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Evaluation of GBS Detect™ and PNA FISH™ for the Detection of Group B Streptococci in Subcultures of LIM Broth

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Revised Abstract

Group B Streptococci (GBS) remains a leading cause of morbidity and mortality in newborn populations. The Centers for Disease Control and Prevention (CDC) recommends the screening of all pregnant women at 35 to 37 weeks gestation. A vaginal and rectal BBL™ CultureSwab™ (Becton Dickinson and Company, MD) were collected and inoculated to an enrichment broth, BBL™ LIM broth (Becton Dickinson and Company, MD) and a BBL™ TSA II (Becton Dickinson and Company, MD) 5% Sheep Blood Agar Plate (primary BAP). Subculture of the broth is routinely performed after 24 hours of incubation, when GBS is not detected on the primary BAP. For this study, subculture of the LIM broth was performed regardless of the results on the primary plate. Non-beta hemolytic strains of GBS may be indistinguishable from other types of bacteria present on conventional media, such as a BAP. To prevent false-negative results, the GBS Detect™ (Hardy Diagnostics, CA) media incorporates supplements to enhance the hemolysis of weakly or non-hemolytic strains. The PNA FISH™ (AdvanDx, Inc., MA) method also detects hemolytic and non-hemolytic strains of GBS. The goal of this study was to compare conventional subculture from LIM broth to BAP and GBS Detect™ with the PNA FISH™ test. A total of 45 vaginal-rectal specimen culture swabs were inoculated to a BAP and LIM broth. After 24 hours of incubation, the LIM broth was subcultured to BAP and GBS Detect™ media and a slide was prepared for PNA FISH™ testing. Sixteen specimens were positive for GBS using one or more methods **(35.6%)**. One specimen was positive for beta-hemolytic GBS after 48 hours on the primary BAP and by all other methods. Another specimen was positive on the GBS Detect™ plate only. One specimen was positive on the BAP and GBS Detect™ plate, but negative using the PNA FISH™ method. In conclusion, use of the GBS Detect™ medium provides increased sensitivity for the detection of GBS compared to the other methods, and especially for the detection of non-beta-hemolytic GBS.

Introduction

Approximately 10 to 30% of pregnant woman are colonized with GBS in the vagina or rectum[3]. The Centers for Disease Control and Prevention (CDC) recommends vaginal and rectal screening of all pregnant women at 35 to 37 weeks of gestation by using an enrichment broth followed by subculture to a blood agar plate (BAP).[4] A limitation of subculturing to a BAP is that non-hemolytic strains of GBS may be missed due to the presence of other non-hemolytic vaginal flora or masking by other beta-hemolytic organisms.

At least 2% of GBS isolates are non-hemolytic[1]. The Hardy GBS Detect™ (Hardy Diagnostics, CA) plate allows for the detection of non-hemolytic strains and beta-hemolytic colonies masked by other non-GBS bacteria. The goal of this study was to compare conventional subculture of LIM broth to a blood agar plate with subculture to a GBS Detect™ plate and the GBS PNA FISH™ (AdvanDx, Inc., MA) test.

Materials And Methods

Study duration

This study was conducted between April 2010 and July 2010.

Sample collection

Vaginal and rectal swabs from pregnant women at 35 to 37 weeks gestation were collected using the dual BBL™ CultureSwab™ (Becton Dickinson and Company, MD) containing liquid Stuart transport medium. The specimens were transported to the laboratory at room temperature within 24 hours of collection. Both swabs were used to first inoculate a BBL™ TSA II (Becton Dickinson and Company, MD) 5% Sheep Blood Agar Plate (primary BAP), followed by inoculation to the BBL™ LIM broth (Becton Dickinson and Company, MD).

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Microbiological Analysis

LIM Broth

For this study, subculture of the LIM broth was performed even when GBS was detected on the primary BAP.

