

Evaluation of GBS Detect™ as a Subculture Medium from LIM Broth for the Detection of Group B Streptococcus: A Multi-Centric Study

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A. Robertson¹, N. A. Ledebor², M. L. Faron², B. W. Buchan², J. Connolly², H. Seliger², L. Westblade¹,
A. Schuetz^{1,5}, K. Fauntleroy¹, M. Sarina³, D. Wilson⁴, G. W. Procop⁴, S. Jenkins¹

¹Cornell Medical Center, New York, NY; ²Medical College of Wisconsin, Milwaukee, WI; ³Central Coast Pathology, San Luis Obispo, CA, ⁴Cleveland Clinic, Cleveland, OH; ⁵Current Affiliation – Mayo Clinic, Rochester, MN

Abstract

Background: The purpose of this study was to evaluate the performance of GBS Detect™ for the detection of Group B Streptococcus (GBS) after LIM Broth enrichment compared to LIM Broth cultured onto sheep blood agar. GBS Detect™ induces beta-hemolysis in non-hemolytic GBS strains, which encompass approximately 3-5% of all GBS and are often missed by traditional culture methods.

Methods: Any vaginal/rectal swab specimen from asymptomatic patients screened for GBS colonization submitted to the clinical laboratory for regular culture were enrolled and tested at four geographically distinct sites in the United States. Each site enrolled specimens that were stored in a liquid-based transport system or a sponge-based transport system and kept refrigerated until processed. Specimens were processed by inoculating LIM broth with 30µl of either the liquid from specimens stored in a liquid-based system, or the residual liquid collected from the sponge if the specimen was stored in a sponge-based system. After 24 hours of incubation, LIM broth tubes were subcultured to GBS Detect™ and to sheep blood agar. All isolates were confirmed via traditional methods such as Gram-stain, followed by catalase reaction and latex agglutination.

Results: A total of 884 specimens across the four sites were enrolled for this study from November 2015 to October 2016. There were 245 isolates recovered on sheep blood agar and 257 isolates recovered by GBS Detect™. GBS Detect™ demonstrated 99.6% sensitivity and 98.0% specificity. There were 13 specimens that were positive by GBS Detect™ and negative by the reference method. In addition, 7 GBS strains were recovered in this study that exhibited no hemolysis on sheep blood agar via the reference method, but that appeared beta-hemolytic on GBS Detect™. This demonstrates the ability of GBS Detect™ to easily recover non-hemolytic GBS strains that could potentially be overlooked in a traditional LIM Broth to sheep blood agar method.

Conclusion: This study shows the equivalency of GBS Detect™ and the performance advantages over a traditional LIM broth subcultured to sheep blood agar culture method.

Introduction

Approximately 10-35% of women are asymptomatic carriers of Group B Streptococci (GBS) in the genital and gastrointestinal tracts.(1) Group B Streptococci (GBS) remains a leading cause of serious illness and death in newborn populations and, therefore, the detection of Group B Streptococci in the vaginal-anorectal area is critical to the prevention of neonatal GBS disease. Several surveys have been conducted that show the incidence of neonatal sepsis and meningitis due to GBS is currently 0.5-3 cases per 1,000 live births, although there are substantial geographical and racial differences.(2) The case-fatality ratios are now declining due to prompt recognition and proper treatment.(3) The Centers for Disease Control and Prevention (CDC) recommends the screening of all pregnant women for vaginal and rectal Group B Streptococci colonization between 35 and 37 weeks of gestation using an enrichment broth followed by subculture.(4)

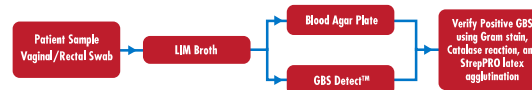
GBS Detect™ enhances hemolysis of β-hemolytic and non-hemolytic GBS, allowing for increased ease of detection of non-hemolytic GBS. With increased visibility on the GBS Detect™ plate due to enhanced hemolysis, GBS can be isolated and differentiated from a mixed culture with more ease than from a standard blood plate where mixed culture can often hide a positive GBS result or require additional testing.

