Evaluation of Granada Medium as a Subculture Method from LIM Broth for the Detection of Group B *Streptococcus*: A Multi-Centric Study

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Abstract

Background: The purpose of this study was to evaluate the performance of Granada Medium for the detection of Group B *Streptococcus* (GBS) after LIM Broth enrichment compared to plating to sheep blood agar. Granada Medium is a selective medium that incorporates chromogenic pigments resulting in the development of orange, red, or brick red colored colonies in the presence of β-hemolytic GBS.

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. Specimens were stored in either 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 Granada Medium and to sheep blood agar. All isolates, regardless of color, were confirmed via traditional methods such as Gram-stain, followed by catalase reaction and Lancefield latex agglutination. Because the color reaction of Granada Medium is limited solely to the detection of β-hemolytic GBS, all white colonies were tested for GBS.

Results: A total of 884 specimens across the four sites were enrolled for this study from November 2015 to October 2016. A total of 245 isolates were recovered on sheep blood agar and 247 isolates were recovered by color development on Granada Medium. A total of 237 isolates were recovered by both methods simultaneously. Overall, the recovery of GBS isolates from Granada Medium, by the orange/red color reaction, demonstrated 96.7% sensitivity and 98.4% specificity in comparison to the reference method where both hemolytic and non-hemolytic strains were accounted for. Five of the white colonies recovered were determined to be non-hemolytic GBS strains. Following full identification of non-color producing colonies, the overall sensitivity increased to 99.2%. Additionally, there were 10 specimens that were positive by Granada medium and negative by the reference method.

Conclusion: This study shows the equivalency of Granada Medium to a traditional LIM Broth/BAP method, with quicker visualization of positive specimens, and requiring less work-up of the breakthrough organisms.

Introduction

Approximately 10-35% of women are asymptomatic carriers of group B Streptococci (GBS) in the genital and gastrointestinal tracts.(1) GBS remains a leading cause of serious illness and death in newborn populations and, therefore, the detection of GBS carriage 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)

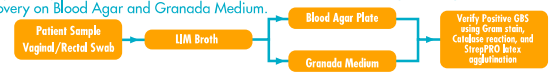
Granada Medium Agar is a selective medium that incorporates chromogenic pigments resulting in the development of orange, red, or brick red colored colonies in the presence of β-hemolytic GBS. The production of orange, red, or brick red pigment is a unique characteristic of hemolytic GBS due to reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. These components serve as the basis for culture media used to detect and identify these organisms. Since the original description of starch serum agar by Islam in 1977, there have been many improvements to the original formula.(5) Presently, Granada Medium is a reliable method for the detection and identification of beta-hemolytic GBS. This medium is limited in its ability to detect non β-hemolytic GBS.

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.

LIM broth was inoculated with 30µL of the specimen sample and incubated 18-24 hours at 35°C. After incubation, broth was subcultured to Granada Medium and to 5% Sheep Blood Agar. All isolates recovered on Blood Agar were confirmed as GBS via traditional methods such as Gram-stain, 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 were non-hemolytic on Blood Agar, Gram positive, catalase negative, and Group B Streptococci by latex agglutination were considered positive "NH Group B Strep." All isolates recovered on Granada Medium, regardless of color, were confirmed via traditional methods such as Gram-stain, catalase reaction, and Lancefield latex agglutination. Because the color reaction of Granada Medium is limited solely to the detection of β-hemolytic GBS, all white colonies were tested for GBS.

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 (10^3 CFU/mL) in donated negative-vaginal rectal matrix for their recovery on Blood Agar and Granada Medium.



Participating Study Sites

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

Results

Table 1. Blood Agar Reference Method vs. Granada Medium Color Reaction

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
CCP	47	1	5	142	90.4	79.4	95.8	99.3	96.1	99.9
NY	72	6	2	141	97.3	90.7	99.3	95.9	91.4	98.1
CC	78	1	0	104	100.0	95.3	100.0	99.0	94.8	99.8
MCW	40	2	1	242	97.6	87.4	99.6	99.2	97.1	99.8
Overall	237	10*	8**	629	96.7	93.7	98.3	98.4	97.1	99.1

*There were 10 False Positives overall. All isolates were confirmed to be β Group B Strep and considered true positives in discrepant analysis.

**There were 8 False Negatives overall. Two of the isolates were confirmed as β Group B Strep and subsequently confirmed as positive by Granada Medium color reaction. Six isolates were confirmed to be non-hemolytic Group B Strep.

Table 2. Blood Agar Reference Method vs. Granada Medium Identification

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
CCP	51	1	1	142	98.1	89.9	99.7	99.3	96.1	99.9
NY	73	6	1	141	98.6	92.7	99.8	95.9	91.4	98.1
CC	78	1	0	104	100.0	95.3	100.0	99.0	94.8	99.8
MCW	41	2	0	242	100.0	91.4	100.0	99.2	97.1	99.8
Overall	243	10*	2**	629	99.2	97.1	99.8	98.4	97.1	99.1

*There were 10 False Positives overall. All isolates were confirmed to be β Group B Strep and considered true positives in discrepant analysis.

**There were 2 False Negatives overall. One isolate was confirmed as β Group B Strep and subsequently confirmed as positive by Granada Medium color reaction. The second isolate was confirmed as a non-hemolytic Group B Strep and was not recovered from Granada Medium from LIM Broth during discrepant testing.

Conclusions

Discussion:

- Granada Medium demonstrated 96.7% sensitivity and 98.4% specificity compared to the reference method based on color reaction alone.
- Sensitivity of Granada Medium was enhanced to 99.2% upon latex confirmation of non-hemolytic Group B Strep strains (white colonies on Granada).
- Ten strains of beta-hemolytic GBS were undetected by LIM-BAP while these were recovered by the LIM-Granada agar.
- Due to the enhanced selectivity and distinct color reaction, LIM broth overnight enrichment followed by Granada subculture can be deemed as a reliable method for the recovery of GBS, in lieu of traditional subculture to BAP.

References:

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