Evaluation of New Strep B Carrot Broth™ One-Step in the Detection of Group B *Streptococcus*: A Multi-Center Study

Poster Number: 438

M. L. Faron[1], N. A. Ledeboer[1], B. W. Buchan[1], J. Connolly[1], H. Seliger[1], L. Westblade[2], A. N. Schuetz[2,5], A. Robertson[2], K. Fauntleroy[2], M. Sarina[3], D. Wilson[4], G. W. Procop[4], S. Jenkins[2]

[1]Medical College of Wisconsin, Milwaukee, Wisconsin; [2]Cornell Medical Center, New York, New York; [3]Central Coast Pathology, San Luis Obispo, California, [4]Cleveland Clinic, Cleveland, Ohio; [5]Current Affiliation – Mayo Clinic, Rochester, Minnesota

Abstract

Background: The aim of this study was to evaluate the performance of the newly developed Strep B Carrot Broth™ One-Step and the already well-established Strep B Carrot Broth™ Kit against the reference LIM broth method in the enrichment and detection of Group B Streptococci (GBS). Carrot Broth™ Kit and Carrot Broth™ One-Step are selective enrichment broths which incorporate chromogenic pigments that result in the development of orange, red, or brick red color in the presence of β-hemolytic GBS.

Methods: A total of 884 clinical 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. LIM broth, Carrot Broth™ Kit, and Carrot Broth™ One-Step were inoculated with 30µL of the specimen sample and incubated overnight at 35°C. After incubation, all broths were subcultured to 5% Sheep Blood Agar. All isolates recovered on Blood Agar were confirmed as GBS via traditional methods such as Gram-stain, followed by catalase reaction and Lancefield group latex agglutination.

Results: The LIM broth sub cultured to Blood Agar reference method recovered 245 GBS isolates. 210 isolates from the Carrot Broth™ Kit and 207 isolates from Carrot Broth™ One-Step were detected by color development with concordant results obtained by the LIM Broth reference method. Due to the fact that Carrot Broth, by color reaction itself, is limited solely to the detection of β-hemolytic GBS, overall Carrot Broth™ Kit demonstrated 85.7% sensitivity and 98.9% specificity prior to subculture. After subculture, Carrot Broth™ Kit demonstrated 98.8% sensitivity by detecting both hemolytic and non-hemolytic strains in comparison to the LIM broth methodology. Carrot Broth™ One-Step demonstrated 84.5% sensitivity and 98.6% specificity prior to subculture, and the sensitivity was enhanced to 98.8% upon subculture of non-orange/red tubes. There were seven instances that the Carrot Broth™ Kit and nine instances that Carrot Broth™ One-Step recovered GBS based on orange color reaction while the LIM Broth method did not. In addition, there were nine isolates from the Carrot Broth™ kit and six isolates from Carrot Broth™ One-Step that were recovered upon subculture that the LIM Broth method did not detect.

Conclusion: This study shows the equivalency of both Carrot Broth products and their performance advantages over the traditional LIM Broth method.

Introduction

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

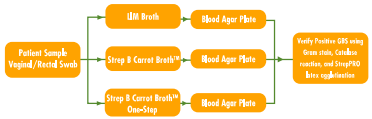
Strep B Carrot Broth™ and Strep B Carrot Broth™ One-Step are selective and differential enrichment broths with selective components designed to enrich for Group B Streptococci. The production of a peach, orange, red, or brick red pigment is a unique characteristic of hemolytic GBS due to reaction with substrates such as starch, peptone, serum, and folate pathway inhibitors. GBS detection with Strep B Carrot Broth™ and Strep B Carrot Broth™ One-Step is only possible with β-hemolytic Group B Streptococci colonies, providing evidence of a direct genetic linkage between pigment production in this media and hemolysin production.

