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 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 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, the presence of *GBS* is recommended by the Centers for Disease Control and Prevention (CDC) (1). The use of LIM broth for the detection of *GBS* has been described by D. A. Wilson, G. S. Hall, and G. W. Procop*.

A total of 206 LIM enrichment broth samples were cultured by subculture (i.e., subculture with sheep’s blood agar and identification using *GBS* antigen-specific latex agglutination). The presence or absence of *GBS* was determined by LIM broth subculture; this was performed for the detection of *S. agalactiae* and negative (noninfectious DNA from *S. agalactiae*) and negative (noninfectious DNA from *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).

*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 important during pregnancy, since prophylactic measures may be taken to diminish the likelihood of early-onset GBS neonatal disease. 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).
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 for 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 molecular methods (IDI-MRSA PCR assay and GenoType MRSA Direct PCR assay) with three selective MRSA agars (MRSA ID, MRSA Direct ID, and Geno-probe AccuProbe group B streptococcus culture identification test with CHROMagar MRSA) for use with infection-control swabs. J. Clin. Microbiol. 45:2486–2490.

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.

REFERENCES