C-317. Comparison of Commercially Available Methods (StrepB Carrot Broth™ and GBS Medium) for the Detection of Group B Streptococcus

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GBS remains one of the leading causes of sepsis and meningitis in newborns despite recent advances in the prevention of neonatal group B streptococcal (GBS) disease. Although the current gold standard method is the LIM broth method, as recommended by the Centers for Disease Control and Prevention (CDC), several alternative methods have been developed with comparable sensitivity, specificity, and reduced turnaround time. The purpose of this study was to determine the accuracy of two recently launched products: StrepB Carrot Broth™ (Hardy Diagnostics, Santa Maria, CA) and GBS Medium (Northeast Laboratory Services, Winslow, ME).

A total of 100 vaginal-rectal specimens were collected during the second half of 2005 and tested in parallel by both methods. Overall, 25 (25.0%) specimens were detected as positive for GBS. StrepB Carrot Broth™ detected 23 of 25 while GBS Medium detected seven of 25. Overall results are shown in the table below.

	StrepB Carrot Broth™	GBS Medium
Number of specimen tested	100	100
Number of positives	23	7
Strong positive	23	3
Weak positive	0	4
Number of positive after subculture	2*	18**
Number of negative after subculture	75	75

*Non-hemolytic strains of GBS.**Two were non-hemolytic.

As described elsewhere, detection of GBS based on development of orange-pigment reaction (as demonstrated in Granada media) is only effective against beta-hemolytic strains of GBS. In the present study, StrepB Carrot Broth showed 100% sensitivity and specificity against beta-hemolytic strains of GBS with strong color reactions without need for further confirmation while GBS medium only detected 28%. Based on this evaluation, StrepB Carrot Broth™ can be used as a reliable tool for detection of hemolytic GBS in pre-natal screening.

C-316. Evaluation of Accuracy of StrepB Carrot Broth™ in the Detection of Different Serotypes of Group B Streptococci (GBS)

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Detection of GBS in the vaginal-anorectal area is critical for the prevention of neonatal GBS disease. Several microbiological assays employing different methods have been developed worldwide with increased sensitivity, reduced costs, and shorter turnaround time for the detection of GBS. However, very little has been documented concerning the accuracy of each methodology in the detection of different serotypes of GBS. The goal of this study was to evaluate Strep B Carrot Broth™ against several serotypes of GBS and a few rare pigmented species of *Enterococci* and *Streptococci*. A total of 50 isolates (45 GBS of several serotypes, four pigmented *Enterococcus*, and one *Streptococcus porcinus*) were retrieved from Centers for Disease Control and Prevention's collection and inoculated into StrepB Carrot Broth™, in order to evaluate the ability of this medium to accurately identify GBS. Overall results are shown in the table below.

	Hemolysis	*Serotype*	*Number of isolates tested*	*StrepB Carrot Broth™ Positive*	*StrepB Carrot Broth™ Negative*
S. agalactiae	Positive	Ia	6	6	0
S. agalactiae	Positive	Ib	4	4	0
S. agalactiae	Positive	II	4	4	0
S. agalactiae	Positive	III	4	4	0
S. agalactiae	Positive	IV	4	4	0
S. agalactiae	Positive	V	8	8	0
S. agalactiae	Positive	VI	1	1	0
S. agalactiae	Positive	VII	1	1	0
S. agalactiae	Positive	VIII	2	2	0
S. agalactiae	Positive	Non-typeable	2	2	0
S. agalactiae	Negative	N/A	9	0	9
S. porcinus	N/A	N/A	1	0	1
E. casseliflavus	N/A	N/A	1	0	1
E. faecalis variant	N/A	N/A	3	0	3

As expected, all non-hemolytic GBS produced negative results. StrepB Carrot Broth™ demonstrated 100% sensitivity and 100% specificity against all the beta-hemolytic GBS, and produced no false positives against pigmented *Streptococci* and *Enterococci*. Based on these findings, Strep B Carrot Broth™ can be employed as reliable method for detection of beta-hemolytic Group B Streptococci.