Methods

A total of 884 vaginorectal clinical 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, Carrot Broth™ Kit, and Carrot Broth™ One-Step were inoculated with 30µL of the specimen sample and incubated 18-24 hours at 35°C. After incubation, all broths were subcultured to 5% Sheep Blood Agar. All isolates recovered on Blood Agar were confirmed as GBS via traditional methods such as Gram-stain, followed by 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 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 from the LIM reference method, color development in Carrot Broth™ Kit, and recovery from the Carrot Broth™ Kit to Blood Agar System.



Participating Study Sites

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

Results

Table 1. LIM Reference Method vs. Strep B Carrot Broth™ One-Step Color reaction

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
CCP	48	1	4	142	92.3	81.8	97.0	99.3	96.1	99.9
NY	54	4	20	143	73.0	61.9	81.8	97.3	93.2	98.9
CC	70	1	8	104	89.7	81.0	94.7	99.0	94.8	99.8
MCW	35	3	6	241	85.4	71.6	93.1	98.8	96.4	99.6
Overall	207	9*	38**	630	84.5	79.4	88.5	98.6	97.3	99.3

*There were 9 False Positives overall. Of these, seven isolates were confirmed to be β Group B Strep and considered true positives in discrepant analysis. One specimen had a Proteus species swarm the Blood Agar and β-hemolysis could not be seen. One was not able to be confirmed because no GBS isolate was frozen.
**There were 38 False Negatives overall. All isolates were re-tested and confirmed. Thirty-two of the isolates were confirmed as β Group B Streptococci and subsequently confirmed as positive by Strep B Carrot Broth™ One-Step color reaction at LoD. Two isolates of β Group B Streptococci showed very weak β-hemolysis on the Blood Agar and did not produce the expected color reaction in Strep B Carrot Broth™ One-Step. Four isolates were confirmed to be non-hemolytic Group B Strep.

When comparing the number of Group B Streptococci positive specimens recovered by the LIM reference method to the number identified by Strep B Carrot Broth™ One-Step color change in conjunction with the subculture of presumptive negatives to the Blood Agar, an additional 35 specimens showed concordant positive results for a total of 242 true positive results. The LIM reference method included the identification of both β-hemolytic and non-hemolytic GBS from samples by culture. Those results are shown in Table 2.

Table 2. LIM Reference Method vs. Strep B Carrot Broth™ One-Step (color), plus Subculture of Presumptive Negatives to Blood Agar with Biochemical Testing

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
CCP	51	0	1	143	98.1	89.9	99.7	100.0	97.4	100.0
NY	74	6	0	141	100.0	95.1	100.0	95.9	91.4	98.1
CC	77	2	1	103	98.7	93.1	99.8	98.1	93.3	99.5
MCW	40	5	1	239	97.6	87.4	99.6	98.0	95.3	99.1
Overall	242	13*	3**	626	98.8	96.5	99.6	98.0	96.6	98.8

*There were 13 False Positives overall. All isolates were confirmed to be β Group B Strep and considered true positives in discrepant analysis. Of the thirteen, six did not produce the expected color reaction in Strep B Carrot Broth™ One-Step in the original clinical data but did upon discrepant analysis.
**There were 3 False Negatives observed. All three isolates were confirmed as β Group B Strep and subsequently confirmed as positive by Strep B Carrot Broth™ One-Step system at the LoD.

Strep B Carrot Broth™ One-Step (Positive)

Table 3. LIM Reference Method vs. Strep B Carrot Broth™ Kit Color reaction

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
CCP	48	1	4	142	92.3	81.8	97.0	99.3	96.1	99.9
NY	56	3	18	144	75.7	64.8	84.0	98.0	94.2	99.3
CC	70	0	8	105	89.7	81.0	94.7	100.0	96.5	100.0
MCW	36	3	5	241	87.8	74.5	94.7	98.8	96.4	99.6
Overall	210	7*	35**	632	85.7	80.8	89.5	98.9	97.8	99.5

*There were 7 False Positives overall. One specimen had a swarming Proteus and no β-hemolytic colonies could be isolated for confirmation. Two isolates were not recoverable from the frozen samples and therefore could not be confirmed. The remaining four discrepant isolates were confirmed to be β Group B Streptococci and considered true positives in discrepant analysis.

