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Detection of Group B Streptococci in Lim Broth by Use of Group B *Streptococcus* Peptide Nucleic Acid Fluorescent In Situ Hybridization and Selective and Nonselective Agars[▽]

Naomi S. Montague,[1] Timothy J. Cleary,[1] Octavio V. Martinez,[2] and Gary W. Procop[3]*

Department of Pathology, Jackson Memorial Hospital, and the University of Miami Miller School of Medicine, Miami, Florida[1]; Department of Orthopedics, University of Miami Miller School of Medicine, Miami, Florida[2]; and Section of Clinical Microbiology, Department of Clinical Pathology, Cleveland Clinic, Cleveland, Ohio[3]

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The sensitivity, specificity, and positive and negative predictive values for the detection of group B streptococci from Lim enrichment broth with sheep blood agar (SBA), with selective *Streptococcus* agar (SSA), and by a peptide nucleic acid fluorescent in situ hybridization (PNA FISH) assay were as follows: for culture on SBA, 68.4%, 100%, 100%, and 87.9%, respectively; for culture on SSA, 85.5%, 100%, 100%, and 94.1%, respectively; and for the PNA FISH assay, 97.4%, 98.3%, 96.1%, and 98.9%, respectively.

Streptococcus agalactiae, which is a member of the group B streptococci (GBS), is a gram-positive bacterium that can cause invasive disease in newborns (1, 2, 8). The aim of this study was to compare two types of media and the peptide nucleic acid (PNA) fluorescent in situ hybridization (FISH) assay for their abilities to detect GBS from Lim enrichment broth.

Two hundred fifty-one genital swab specimens were obtained over a 6-week period from pregnant women as part of their routine diagnostic evaluation (8). All swabs were collected by the clinical staff by the use of routine protocols and sterile double swabs. For this institutional review board-approved study, the following assays were performed in a blinded manner (i.e., the results from each subsequently performed assay were unavailable to the person performing the next assay).

Culture on sheep blood agar (SBA) was performed by laboratory staff by the following protocol. The swab specimens (both swabs in the dual swab specimen were used) were directly inoculated onto an SBA plate (plate 1) and then into a vial of Lim selective enrichment broth (which was incubated in 5 to 10% CO_2 at 37°C). Beta-hemolytic colonies were sought the next day, and if any were present, they were subcultured for purity. At this time, the Lim broth was also subcultured onto an SBA plate (plate 2). On the following day (day 3), the cultures plates were again examined and tested to determine if they contained GBS (see below). If GBS were not found on plate 1, then suspect colonies were sought on plate 2; and if any were present, they were subcultured for purity. Any potential GBS from the purity plate that were derived from plate 2 (day 4) were tested to determine if they were GBS.

The Lim broth, following the initial phase of incubation described above, was also subcultured onto group A-selective *Streptococcus* agar (SSA) with 5% sheep blood (no. 221780; BD, Sparks, MD). The package insert indicates that this medium also supports the growth of GBS. This subculture was incubated as described above. On the first day following incubation, beta-hemolytic colonies were sought, and if any were present, they were subcultured for purity onto SBA (and incubated in 5 to 10% CO_2 at 37°C). These were subcultured for purity onto SBA rather than SSA because in our experience the performance of the GBS latex agglutination assay has been less reliable when it is done with colonies from the SSA plates than when it is done with colonies from SBA plates (data not shown). Following another 24 h of incubation, any colonies thought to possibly represent GBS were tested by a latex agglutination assay (Streptocard acid latex test, no. 240951; BD).

The PNA FISH assay (AdvanDx Inc., Woburn, MA) for GBS employs a PNA probe, which is a synthetic mimic of DNA. The molecular design of the PNA probes affords superior penetration of the probes through the cell wall and hydrophobic lipid bilayer of the target microorganism. This retains the morphological integrity of the microorganism and decreases the hybridization time (11, 14). This assay was performed according to the manufacturer's instructions. Briefly, 1 drop of Lim enrichment broth culture, following 24 h of incubation, was mixed with 1 drop of fixation solution on a microscope slide, and the slide was heated at 55°C for 20 min. The slides were then immersed in absolute ethanol for 10 min and air dried for 10 min. One drop of the GBS-specific PNA probe was added and allowed to hybridize in a slide warmer at 55°C for 30 min. The slides were washed in stringent buffer at 55°C for 30 min. The entire PNA FISH procedure required approximately 2 h. The slides were analyzed under an Olympus BH2-RFCA fluorescence microscope with a fluorescein isothiocyanate (FITC) filter and a FITC-Texas Red filter. The slides were analyzed at magnifications of ×200 and ×400. Positive (*S. agalactiae*) and negative (*S. pyogenes*) controls (50 total) were included with each run and consistently stained appropriately. The results were determined on the basis of the combination of the fluorescence and morphology results.

Aliquots of Lim broths that could not be determined to definitively contain GBS by the three test methods (i.e., those

* Corresponding author. Mailing address: Clinical Pathology, Cleveland Clinic, 9500 Euclid Avenue/L11, Cleveland, OH 44195. Phone: (216) 444-5879. Fax: (216) 444-4414. E-mail: procopg@ccf.org.

