

Evaluation of Three Methods (StrepB Carrot Broth[™], LIM Broth, and Granada Agar) for Recovery of Group B Streptococci. G. Peterson¹, A. Hsiung¹, M. Blevins¹, C. Mendes², C. Oplustil², E. Sakagami², S. Sinto², J. Hardy¹. Hardy Diagnostics, Santa Maria, California, USA¹ | Fleury - Medical Diagnostics Center, São Paulo, Brazil²

Abstract

Although many adult females are colonized with Group B Streptococci (GBS) in their genital and gastrointestinal tracts without symptoms, detection of GBS in vaginal-anorectal area is critical for the prevention of neonatal GBS disease. Several microbiological assays employing different approaches and media have been previously conducted worldwide, however more data is still needed to assess the sensitivity and shorten the turn around time of the most commonly used methods for GBS detection. The goal of this study was to evaluate and compare two commercially available methods (the new StrepB Carrot Broth[™] and Granada Agar) against the CDC recommended LIM Broth protocol. Eighty-one clinical isolates were tested with the three methods at seven dilutions (10⁷ through 10¹ CFU/mL). As shown in Table 1, all three methods presented 100% sensitivity in dilutions of 10⁷ through 10³. At a concentration of 10² CFU/mL, StrepB Carrot Broth[™] and Granada Agar were able to recover GBS in 80 of 81 (99%) of samples followed by the LIM Broth method (94%). At a concentration of 10¹ CFU/mL, StrepB Carrot Broth[™] was the most sensitive (82%), followed by LIM Broth (67%) and Granada Agar (64%). When compared individually, LIM Broth outperformed the Granada Agar in sensitivity by one or two dilutions in 12 (15%) instances whereas Granada Agar was superior in 6 (7%) samples. StrepB Carrot Broth[™] out-performed LIM Broth in 18 (22%) samples while LIM Broth was superior in only four cases (5%). The StrepB Carrot Broth[™], 24 hour broth method, was shown to be the most sensitive of three methods tested. Based on these findings and considering the advantage of both StrepB Carrot Broth[™] and Granada agar methods having shorter test periods (24 hours versus 48 hours for the LIM method), StrepB Carrot Broth[™] and Granada Agar can be employed as reliable methods 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. 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. 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. Currently, the method recommended by Centers for Disease Control and Prevention (CDC) is considered to be the "gold-standard" method. This method recommends 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. In order to improve upon CDC recommendations, 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. 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 new Hardy Diagnostics' StrepB Carrot Broth[™] is designed to surpass all currently available culture methods in sensitivity and specificity. Additional enrichment factors and supplements are added to enhance the pigment intensity. The intent of this study is to compare performances of Hardy Diagnostics' StrepB Carrot Broth[™], Granada agar, and CDC recommended GBS screening protocol in pregnant women between 35 to 37 weeks of gestation.

I Comparison of sensitivity among methods evaluated (StrepB Carrot Broth[™], Table ' LIM Broth, and Granada Agar).

| | Group B Streptococci Recovery Rate | | |
|----------------------|---|------------------------|------------------------|
| | 10 ⁷ -10 ³ CFU/mL | 10 ² CFU/mL | 10 ¹ CFU/mL |
| StrepB Carrot Broth™ | 100% (81/81) | 99% (80/81) | 82% (66/81) |
| LIM Broth | 100% (81/81) | 94% (76/81) | 67% (54/81) |
| Granada Agar | 100% (81/81) | 99% (80/81) | 64% (52/81) |



Materials & Methods Isolates

- A total of 81 clinical isolates of Group B Streptococci were analyzed in this study. • Thirty (n=30) are part of Hardy Diagnostics' microorganism collection previously contributed by several hospitals and clinics across central and southern California.
- Fifty one (n=51) were kindly provided by Fleury Medical Diagnostics Center, Sao Paulo, Brazil.
- Among 81 isolates, two were known to be weak-hemolytic GBS and purposely selected to challenge the methods.

Sample Preparation and Inoculation

- Granada Agar).
- recommendations.

Interpretation

StrepB Carrot Broth[™]

• All tubes that did not turn orange to red color were considered as false-negatives.

LIM Broth

Granada Agar

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.

Results

- and Granada Agar (64%).
- Carrot Broth[™] in 6 (7%) cases.

Discussion

References

- 6:425-426.

Jul-Aug;7(4):172-6.



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

• A suspension equivalent to 0.5 McFarland was prepared for each isolate. • Serial dilution from 1:10¹ (equivalent to 1.5 x 10⁷ CFU/mL) through 1:10⁷ (1.5 x 10¹ CFU/mL) was prepared.