- All LIM Broths incubated for 18 to 24 hours at 37 °C in 5 to 7% CO_2 with loose caps.
- After incubation, all LIM Broths were subcultured to a BAP and a GBS Detect™ plate.[2] A GBS PNA FISH™ slide was also prepared.[1]
- Both subcultured plates were then incubated for 18 to 24 hours at 37 °C in 5 to 7% CO_2.[2]
- All colonies of GBS were confirmed by latex agglutination using the BD BBL™ Streptocard™ Acid Latex Test (Becton Dickinson and Company, MD).

GBS peptide nucleic acid (PNA) fluorescent in-situ hybridization (FISH) assay[1]

- A slide was prepared using 10 to 50 µL of the incubated LIM broth samples mixed with one drop of Fixation Solution.
- Smears were fixed by allowing them to air-dry, followed by flame-fixation.
- Slides were immersed in 96% ethanol for 5-10 minutes and air-dried.
- One drop of GBS PNA hybridization solution was added to the slide well, along with a coverslip. The slide was incubated for 30 ± 5 min. at 55 ± 1 °C.
- Slides were immersed in a preheated Wash solution at 55 °C to remove the coverslip.
- The slides were again incubated for 30 ± 5 min. at 55 ± 1 °C and allowed to air dry.
- One drop of Mounting Medium was added to the slides, along with a coverslip.
- Slides were examined within 2 hours using a fluorescence microscope.
  Positive for *S. agalactiae*: multiple bright green fluorescent cocci in multiple fields
  Negative: non-fluorescent.

RESULTS

Table 1. Result Summary

	Total	LIM + SBA	LIM + GBS Detect	LIM + PNA FISH
No. of Specimens	45	45	45	45
GBS Detected	16	15	16	14
Negatives	29	30	29	31
Sensitivity (%)		93.8	100	87.5
Specificity (%)		100	100	100
PPV (%)		100	100	100
NPV (%)		96.7	100	93.4

- Overall 35.6 % of specimens included in the study were positive for GBS.
- Among the positive samples, one non-hemolytic GBS isolate was detected by GBS Detect™ only.

Discussion

Of the 45 samples positive for GBS, 44 (97.8%) were identified as hemolytic on both GBS Detect™ and conventional plating methods. One non-beta hemolytic isolate was identified using GBS Detect™ only. One isolate did not appear beta-hemolytic on the BAP subculture, but appeared beta hemolytic on the primary BAP after 48 hours of incubation. The specimen was positive using the GBS Detect™ and PNA FISH™ methodologies. One specimen grew a slightly hemolytic colony on the GBS Detect™, but was negative by all other methods. This isolate was confirmed negative using latex agglutination. Only colonies that have large and clear zones of hemolysis on GBS Detect™ should be evaluated for GBS according to the manufacturer's instructions.[2] Other bacteria, such as *Enterococcus faecalis* can produce faint or incomplete zones of hemolysis, and is one of the recommended GBS Detect™ QC strains.

Conclusion

Based on the results of this study, GBS Detect™ is a reliable method to increase the sensitivity of traditional culture methods for GBS detection, especially for non-beta hemolytic strains. The GBS Detect™ method is significantly less expensive and much less labor-intensive than the PNA FISH™ method. Since GBS screening is recommended at 35-37 weeks of gestation, and not at the time of delivery, a rapid test is not necessary for patient management.

References

1. AdvanDx, Inc. *GBS PNA FISH® Streptococcus agalactiae* Culture Identification Kit Package Insert. May 15, 2009 Rev. C
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3. Overman, S.B., et al., Evaluation of methods to increase the sensitivity and timeliness of detection of *Streptococcus agalactiae* in pregnant women. *J Clin Microbiol*, 2002. 40(11):4329-31.
4. Verani, J., et al., Prevention of Perinatal Group B Streptococcal Disease. Revised Guidelines from CDC. *MMWR Recomm. & Rep.* 2010. 59(RR10):1-32.

Streptococcus agalactiae ATCC® 13813 growing on GBS Detect™

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