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Evaluation of GBS Detect™: a New Medium for the Detection of Non-Hemolytic Group B Strep in Subcultures of Carrot Broth™ and LIM Broth. Results of a Multi-Center Trial

R. Clasen*[1], V. Cuna[1], S. Dolan[2], J. Bartos[3], J. Spoerke[4], G. Peterson[5], B. Peterson[6], Mary Colson-Burns[6], I. Yeung[7], J. Hardy[1]

[1*]Hardy Diagnostics, Santa Maria, California, [2]Bethesda Oak Hospital, Cincinnati, Ohio, [3]Aurora Health Care, Milwaukee, Wisconsin, [4]Columbia -St. Mary's, Milwaukee, Wisconsin, [5]Central Coast Pathology, San Luis Obispo, California, [6]Allina Laboratories, Abbott-Northwestern Hospital, Minneapolis, Minnesota, [7]Kaiser Berkeley Central Laboratory, Berkeley, California.

HARDY DIAGNOSTICS

Revised Abstract

Detection of Group B Streptococci (GBS) colonization in perinatal women is critical for the prevention of postpartum neonatal and maternal disease. The most sensitive culture based methods utilize enrichment broth procedures and the most commonly used methods are Strep B Carrot Broth™ and LIM Broth. Strep B Carrot Broth™ detects GBS by utilizing the organism's ability to produce a visible orange pigment. Pigment production is possible only with hemolytic strains of GBS; therefore negative Strep B Carrot Broth™ specimens must be subcultured to a Blood Agar plate or equivalent medium and observed for the presence of beta hemolytic and/or non-hemolytic colonies typical of GBS. All specimens cultured in LIM Broth require subculture for the detection of hemolytic and non-hemolytic strains of GBS. The limitation of plating subcultures to conventional media is that non-hemolytic or gamma strains of GBS may be missed because the colonies are not readily distinguishable from other small non-hemolytic colonies. Special supplements contained in the novel GBS Detect™ plate cause normally weakly or non-hemolytic strains to appear strongly beta hemolytic. The goal of this study was to evaluate the GBS Detect™ medium against conventional plating methods in a multi-center trial format. Six different laboratories plated subcultures from either LIM Broth or negative Strep B Carrot Broth ™, using GBS Detect ™ and a Blood agar plate or equivalent. A total of 619 samples were tested, with 107 positive for GBS on one or more media (17.2%). Of the 107 samples containing GBS, 83 (77.5%) were identified as hemolytic on both GBS Detect and conventional plating methods and 24 (28.9%) were identified as hemolytic only when using GBS Detect™ plates. No positives were detected on standard media without being detected using the GBS Detect ™ plate. All isolates were confirmed as GBS using latex agglutination. Compared to GBS Detect™, conventional media demonstrated sensitivity of only 77.5%. GBS Detect™ was able to detect 24 additional strains of Group B Streptococcus that would otherwise have been missed using conventional methods. Based on these findings, GBS Detect™ can be utilized as a reliable method to increase the sensitivity of traditional culture based GBS detection methods. In addition, GBS Detect ™ is a useful compliment to Strep B Carrot Broth™ and ultimately reduces the work load and the time necessary to accurately identify GBS in pregnant women.

Introduction

Intrapartum colonization of group B Streptococci (GBS) is a major risk factor for early-onset neonatal sepsis and remains 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. Currently, the method recommended by Centers for Disease Control and Prevention (CDC) is considered the "gold-standard" [5]. 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. Parallel to CDC recommendations, several alternative methods (molecular approach or culture media) have long ago 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 and Strep B Carrot Broth™ [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. Pigment production is possible only with hemolytic strains of GBS; therefore negative Strep B Carrot Broth™ specimens must be subcultured to a Blood Agar plate or equivalent medium and observed for the presence of beta hemolytic and/or non-hemolytic colonies typical of GBS. All specimens cultured in LIM Broth require subculture for the detection of hemolytic and non-hemolytic strains of GBS. The limitation of plating subcultures to conventional media is that non-hemolytic or gamma strains of GBS may be missed because the colonies are not readily distinguishable from other small non-hemolytic colonies. Special supplements contained in the novel GBS Detect™ plate cause normally weakly or non-hemolytic strains to appear strongly beta hemolytic. In addition, GBS Detect™ contains selective agents to assist the microbiologist in selecting only GBS colonies. The goal of this study was to evaluate the GBS Detect™ medium against conventional plating methods in a multi-center trial format.

Materials and Methods

Participating centers

Six centers that currently provide testing for GBS in pregnant women either by using LIM Broth or Carrot Broth.

- Bethesdsa Oak Hospital, Cincinnati, Ohio
- Aurora Health Care, Milwaukee, Wisconsin
- Columbia – St. Mary's, Milwaukee, Wisconsin
- Central Coast Pathology, San Luis Obispo, California
- Allina Healthcare Systems, Abbott-Northwestern Hospital, Minneapolis, Minnesota
- Kaiser Berkeley, Berkeley Central Laboratory, Berkeley, California

Study duration

This study was conducted between November 2007 and April 2008.

Patient eligibility and sample collection

- Pregnant patients between 35 – 37 weeks gestation that were eligible for GBS screening.
- Specimen from the lower vagina (vaginal introitus) and rectum were collected, transported to the laboratory with appropriate transport medium (e.g., Amies or Stuart's) and inoculated into either LIM Broth or Strep B Carrot Broth™.

