Evaluation of StrepB Carrot Broth™ and LIM Broth for Recovery of Group B Streptococci

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Abstract

Group B Streptococci (GBS) remains one of the leading causes of sepsis and meningitis in newborns despite recent advances in the prevention of neonatal group B streptococcal (GBS) disease. Although the current gold standard method is the LIM broth method, as recommended by the Centers for Disease Control and Prevention (CDC), several alternative methods such as StrepB Carrot Broth™ and Granada™ Biphasic broth have been developed with comparable sensitivity, specificity, and reduced turnaround time. The goal of this study was to evaluate and compare the new commercially available method - StrepB Carrot Broth™ against the LIM Broth protocol.

GBS and GAS reference strains and Eighteen clinical isolates were tested with the two methods at three dilutions (1.5 x 10^4 CFU/mL, 1.5x10^2 CFU /mL, 1.5x10^1 CFU / mL) for evaluating the detection of limit. 100 clinical specimens were collected and tested in parallel by both StrepB Carrot Broth™ and traditional LIM broth protocols. Both the methods presented 100% sensitivities in dilutions of 1.5 x 10^4 CFU /mL and 1.5x10^2 CFU /mL. At a concentration of 1.5x10^1 CFU /mL, StrepB Carrot Broth™ was able to recover GBS in 16 of 19 of samples, while LIM Broth was able to cover GBS in 15 of 19 samples. 20 (20.0%) clinical specimens were detected as positive for GBS by StrepB Carrot Broth™ and 19 (19.0%) by LIM Broth enrichment protocols while direct plating only detect 13.

Based on the evaluation, the StrepB Carrot Broth™ 24 hour broth method was shown to be more sensitive comparing to LIM broth and has equivalent clinical performance against LIM broth protocol. Besides, the application of the StrepB Carrot Broth™ can shorten the time to identify beta hemolysis GBS from at least 48 hours to 24 hours without further identification testing such as streptococcal grouping latex agglutination test, presumptive identification testing by CAMP test, and etc.. Strep B Carrot Broth™ can be employed as reliable method for detection of beta-hemolytic Group B Streptococci.

Introduction

Approximately 10% to 30% of pregnant women are colonized with GBS in the vaginal-rectal region. Neonates may acquire GBS from their mothers during delivery and may lead to sepsis, meningitis, pneumonia, or other serious conditions. To prevent the possible GBS infections in neonates, the US CDC recommends screening all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation. Once women identified as carriers of GBS, neonates GBS infection can be prevented by administering interpartum antibiotics during delivery. US CDC has measured that the incidence of GBS illness in neonates has been greatly reduced.

The current recommended standard method for GBS detection is to inoculate specimen swab into LIM broth and followed by subculture onto a blood agar plate. Recently, alternative methods have been developed for easier identifying GBS and shorten the turnaround time. Among the alternative methods, the most straightforward method of detection is based on pigment development as seen in Granada Agar. The production of orange to brick red pigments is a unique characteristic of hemolytic GBS due to reaction with ingredients such as starch, proteose peptone, serum, and folate pathway inhibitors.

StrepB Carrot Broth™ (Hardy Diagnostics, Santa Maria, CA) is a tube assay designed to surpass all currently available culture methods in sensitivity and specificity via adding additional enrichment factors and supplements for enhancing the pigment intensity. The intent of this evaluation is to compare the performance of StrepB Carrot Broth™ and LIM broth for recovery of Group B streptococcus.

Materials and Methods

Sample Preparation and Inoculation

Stage I

- 18 Group B Streptococcus isolates and reference strains of Group B Streptococcus and Group A Streptococcus were analyzed in this evaluation.
- A suspension equivalent to 0.5 McFarland was prepared for each isolate. Serial dilution equivalent to 1.5 x 10^4CFU/mL through 1.5 x 10^1 CFU/mL was prepared.
- An amount of 100µL of each dilutions was inoculated to StrepB Carrot Broth™ and LIM Broth.
- StrepB Carrot Broth™ were inoculated, incubated, and interpreted according to manufacturer's recommendations.

Stage II

- 100 clinical GBS screening specimens were collected since June 1, 2011.
- Specimens were collected using swabs, inoculated on blood agar plate, and vortexed in saline. The suspension was evenly pipetted into StrepB Carrot Broth™ and LIM Broth.
- StrepB Carrot Broth™ and LIM Broth were then subcultured on to blood agar plate after overnight culturing.

Interpretation

StrepB Carrot Broth™

- All tubes that turned orange to red were considered to be positive.
- All tubes that did not turn orange to red color were considered as negative.

LIM Broth

- Samples that yielded GBS after subculture to blood agar plate and identified by latex assay for serotyping were considered as positives.
- Samples that did not yield GBS after subculture to blood agar plate were considered as negatives.

Results

Stage I

- Both methods resulted in 100% sensitivity in concentrations of 10^4 CFU/mL and 10^2 CFU/mL.
- At concentration of 10 CFU/mL, StrepB Carrot Broth™ was able to recover GBS in 16 of 19 (84%) of samples followed by the LIM Broth method (recover GBS in 15 of 19, 79%).