** There were 35 False Negatives overall. Twenty-nine of the isolates were confirmed as β Group B Strep and subsequently confirmed to have a positive Strep B Carrot Broth™ Kit color reaction at LoD. Two isolates were identified as a very weak β Group B Streptococci and did not produce the expected color reaction in Strep B Carrot Broth™ Kit. Four isolates were confirmed as non-hemolytic Group B Strep.

When comparing the number of Group B Streptococci positive specimens recovered by the LIM reference method to the number identified by Strep B Carrot Broth™ color change in conjunction with the subculture of presumptive negatives to the Blood Agar, an additional 32 specimens showed concordant positive results for a total of 242 true positive results. The Lim reference method included the identification of both β-hemolytic and non-hemolytic GBS from samples by culture. Those results are shown in Table 4.

Table 4. LIM Reference Method vs. Strep B Carrot Broth™ Kit (color), plus Subculture of Presumptive Negatives to Blood Agar with Biochemical Testing

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	74	7	0	140	100.0	95.1	100.0	95.2	90.5	97.7
CC	77	1	1	104	98.7	93.1	99.8	99.0	94.8	99.8
MCW	40	5	1	239	97.6	87.4	99.6	98.0	95.3	99.1
Overall	242	14*	3**	625	98.8	96.5	99.6	97.8	96.4	98.7

*There were 14 False Positives overall. All fourteen isolates were confirmed to be β Group B Strep and considered true positives in discrepant analysis. Of the fourteen, ten did not produce the expected color reaction in Strep B Carrot Broth™ Kit in the original clinical data but did upon discrepant analysis.

**There were 3 False Negatives overall. All three isolates were confirmed as β Group B Strep and subsequently confirmed as positive by Strep B Carrot Broth™ Kit system at LoD.

Conclusions

- All False Positives by both Carrot Broth™ products were later confirmed as β-hemolytic GBS: More positive cultures were detected by Carrot Broth™ products than the reference method.
- Of the 73 False Negatives by color reaction (38 from CB One-Step and 35 from CB Kit), 67 were subsequently detected by subculture.
- Carrot Broth™ One-Step demonstrated 84.5% sensitivity and 98.6% specificity prior to subculture compared to the reference method. Sensitivity was enhanced to 98.8% upon subculture of non-orange/red tubes.
- Carrot Broth™ Kit demonstrated 85.7% sensitivity and 98.9% specificity prior to subculture compared to the reference method. After subculture, Carrot Broth™ Kit demonstrated 98.8% sensitivity.
- This study demonstrates that Carrot Broth™ One-Step is equivalent in performance to Carrot Broth™ Kit, and is a superior testing option than the Carrot Broth™ Kit because no tile is required, thus reducing specimen processing time.

References

1. Regan, J.A., Klebanoff, M.A., Nugent, R.P. 1991. *The epidemiology of group B streptococcal colonization in pregnancy.* Vaginal Infections and Pregnancy Study Group. Obstet. Gynecol.; 77:604-10.

2. Schrag, S.J., E.R. Zell, R. Lynfield, et al. 2002. *A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates.* N. Engl. J. Med. 25;347(4):233-9.

3. Schuchat, A. 2001. *Group B streptococcal disease: from trials and tribulations to triumph and trepidation.* Clin. Infect. Dis. 5;33(6):751-6.

4. The Centers for Disease Control and Prevention. 2010. *Prevention of Perinatal Group B Streptococcal Disease.* Revised Guidelines. MMWR 59 (RR-10). Internet: http://www.cdc.gov/mmwr/pdf/rr/rr5910.pdf