• From each dilution, an amount of 100mL was inoculated to each method (StrepB Carrot Broth™, LIM Broth, and

• StrepB Carrot Broth[™] and Granada agar were inoculated, incubated, and interpreted according to manufacturer's

• LIM Broth was inoculated, incubated, and interpreted according to CDC's recommendations.

• All tubes that turned orange to red were considered to be positive.

• Samples that yielded GBS after subculture to blood agar plate were considered as positives. • Samples that did not yield GBS after subculture to blood agar plate were considered as false-negatives.

• Samples that yielded orange to red colonies were considered as positives. • Samples that did not yield orange to red colonies were considered as false-negatives.

• All methods resulted in 100% sensitivity in concentrations of 10⁷ through 10³.

 At concentration of 10² CFU/mL, StrepB Carrot Broth[™] and Granada Agar were able to recover GBS in 80 of 81 (99%) of samples followed by the LIM Broth method (94%).

• At low concentration of 10¹ CFU/mL, StrepB Carrot Broth[™] was the most sensitive (82%), followed by LIM Broth (67%)

• StrepB Carrot Broth[™] out-performed LIM Broth in 18 (22%) samples while LIM Broth was superior in only four cases (5%). • LIM Broth out-performed the Granada Agar in sensitivity by one or two dilutions in 12 (15%) instances whereas Granada Agar was superior to LIM Broth in 6 (7%) samples.

• StrepB Carrot Broth[™] out-performed Granada Agar in 22 (27%) instances while Granada Agar was superior to StrepB

• Weak-hemolytic isolates were successfully detected by StrepB Carrot Broth[™] and Granada Agar by producing faintly orange color reactions. These isolates did not have the typical characteristics of GBS on the blood agar plate.

• Based on these findings, all three methods 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 10³ CFU/mL.

• In simulations of low GBS count, StrepB Carrot Broth[™] was the most sensitive method.

• Weak-hemolytic isolates of GBS were successfully detected by StrepB Carrot Broth[™] and Granada Agar. These isolates could easily be overlooked by inexperienced microbiologists using other methods.

Both StrepB Carrot Broth[™] and Granada Agar are easy to read-out based on color development and do not require further subculture or testing for positive samples. Positive samples can develop a color change in as early as 6 hours of incubation in StrepB Carrot Broth[™].

• 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.

Overman SB, DD Eley, BE Jacobs, JA Ribes. 2002. Evaluation of methods to increase the sensitivity and timeliness of detection of Streptococcus agalactiae in pregnant women. J Clin Microbiol. 40(11):4329-31.

3. Schuchat A. Group B streptococcal disease: from trials and tribulations to triumph and trepidation. 2001. Clin Infect Dis. 5;33(6):751-6. 4. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. 2002. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep. 6;51(RR-11):1-22.

5. de la Rosa, M., M. Perez, C. Carazo, L. Pareja, J.I. Peis and F. Hernandez. 1992. New Granada Medium for detection and identification of group B streptococci. J. Clin. Microbiol.; 30:1019-1021.

based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. 2002. N Engl J Med. 25:347(4):233-9.

6. Garcia Gil, E., M.C. Rodriguez, R. Bartolome, B. Berjano, L. Cabero and A. Andreu. 1999. Evaluation of the Granada Agar plate for detection of vaginal and rectal group B streptococci in pregnant women. J. Clin. Microbiol.; 37:2648-2651. 7. Rosa-Fraile Manuel, J. Rodriguez-Granger, M. Cueto-Lopez, A. Sampedro, E. Biel Gaye, J.M. Haro and A. Andreu. 1999. Use of Granada Medium to detect group B streptococcal colonization in pregnant women. J. Clin. Microbiol.; 37:2674-2677. 8. Rosa-Fraile Manuel, A. Sampedro, J. Varela, M. Garcia-Pena, and G. Gimenez-Gallego. 1999. Identification of a peptide pigment from mammal albumins responsible for enhanced pigment production by group B streptococci. Clin. Diag. Lab. Imm.;

9. Islam, AKMS. 1977. Rapid recognition of group B streptococci. Lancet i:256-257.

10. B. Spellerberg, B. Pohl, G. Haase, S. Martin, J. Weber-Heynemann and R. Lütticken. 1999. Identification of Genetic Determinants for the Hemolytic Activity of Streptococcus agalactiae by ISS1 Transposition. J. Bacteriol.; 181: 3212-3219. 11. Nguyen TM, Gauthier DW, Myles TD, Nuwayhid BS, Viana MA, Schreckenberger PC.1998. Detection of group B streptococcus: comparison of an optical immunoassay with direct plating and broth-enhanced culture methods. J Matern Fetal Med. 1998