Results (Continued)

Stage II

- 20 of 100 (20%) of clinical specimens tested were positive for GBS.
- StrepB Carrot Broth™ successfully detected all GBS strains with 18 strong positive reactions and 2 weak positive reactions. All positive reactions are confirmed as growth of GBS after subculture onto blood agar plate.
- Direct plating was failed to detect 7 GBS isolates.
- No non-hemolytic GBS strains was found during the evaluation period of time.

Table 1.

Stage I Comparison of Sensitivity between StrepB Carrot Broth™ and LIM Broth on Given Isolates

Group B Streptococci Recovery Rate			
	10^4 CFU/mL	10^2 CFU/mL	10 CFU/mL
StrepB Carrot Broth™	100% (19/19)	100% (19/19)	84% (16/19)
LIM Broth	100% (19/19)	100% (19/19)	79% (15/19)

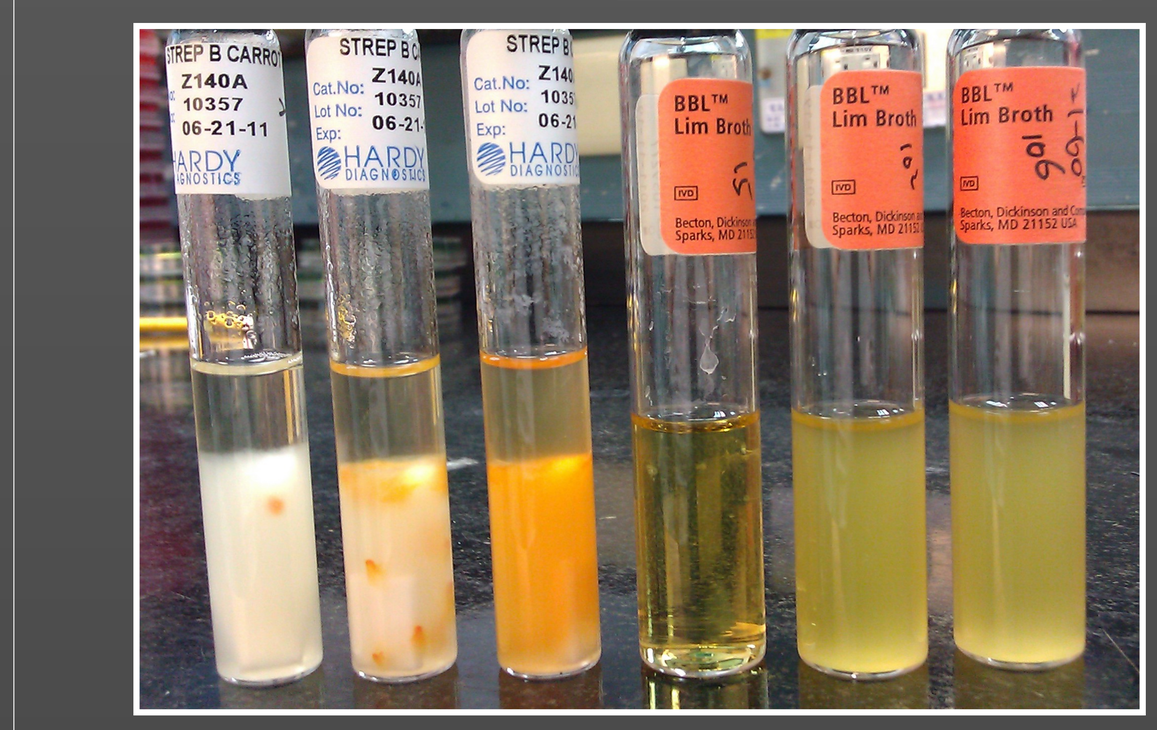

Table 2.

Stage II Comparison of Sensitivity among StrepB Carrot Broth™, LIM Broth, and Blood Agar Plate on Clinical Specimens Tested

Group B Streptococci Recovery Rate			
	StrepB Carrot Broth™	LIM Broth	Blood Agar Plate
GBS Positive	20	19	13
GBS Negative	80	81	87
Total	100	100	100

Discussion

- Based on the Stage I findings, StrepB Carrot Broth™ are very reliable in GBS detection considering the fact that most clinical specimens, when positive for GBS, will most likely have a GBS load above 10^3 CFU/mL.
- In simulations of low GBS count, StrepB Carrot Broth™ was more sensitive than LIM Broth in this evaluation.
- Beta-hemolytic isolates of GBS were successfully and easily detected by StrepB Carrot Broth™. These isolates could easily be overlooked by inexperienced microbiologists using other methods.
- StrepB Carrot Broth™ is easy to read-out based on color development and do not require further subculture or identification testing for positive samples.
- StrepB Carrot Broth™ was100% sensitive and specific against all isolates tested.
- StrepB Carrot Broth™ is an improvement over conventional methods by increasing sensitivity, decreasing turn around time, and cutting costs by reducing need for further testing.
- Non-hemolytic GBS was not found during the evaluation period of time, however, to investigate the clinical non-hemolytic GBS positive rate, more clinical specimens should be evaluated.
- StrepB Carrot Broth™ revealed the best performance among the three culture medium in this evaluation.

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