

Detection of Group B *Streptococcus* Bacteria in LIM Enrichment Broth by Peptide Nucleic Acid Fluorescent *In Situ* Hybridization (PNA FISH) and Rapid Cycle PCR[∇]

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The sensitivity, specificity, and negative and positive predictive values for the detection of group B *Streptococcus* (GBS) in 206 LIM enrichment broths by the use of subculture, GBS peptide nucleic acid fluorescent *in situ* hybridization (PNA FISH), and GBS PCR were 96.9%, 100%, 98.6%, and 100%; 98.4%, 100%, 99.3%, and 100%; and 100%, 100%, 100%, and 100%, respectively.

Streptococcus agalactiae, a group B *Streptococcus* (GBS), is a Gram-positive bacterium that may cause invasive disease in newborns (1). The detection of GBS recto-vaginal colonization is important during pregnancy, since prophylactic measures may be taken to diminish the likelihood of early-onset GBS neonatal disease. The screening of pregnant women for the presence of GBS is recommended by the Centers for Disease Control and Prevention (CDC) (1).

The presence of GBS has been traditionally detected through culture, the sensitivity of which is notably improved through broth enrichment. A number of methods, both culture-based and molecular, have been described over the past decade for the detection of GBS (2, 4, 5, 7). These include various enrichment broths and agar-based culture methods, antigen testing, nucleic acid amplification, and, more recently, peptide nucleic acid fluorescent *in situ* hybridization (PNA FISH). LIM enrichment broth has been used prior to culture, PNA FISH, and PCR for the enhanced detection of GBS, but a comparison of PNA FISH and PCR following LIM broth enrichment has not been performed. We, therefore, sought to perform this comparison.

Two hundred six LIM enrichment broth samples that had been inoculated with swabs from patients routinely screened for the presence of GBS were used for this study following institutional review board (IRB) approval. This study occurred over a 1-month period; 190/206 swabs were labeled rectal/vaginal, 11 vaginal, and five genital, without further designation. Copan liquid Amies double plastic swabs for collection were used per routine. LIM broths were incubated for a minimum of 18 h. Aliquots of LIM broth were tested for the presence of GBS by rapid cycle PCR (Smart GBS; Cepheid, Sunnyvale, CA), GBS PNA FISH (AdvanDx, Inc., Wolburn, MA), and subculture. Both molecular assays were performed according to manufacturers' recommendations, as briefly described below.

The GBS PNA FISH assay (AdvanDx Inc., Wolburn, MA) was performed using one drop of LIM broth that was mixed

with one drop of fixation solution on a microscope slide and heated at 55°C for 20 min. Slides were then immersed in absolute ethanol for 10 min and air dried for 10 min. One drop of GBS PNA probe was added and hybridized in a slide warmer at 55°C for 30 min. Slides were washed in stringent buffer at 55°C for 30 min. The entire PNA FISH procedure required approximately 2 h to perform. Slides were analyzed using an Olympus BX41 fluorescence microscope with a fluorescein isothiocyanate (FITC)-Texas Red filter. Slides were analyzed using a 60× oil objective. Positive results were based on interpretation of a combination of fluorescent signal and morphology (i.e., chaining cocci). The interpretation phase required approximately 30 min. Positive (*S. agalactiae*) and negative (*Streptococcus pyogenes*) controls were included with each PNA FISH run.

Briefly, Smart GBS assay is supplied with a lysis reagent (glass beads), diluent, and master mix. LIM broths are incubated for a minimum of 18 h. Growth in the LIM broth was pelleted by adding 200 microliters to the lysis reagent (glass beads) and centrifuged at 10,000 × g for 3 min; the supernatant was discarded. Seven hundred fifty microliters of diluent was added, and then the mixture was shaken vigorously for 5 min to disrupt the cells. The resulting lysate was added to the prepared master mix and transferred to a SmartTube. The SmartTube was then placed into the SmartCycler Dx system for amplification and detection. Positive (i.e., noninfectious DNA from *S. agalactiae*) and negative (noninfectious DNA from *Streptococcus pneumoniae*) controls were included in the kit and tested with each PCR run.