Microbiological Analysis

LIM Broth

- All LIM Broths were subcultured after the 18 to 24 hour incubation period to a 5% Sheep Blood agar plate (or equivalent) and to a GBS Detect Plate™. Plates were incubated for 18 - 24 hours.
- All colonies suggestive of GBS were confirmed by CAMP test or latex agglutination.

Strep B Carrot Broth™

- The Strep B Carrot Broth™ 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.
- All negative (non-orange) Strep B Carrot Broth™ tubes were subcultured to 5% Sheep Blood agar and GBS Detect™ plates.
- Beta-hemolytic colonies and gamma hemolytic colonies from Blood Agar plates and only Beta-hemolytic colonies detected on the GBS Detect™ plate were tested by CAMP test or latex agglutination.

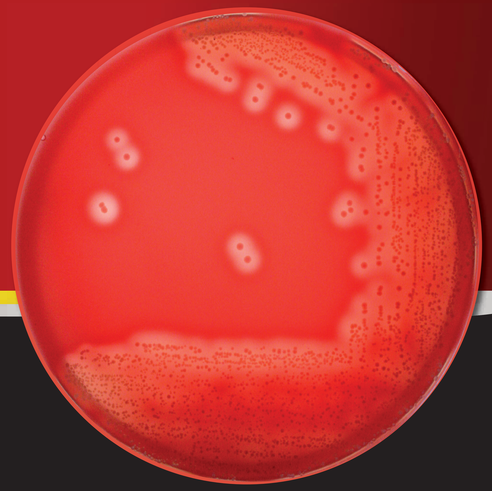
Results and Data Analysis

Results from each method were independently recorded by the different technologists who performed the testing. These results were later analyzed by the study coordinator. Results are summarized in the table below.

Table 1. Overall Results of GBS Detect™ vs. Blood Agar, Multi-Clinical Trial Study

	Samples Contributed	no. (%) of specimens positive for GBS	GBS positives detected by both methods	GBS positives detected on GBS Detect™ only	GBS positives detected only on Blood Agar	Beta hemolysis occuring on GBS Detect™ that were not GBS*
Bethesda Oak Hospital Cincinnati, Ohio	100	19 (19.0)	14	5	0	6
Aurora Health Care Milwaukee, Wisconsin	200	25 (12.5)	20	5	0	42
Columbia - St. Mary's Milwaukee, Wisconsin	76	20 (26.3)	13	7	0	1
Central Coast Pathology San Luis Obispo, California	89	9 (10.1)	5	4	0	1
Allinia Laboratories, Abbot Northwestern Hospital Minneapolis, Minnesota	55	4 (7.3)	2	2	0	9
Kaiser - Berkeley Berkeley, California	99	30 (30.3)	29	1	0	3
Total	**619**	**107 (17.2)**	**83**	**24**	**0**	**62**

- No GBS were detected on Blood Agar (or equivalent) and not detected using GBS Detect
- Overall, 17.2% of specimens were screened as positive for GBS.
- The positive rate ranged from 7.3% to 30.3% between the participating centers.
- Among the positive samples, 83 (77.5%) were detected by both methodologies.
- Twenty four cases (22.4%) were detected by GBS Detect™ only.
- *62 out of 617 specimens produced hemolytic colonies on GBS Detect™ that were not confirmed as GBS. The number of non-GBS hemolytic colonies detected varied at the sampling sites. Subsequent to this study, additional selective agents were added to the GBS Detect ™ formula to reduce the number of false positives.



Discussion

Of the 107 samples containing GBS, 83 (77.5%) were identified as hemolytic on both GBS Detect™ and conventional plating methods. 24 additional GBS positives (22.4%) were identified as hemolytic only when using GBS Detect™. Confirmation of all GBS isolates was done using latex agglutination. Finally, no positives were detected on standard media without being detected using the GBS Detect™ plate. Some study participants expressed concern over the number of isolates that produced hemolytic reactions and were not confirmed as GBS (false positives). The number of hemolytic non-GBS isolates varied between testing centers; some reporting large numbers of isolates and others only a few. Hemolysis, in some instances, was reported as "underneath the colony". Another site reported large numbers of *E. faecalis* isolates showing beta hemolysis on the GBS Detect ™ plate but not on the Blood Agar plate. Since the conclusion of the study, the technical data sheet for the use of the medium has been modified to specify that only colonies that have large and clear zones of hemolysis should be evaluated for GBS by further testing. Additional selective agents have been included in the media to inhibit *E. faecalis*. These changes will increase the utility of this new medium and decrease the occurrence of false positives.

Conclusion

GBS Detect™ medium recovered significantly higher numbers of non-hemolytic GBS isolates from subcultures of traditional enrichment broths than Blood agar plates or equivalent. GBS Detect™ detected 22.4% more strains of Group B Streptococcus that would otherwise have been missed using conventional methods. Based on these findings, GBS Detect™ can be utilized as a reliable method to increase the sensitivity of traditional culture based GBS detection methods. In addition, GBS Detect™ is a useful compliment to Strep B Carrot Broth™ and ultimately reduces the work load and the time necessary to accurately identify GBS colonization in pregnant women.

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