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C-109Evaluation of StrepB Carrot Broth and LIM Broth Methods for Recovery of Group B Streptococci (GBS). Results of a Multi-Center Trial

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Revised Abstract

Detection of GBS in the vaginal-anorectal area is critical for the prevention of neonatal Group B Streptococci (GBS) disease. Several microbiological assays employing different methods have been previously conducted worldwide, however newer approaches may be needed to increase the sensitivity, reduce costs, and shorten the turnaround time for the detection of GBS. The goal of this study was to evaluate and compare two commercially available methods: StrepB Carrot Broth[™] and the Centers for Disease Control and Prevention (CDC) recommended LIM Broth protocol in a multicenter clinical trial format. A total of 932 vaginal-rectal specimens from eight laboratories across the nation were tested in parallel by both methods. One hundred and ninety three (20.7%) were detected as positive for GBS. Of the positive samples, 86.4% (166) were simultaneously detected by both methods, 10.4% (20) were detected only by the Carrot Broth method, and 3.6% (7) by the LIM broth method only. The overall the sensitivity/specificity for Carrot Broth was 96.8%/100%, and for the LIM Broth method the overall sensitivity/specificity was 93.2%/99.4%. One specimen was correctly identified by the Carrot Broth as a non-GBS and considered as false positive on LIM Broth since it was latex agglutination positive for GBS. Further testing confirmed this isolate as Streptococcus porcinus. This species is known to cross-react with group B antisera. Overall, StrepB Carrot Broth[™] demonstrated greater sensitivity and shorter turnaround time than the LIM Broth method

Furthermore, the StrepB Carrot Broth[™] method uses only one tube of media for positives, without the need for further testing or subculture. Based on these findings, StrepB Carrot Broth[™] can be employed as a reliable method for GBS detection.

Introduction

Intrapartum colonization of group B Streptococci (GBS) is a major risk factor for earlyonset neonatal sepsis and remains as a significant source of morbidity in perinatal care despite great strides in prevention and detection. The gastrointestinal tract serves as the natural reservoir for GBS and is the likely source of vaginal colonization. Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum [1]. GBS colonization can be transient, chronic, or intermittent. Although colonization in early pregnancy is not predictive of neonatal sepsis, culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect women who are likely to be colonized with GBS at the time of delivery and are thus at higher risk of perinatal transmission of GBS [2-5]. Numerous studies employing several methods have documented that the accuracy of prenatal screening cultures in identifying intrapartum colonization status can be enhanced by careful attention to the timing of cultures, the anatomic sites swabbed, and the precise microbiologic methods used for culture and detection of GBS. The current guidelines published by Centers for Disease Control and Prevention (CDC) [5]. recommend screening of all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation by using an enrichment broth followed by sub-culture to a blood agar plate. Several alternative methods (molecular approach or culture media) have been developed to improve the sensitivity and specificity of GBS detection while reducing the incubation time and need for additional subcultures. Among the alternative methods, the most straightforward method of detection is based on pigment development as seen in Granada Agar [1, 6, 7]. 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. The purpose of this study was to assess the efficacy of Hardy Diagnostics' StrepB Carrot Broth™ in comparison to the LIM broth methodology in pregnant women between 35 to 37 weeks of gestation.

> For more information on StrepB Carrot Broth[™] contact Andre Hsiung, MS (email: hsiunga@hardydiagnostics.com)

Materials & Methods

Participating Centers

Eight centers that provide services to OB/GYN clinics and currently employing the CDC recommended LIM Broth methodology for GBS screening were selected for the study.

- University of Illinois; Chicago, Illinois
- University of North Carolina; Chapel Hill, North Carolina
- Elmhurst Memorial Hospital; Elmhurst, Illinois
- + Health Line Clinical Laboratories; Burbank, California
- Summa Health System; Akron, Ohio
- Parkview Medical Center; Pueblo, Colorado
- French Hospital; San Luis Obispo, California
- Ohristus Spohn Hospital; Corpus Christi, Texas

Study Duration

This study was conducted between March to October 2004.

Patient Eligibility and Sample Collection

- \rightarrow Pregnant patients between 35 37 weeks gestation that were eligible for GBS screening.
- -> Specimens from the lower vagina (vaginal introitus) and rectum were collected using the same swab or two different swabs, and transported to the laboratory with appropriate transport medium (e.g., Amies or Stuart's).

Microbiological Analysis

The specimens were evenly divided by placing the swabs in saline and inoculating into StrepB Carrot Broth™ and LIM Broth according to manufacturer's recommendations, followed by incubation at $35^{\circ}C$ for 18 - 24 hours.