Methods

A total of 884 vaginal/rectal swab specimens were submitted to the clinical microbiology laboratory for routine assessment of GBS colonization from 11/2015 to 10/2016 as part of a multi-centric study. Specimens stored in a liquid-based transport system (i.e. Eswab™ or TransPRO™) were kept refrigerated for a maximum of 5 days if not immediately processed. Specimens stored in a sponge-based transport system (i.e., HealthLink TransPorter®) were kept refrigerated for a maximum of 4 days if not immediately processed. In order to use sponge-based transport systems, the liquid was squeezed from the sponge using a hemostat on the outside of the transport tube, and then aseptically transferred to a sterile vial for easy pipetting.

30 µl of vaginal specimen submitted for GBS testing was inoculated to LIM broth and incubated 18-24 hours at 35°C. After incubation, the broth was subcultured to 5% Sheep Blood Agar and GBS Detect™. The plates were incubated aerobically at 35°C for 24 hours. Colonies that were recovered on Blood Agar and GBS Detect™ were confirmed as GBS by gram stain, a catalase reaction, and Lancefield group latex agglutination. Colonies that were β-hemolytic on Blood Agar, gram positive, catalase negative, and Group B Streptococci by latex agglutination were considered positive "β Group B Strep". Colonies that met the same criteria yet were non-hemolytic on blood agar were considered positive "NH Group B Strep."

All discrepant isolates were frozen in CryoSavers™ with Brucella Broth and returned to Hardy Diagnostics for testing. The identity of each isolate was confirmed (β Group B Streptococci, NH Group B Streptococci, or non-Group B Streptococci). Once the identity was confirmed, positive organisms (β Group B Streptococci or NH Group B Streptococci) were tested at LoD (10³ CFU/mL) in donated negative-vaginal rectal matrix for their recovery on Blood Agar and GBS Detect™.



Participating Study Sites

Site	Site Name	Total Specimens Enrolled
Site 1	Central Coast Pathology Laboratory San Luis Obispo, CA	195 Specimens Enrolled
Site 2	Weill Cornell Medical College New York, NY	221 Total Specimens Enrolled
Site 3	Cleveland Clinic Cleveland, OH	183 Total Specimens Enrolled
Site 4	Medical College of Wisconsin Milwaukee, WI	285 Total Specimens Enrolled

Results

Table 1. LIM Reference Method vs. GBS Detect™ Pre-Discrepant Analysis

Site	TP	FP	FN	TN	Sensitivity	95% CI	Specificity	95% CI
CCP	52	2	0	141	100.00	93.1	100.00	98.6
NY	73	7	1	140	98.6	92.7	99.8	95.2
CC	78	1	0	104	100.00	95.3	100.00	99.0
MCW	41	3	0	241	100.00	91.4	100.00	98.8
Overall	244	13*	1**	626	99.6	97.7	99.9	98.0

*There were 13 False Positives overall. All isolates produced the expected hemolysis reaction on GBS Detect upon subculture from LIM broth inoculated at LoD. They were confirmed to be β Group B Strep and considered true positives in discrepant analysis.

**There was 1 False Negative overall. The isolate was confirmed as β Group B Strep and subsequently confirmed as positive on GBS Detect™ from LIM broth inoculated at the Limit of Detection.

Non-hemolytic GBS on regular Blood Agar

Non-hemolytic GBS with enhanced hemolysis on GBS Detect™

Conclusions

- GBS Detect™ demonstrated 99.6% sensitivity and 98.0% specificity compared to Blood Agar.
- All 13 false positive isolates were confirmed to be Group B Strep that the LIM-BAP reference method did not recover.
- Seven GBS strains recovered in this study were non-β hemolytic on blood agar, but showed clear β-hemolysis on the GBS Detect. These types of strains could be easily missed by the reference method.
- Results of this evaluation support the usage of more GBS-specific products in lieu of subculture to blood agar.

References

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