Additionally, all LIM enrichment broths were cultured to aid in the resolution of discrepancies that could occur between PCR and PNA FISH results. The presence or absence of GBS was determined by LIM broth subculture; this was performed using standard methods (i.e., subculture with sheep's blood agar and identification using GBS antigen-specific latex agglutination).

When GBS was detected by at least two of the three methods, the specimen was considered truly positive; specimens wherein all three tests were negative were considered truly negative for the presence of GBS (Table 1). A similar method of discrepancy analysis and resolution has been described by van Hal et al. (6).

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TABLE 1. Performance characteristics of PCR, PNA FISH, and subculture for the detection of GBS in LIM enrichment broths^a

Detection method	No. of:				Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
	True positives detected	False positives	True negatives appropriately characterized	False negatives				
GBS PCR	64/64	0	142/142	0	100	100	100	100
GBS PNA FISH	63/64	0	142/142	1	98.4	100	99.3	100
LIM broth subculture	62/64	0	142/142	2	96.9	100	98.6	100

^a NPV, negative predictive value; PPV, positive predictive value.

Of the 206 LIM broth samples tested, 64 were characterized as containing GBS, whereas 142 were characterized as negative for the presence of GBS. Sixty-one of the 64 (95.3%) LIM broth samples that contained GBS were concordant for the presence of GBS by all three methods. Two specimens that were deemed truly positive were positive by concordant PCR and PNA FISH but were negative by culture (i.e., false-negative culture). One specimen was positive by PCR and culture, but negative by PNA FISH (i.e., false-negative PNA FISH); interestingly, this culture had only a single colony of GBS present in the LIM broth subculture. There were 142 LIM broth samples that were negative for the presence of GBS by all three methods. The sensitivity, specificity, negative and positive predictive values for the GBS PNA FISH assay and LIM broth subculture were 98.4%, 100%, 99.3%, and 100% and 96.9%, 100%, 98.6%, and 100%, respectively; these values for the GBS PCR were all 100%.

A variety of culture-based methods have been used for the detection of GBS in pregnant women, many of which have included the use of broth enrichment. We have previously demonstrated the utility of LIM broth enrichment prior to PCR (data not shown) and demonstrated the feasibility of performing GBS PNA FISH on LIM enrichment broth (3). The two molecular methods evaluated here demonstrated similar performances. There have been several studies that have demonstrated the utility of PCR for detecting GBS. Only our previous study, which compared *in situ* hybridization with two methods of culture following LIM broth enrichment, has demonstrated the utility of PNA FISH for the detection of GBS. This study suggests that PNA FISH is a reasonable alternative to culture or PCR for the detection of GBS following LIM broth enrichment. The sensitivity of LIM broth subculture in this study was substantially higher than in other reports. It is possible that this is because the medical technologist who performed this study was very experienced and thorough; in addition, the examination of the cultures was performed in a research setting, without the pressures experienced by a busy routine bench technologist. This does, however, suggest that

LIM broth subculture may not be as poor as previously described for the recovery of GBS; additional training and a thorough examination of the subculture may increase the yield of GBS from LIM broth subculture. Although PCR was slightly more sensitive, thermocycling instrumentation is not needed for PNA FISH, and issues with amplicon contamination are not applicable to FISH technology, albeit these are greatly reduced with modern rapid cycle PCR methods.

In conclusion, the combination of LIM broth enrichment and a highly sensitive molecular diagnostics method, such as rapid cycle PCR or PNA FISH, is an acceptable alternative to LIM broth subculture. Additional studies may establish that molecular diagnostic methods represent the new gold standard for the detection of GBS following LIM broth enrichment.

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