C-138. Comparison of Real-Time PCR (Cepheid SmartCycler) with Standard LIM Broth Culture and StrepB Carrot Broth™ for the Detection of *Group B Streptococcus* in Pre-Natal Vaginal/Rectal Specimens

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Background: Group B Streptococcus (GBS) continues to be a leading cause of neonatal sepsis and meningitis despite the implementation of revised guidelines in 2002 that recommend screening pregnant women at 35-37 weeks gestation for GBS. This approach does not address women who do not receive prenatal care, have pre-term labor, or whose carrier status may fluctuate. In an attempt to better serve this patient population and identify non-hemolytic GBS strains that may be missed in standard culture methods, two new methods of GBS detection (real-time PCR & color-change media) were compared with standard LIM broth culture. **Methods:** 61 vaginal/rectal swabs from pre-natal patients were analyzed for GBS using three different methods; 1) Direct swab real-time PCR using the Cepheid Strep B SmartCycler assay, 2) LIM broth culture, 3) Detection of color change in StrepB Carrot Broth ™ (Hardy Diagnostics) after 24h incubation. Additionally, bacterial culture and PCR testing was performed on StrepBCarrot Broth ™ after 24h incubation. True positives (TP) were defined as those specimens confirmed to have GBS by serotyping of suspicious organisms seen on blood agar plates inoculated from 24h LIM and StrepB Carrot Broth™ and examined after 24 and 48 h incubation.

Results:

n=61	Direct Swab PCR	Carrot Broth Culture	Carrot Broth PCR	LIM Broth Culture
TP	12	14	14	12
FP	2	0	6	0
TN	45	47	41	47
FN	2	0	0	2
Sensitivity (%)	85.7	100	100	85.7
Specificity (%)	95.7	100	87.2	100
PPV (%)	85.7	100	70	100
NPV (%)	95.7	100	100	95.9
Accuracy (%)	93.4	100	90.2	96.7

Conclusion: StrepB Carrot Broth™ was the most sensitive of the 3 methods studied. All GBS positive specimens produced an orange color in StrepB Carrot Broth™ in 24 hrs. However, the broth still depends on beta-hemolysis for production of orange pigment and therefore requires subculture of negative results to agar plates and examination after 48-h for non-hemolytic GBS. Direct specimen real-time PCR using the Cepheid SmartCycler could be performed in 2 h with results equivalent to LIM broth culture. Use of the SmartCycler, real-time PCR testing for GBS from negative StrepB Carrot Broth™ cultures did not detect any additional GBS and resulted in 6 false-positive tests.

C-111. Evaluation of Strep B Carrot Broth™ and LIM Broth for Long Term Storage/Recovery of Group B *Streptococcus*

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Background: Group B *Streptococcus* (GBS) remains one of the leading causes of sepsis and meningitis in newborns despite recent advances in the prevention of neonatal group B streptococcal disease. The current CDC recommended OBGYN screening method involves use of a selective broth such as LIM or Trans-Vag, but several alternative methods such as Strep B Carrot Broth have been developed with comparable sensitivity, specificity, and reduced turnaround time. **Purpose:** This study evaluated the ability of Strep B Carrot Broth and standard LIM Broth to maintain the viability of GBS over time following specimen inoculation. Use of these broths as a storage medium would facilitate antimicrobial susceptibility testing at a later date if penicillin-allergic patients are not identified at the time of culture. **Methods:** One hundred vaginal/rectal patient specimens were cultured in parallel using both broth methods. Each broth system detected the same 21 GBS positive patients (using manufacturers' recommended procedures). Positive broths were held at room temperature and further evaluated for long-term GBS recovery by subculturing onto SBA for semi-quantitation of growth. **Results**: GBS were recovered from either broth system for a minimum of 15 days and usually much longer. GBS survival in Strep B Carrot Broth averaged 51 days (range 15 to 113 days) and GBS survival in LIM Broth averaged 32 days (range 13 to 90 days). GBS survival in Strep B Carrot Broth was longer than in LIM broth for 19 of 21 positive specimens. In 2 of 21 specimens, GBS survived longer in LIM Broth. **Conclusion**: Both broth systems maintained the viability of GBS for at least 2 weeks and usually much longer, but Strep B Carrot Broth maintained viability longer than LIM broth for most GBS isolates. Use of these broths as a storage medium should allow adequate time for follow-up testing when needed.