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specimens positive by only one of the three methods) were tested by two PCR assays. These were aliquots of the original Lim broth culture after 24 h of incubation that were stored at 6°C. Lysates of Lim broth were prepared by using sputum lysis reagent (Amplicor PCR diagnostics sputum specimen preparation; Roche Molecular Systems, Branchburg, NJ). One assay targeted the CAMP gene and used SYBR green dye I and melting curve analysis for amplicon detection (6). The post-amplification melting curve temperatures were determined for replicates of the positive control, and melting temperature bins were set to include the 50% quartile surrounding the mean. These were temperatures in the range from 81.1 to 84.4°C. Any Lim broth lysate that demonstrated amplification in the quantitation mode and a melting temperature whose peak was within the 81.1 to 84.4°C range was considered positive for the presence of *S. agalactiae* by PCR. The other PCR assay was the FDA-approved SmartCycler Smart GBS assay (Cepheid, Sunnyvale, CA), which was utilized according to the manufacturer's instructions. Positive and negative determinations were made by use of the proprietary FDA-approved software. Positive (*S. agalactiae*) and negative (*S. pyogenes*) controls were included with each PCR run, and the results were appropriate and concordant between assays.

A Lim broth sample was defined as containing GBS (i.e., a true-positive result) if GBS was recovered or its presence was demonstrated by two or more test methods (with SBA and SSA and by the PNA FISH assay). A Lim broth sample was defined as truly negative if all three methods were GBS negative. When one of three test methods recovered GBS, the PCR assays were performed (see above). Two positive PCR results defined these Lim broths as true positive, whereas negative PCR results defined the Lim broth as true negative. Accordingly, the adjudication of discrepant test results was defined on the basis of the PCR results. This design is similar to the discrepancy analysis method described by von Hal et al. (13).

Seventy-six specimens were true positive, whereas 175 were true negative. The prevalence of GBS in our study group was 30.3% (76/251 samples), which is the upper limit of previously published values (4 to 30%) but which is consistent with our patient population (8, 10). Ten specimens required discrepancy analysis, resulting in three false-positive and two false-negative PNA FISH results.

The sensitivity, specificity, and positive and negative predictive values for each method of detection described were as follows: for culture on SBA (routine method), 68.4%, 100%, 100%, and 87.9%, respectively; for culture on SSA, 85.5%, 100%, 100%, and 94.1%, respectively; and for the PNA FISH assay, 97.4%, 98.3%, 96.1%, and 98.9%, respectively.

This study demonstrates that the PNA FISH assay is a more sensitive method for the detection of GBS in Lim enrichment broth than Lim broth subculture onto either SBA or SSA. Importantly, when GBS are cultured from the Lim enrichment broth, the process can take 3 to 4 days, and the technologist must handle the culture up to five times. This is in contrast to the PNA FISH procedure, which requires approximately 2 to 3 h and which requires the technologist to handle the specimen only twice. Several studies have evaluated various culture, antigen, and molecular assays for the detection of GBS; the PNA FISH assay appears to be comparable to other molecular methods for the detection of GBS (Table 1).

TABLE 1. Comparison of various methods for the detection of GBS

Test (reference)	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)
Culture				
Routine culture (6)	42.3	100	100	74.3
Routine culture (3)	54.3	97.1	79.7	91.1
Routine culture (this study)	68.4	100	100	87.9
Selective Strep (this study)	85.5	100	100	94.1
Antigen detection, PathoDx (6)	57.3	99.5	98.5	79.5
Molecular methods				
cfb gene (6)	75.3	100	100	87.1
GBS AccuProbe assay (15)	90.1	97.5	91.1	97.2
GBS LightCycler assay with MagNA Pure Compact (12)	92.5	99.1	97.4	97.5
IDI-Strep B assay (3)	94.0	95.9	83.8	98.6
PCR for *scpB* gene (6)	99.6	100	100	99.7
GBS LightCycler assay with MagNA Pure LC (12)	100	96.9	88.6	100
GBS PNA FISH assay (this study)	97.4	98.3	96.1	98.9

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

The SBA culture sensitivity of 68.4% is consistent with that reported in other studies (Table 1) (3, 6). The low sensitivity may have been due to the presence of a nonhemolytic GBS phenotype or the masking of the presence of GBS by more rapidly growing microbiota, including intensely beta-hemolytic bacteria, such as *S. aureus*. The sensitivity of a culture-based method was improved if SSA (85.5%) was used for subculture of the Lim broth. When both types of media rather than either one alone were used for Lim broth subculture, then the sensitivity, specificity, and positive and negative predictive values were 93.4%, 100%, 100%, and 97.2%, respectively. The sensitivity achieved when SSA and SBA were used in combination (93.4%) approached that of the GBS PNA FISH assay with Lim broth (97.4%). However, the time and labor (i.e., handling) associated with the culture-based method with both SSA and SBA were considerably greater than those associated with the GBS PNA FISH assay.

In summary, the PNA FISH assay is a molecular identification method that detects a specific rRNA target for microbial identification (4, 5, 7, 9, 16). Use of the PNA FISH assay for the detection of GBS from Lim broth culture showed an increased sensitivity, required decreased specimen handling, and had a shorter turnaround time than culture. Culture-based strategies are highly specific but may have poor sensitivity. If culture-based methods are used, this study suggests that subculture of the Lim broth onto SSA is superior to subculture onto SBA, but the superior strategy for culture would be to employ both these media for subculture following Lim broth enrichment.

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