LIM Broth

- All the LIM broths were subcultured after the incubation period to a 5% sheep blood agar plate and incubated for 18 to 24 hours.
- All colonies suggestive of GBS were confirmed by CAMP test or latex agglutination.
- Plates that did not present suspect colonies were re-incubated for an additional 18 to 24 hours and reexamined for GBS.

Quality Control

Streptococcus agalactiae ATCC 12386 (positive control) and Streptococcus pyogenes ATCC 19615 (negative control) were tested in parallel with all clinical isolates of GBS.







StrepB Carrot Broth[™]

- \rightarrow The StrepB Carrot BrothTM tubes were examined after 24 hours of incubation for any degree of color change. Any orange to brick red color development was considered as positive. In cases that the GBS count is low in the specimen. the development of small orange to red spots on the swab or at the bottom of the tube was also considered a positive result for GBS.
- → All negative (non-orange) StrepB Carrot Broth[™] tubes were subcultured to 5% sheep blood agar to confirm negativity.
- All suspected colonies from the subculture plate were tested by CAMP test or latex agglutination.

Results

Table 1. Overall Results of StrepB Carrot Broth[™] vs. LIM Broth, Multi-Center Clinical Trial Study

| | Samples contributed | No. (%) of positive specimens | Positives detected by both methods | Positives detected by Carrot Broth method only | Positives detected by LIM Broth method only |
|--|---------------------|-------------------------------|------------------------------------|---|--|
| University of Illinois; Chicago, Illinois | 281 | 84 (29.9) | 68 | 13 | 3* |
| University of North Carolina; Chapel Hill, North Carolina | 136 | 28 (20.5) | 23 | 1 | 4 |
| Elmhurst Memorial Hospital; Elmhurst, Illinois | 124 | 16 (12.9) | 15 | 1 | 0 |
| Health Line Clinical Laboratories; Burbank, California | 100 | 12 (12) | 10 | 2 | 0 |
| Summa Health System; Akron, Ohio | 100 | 21 (21) | 21 | 0 | 0 |
| Parkview Medical Center; Pueblo, Colorado | 99 | 12 (12.1) | 12 | 0 | 0 |
| French Hospital; San Luis Obispo, California | 47 | 14 (29.8) | 12 | 2 | 0 |
| Christus Spohn Hospital; Corpus Christi, Texas | 45 | 6 (13.3) | 5 | 1 | 0 |
| Total | 932 | 193* (20.7) | 166 | 20 | 7 |

* One case was considered as false positive on LIM Broth. This isolate was latex agglutination positive for group B Streptococci, but further identification confirmed this isolate as Streptococcus porcinus (non-pathogen). S. porcinus is known to cross-react with group B antisera.

- → Twenty cases were detected by StrepB Carrot Broth[™] only.
- Proteus mirabilis.
- \rightarrow LIM Broth's sensitivity and specificity were 93% and 99.4% respectively.

Discussion

- to improve the sensitivity.
- results were seen within 24 hours of incubation.

Conclusions

- detection of GBS.
- compared to the traditional LIM Broth method.
- increasing sensitivity, decreasing turn around time, and cutting labor and material costs by reducing the need for further testing.

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 \rightarrow Overall, 20.6% (192) of specimen were screened as positive for GBS.

 \rightarrow The positive rate ranged from 12% to 30% between the participating centers.

 \rightarrow Among the positive samples, 166 (86.4%) were detected by both methodologies.

→ Seven cases were detected by LIM Broth method only. Two of these cases were not detected by StrepB Carrot Broth[™] due to overgrowth of

→ 94.3% (186) of positive specimens by StrepB Carrot Broth[™] could have been released as positive results within 24 hours of incubation. The sensitivity and specificity for StrepB Carrot Broth[™] were 96.8% and 100% respectively.

 \rightarrow The rate of detection of GBS in this study is in accordance to recent surveys and publications[5, 9-13]

→ StrepB Carrot Broth[™] showed superior sensitivity and specificity compared to the LIM Broth method. There was one false-positive case with LIM Broth. The isolate was latex agglutination positive for group B Streptococci, however, further identification confirmed this isolate as Streptococcus porcinus (non-pathogen). S. porcinus is known to cross-react with group B antisera[8]. The false positive that occurred in the LIM Broth method may have lead to inappropriate and unnecessary antimicrobial therapy.

→ The overgrowth of Proteus mirabilis in the two cases that StrepB Carrot Broth[™] failed has been corrected with the addition of inhibitory agents

→ A color change was observed in as little as 6 hours in StrepB Carrot Broth[™] for specimens that had a high GBS count. Almost all positive

 \rightarrow Specimens found to be positive for GBS in StrepB Carrot BrothTM required no further culturing or testing.

-> Based on these findings, both methods can be considered reliable for the

→ StrepB Carrot Broth[™] demonstrated superior sensitivity and specificity when

→ StrepB Carrot Broth[™] is an improvement over conventional methods by

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