 284 | 314 |
| Total | 115 | 288 | 403 |

[a] Values calculated for ACCUPROBE compared with overnight standard cultures following an 8-h enrichment were as follows: sensitivity, 73.9%; specificity, 98.6%; positive predictive value, 95.5%; negative predictive value, 90.4%; accuracy, 91.6%.

dure presented in this study utilized mixed cultures rather than pure isolates to reduce the turnaround time substantially, while keeping sensitivity and specificity levels high.

The results presented here illustrated the usefulness of modifying a commercially available test kit for detecting GBS from mixed cultures rather than from purified isolates, which take longer to produce. The presence of high levels of normal flora within the vaginal-rectal cultures did not appear to interfere with the specificity of the test for detecting GBS, nor did the presence of stool on several of the rectal swabs. However, consistent with the manufacturer's package insert, bloody specimens interfered with the accuracy of the assay. This is due to the hemin present within blood, which is known to produce a false-positive chemiluminescent signal (4). In general, this modified approach was successful at detecting GBS from mixed cultures without sacrificing test sensitivity or specificity.

To be useful in a partum population, a rapid test for GBS detection is necessary. To this end, the system described here was used successfully on mixed cultures with a shorter culture enrichment period, along with a sample concentration step to produce a test which detected 73% of all GBS-positive women in as few as 4 h, or over 95% of all GBS-positive women in 9 h. This level of sensitivity was similar to that in a study described by Yancey et al. (19) in which a modified AP system detected 71% of all GBS-positive carriers. The one apparent difference between these two studies was the smaller number of false-positive results seen here, which was probably due to fewer bloody samples being tested.

The results presented here provided a practical and useful approach for GBS screening of women in labor. By concentrating the organisms present in the culture by centrifugation, we effectively increased the clinical sensitivity of the test, enabling the use of a shorter LIM broth enrichment time for detecting the organisms. Utilizing a two-time-point system (testing the broth at 3 and 8 h postenrichment), we were successful at detecting 73 and 95% of all GBS-positive women in a total turnaround time of 4 or 9 h, respectively.

Obstetricians were polled as to their interest in a relatively rapid diagnostic test for GBS, i.e., the 4-h test, which would detect the majority of heavily colonized women, versus a less rapid test whose level of sensitivity would approach 100%, i.e., the 9-h test. The majority of clinicians agreed that they would rather have the test with greater sensitivity, since most labor periods and deliveries are generally longer than 9 h. On the basis of this feedback, our laboratory now offers this DNA probe hybridization test for GBS with the 8-h enrichment protocol for screening samples.

The American College of Obstetricians and Gynecologists and American Academy of Pediatrics along with the Centers for Disease Control and Prevention met jointly in March 1995 to address the problem of GBS infection. The draft entitled "Prevention of Group B Streptococcal Disease: a Public Health Perspective," dated 12 April 1995, contained the following recommendations on addressing the laboratory aspect of the problem. Culture techniques which maximize the likelihood of GBS recovery should be used. This includes a single swab or two separate swabs of the vaginal introitus and the anorectum that are inoculated into selective broth medium. These broth cultures should be incubated for 18 to 24 h, and the growth should be subcultured onto a sheep blood agar plate. The resulting culture should be inspected, and the organisms suggestive of GBS should be identified.

Having an accurate picture of a woman's GBS status at the time of delivery would aid the physician in implementing the necessary steps to treat those infants born to GBS-positive women and thereby reduce the risk of neonatal sepsis, meningitis, and death. In fact, it is this population of women, those most heavily colonized with GBS, in whom neonatal transmission is most likely to occur. Therefore, a rapid assay like the one described here would have its greatest impact in this population. In summary, this study revealed that the use of mixed cultures, as well as the modifications made to the AP assay, accurately detected GBS in pregnant women in a timely manner. This approach, if implemented in the labor suite, would provide the clinician with a very practical test having specificity and sensitivity comparable with that of the gold standard, but whose results would be generated in significantly less time.

## ACKNOWLEDGMENTS

ACCUPROBE reagents and supplies were provided by GEN-PROBE, Incorporated.

We thank the Clinical Microbiology Laboratory for their efforts and support.

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