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

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REVISED ABSTRACT

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:** 120 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 StrepB Carrot Broth™ after 24h incubation. **Results:** Thirty-one of 120 specimens were confirmed positive (25.8%) by one or more assays. Sensitivities for the IDI-Strep B™ Smart Cycler®, standard LIM broth method and Carrot Broth™ were 83.9% (26/31), 87.1% (27/31), 93.5% (29/31) respectively. **Conclusion:** Carrot Broth™ was the best performing antepartum assay. Real-time PCR using the IDI-Strep B™ SmartCycler® assay is an exciting alternative to standard antepartum culture that would allow for detection of GBS intrapartum with results available in 2 hrs. Real-time PCR was not as sensitive as Carrot Broth™ assay and would therefore have a limited use in the antepartum setting without a reliable method of retesting negative results after overnight incubation.

References

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INTRODUCTION

The most common GBS screening method is inoculation of a selective enrichment medium such as, LIM broth, with incubation over night followed by subculture to blood agar with manual identification of beta-hemolytic colonies (1). This two step method with a turn around time of 48-72 hours is considered the gold standard and has a sensitivity of 87%-91%, a specificity of 89%-96% (2), and is currently used by 89.0% of labs across seven states surveyed (1). New methods of GBS identification including the use of color change enrichment media and real-time PCR have recently been introduced (4,5) as alternatives to standard culture. The purpose of this study was to compare the sensitivity of LIM broth with color change media (StrepB Carrot BrothTM) and real-time PCR (Cepheid Strep B SmartCyder® assay) for detection of GBS from antepartum vaginal/rectal swabs.

METHODS

120 vaginal/rectal swabs from pre-natal patients were analyzed for GBS using; 1) IDI-Strep B™ Cepheid Strep B SmartCycler® assay, 2) LIM broth culture, 3) Detection of color change in Carrot Broth™ Kit (Hardy Diagnostics) after 24h incubation. Additionally, bacterial culture and PCR testing was performed on Carrot broths after 24h of incubation. Dual swab vaginal/rectal specimens were sequentially placed in Cepheid buffer, by placing a single swab in buffer for 5 min., vortexing for 15 sec and then removing swab. After both swabs were processed in the same buffer tube, 100 µl aliquots were removed and used as inocula for the LIM broth and Carrot broth cultures. 50µl of buffer was transferred to the lysis tube for PCR testing. Lysis is achieved by both the chemical contents of the tube and a 2 minute heating step at 95 C. A 1.5µl sample of lysate was directly added to the SmartCycler® reaction tube for testing on the SmartCycler® instrument

Statistical Analysis: True positives (TP) were defined as those specimens positive by any method that were confirmed to be GBS by latex agglutination performed of colonies suspicious for GBS detected after intensive scrutiny of blood agar plates inoculated from 24h StrepB Carrot Broth™ and examined after 24 and 48 h incubation. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated manually for each of the methods examined.

RESULTS

Thirty-one of 120 (25.8%) specimens were confirmed to be TP. Sensitivities for the IDI-Strep B™ SmartCycler®, standard LIM broth method and Carrot Broth™ were 83.9% (26/31), 87.1% (27/31), 93.5% (29/31) respectively **(Table 1)**. 107 specimens were in agreement by all three methods (21 positive, 86 negative). 13 specimens gave disparent results among the three methods **(Table 2)**. 10 of the 13 disparent results were confirmed positive for GBS by culture and therefore designated TP: 8 were detected by Carrot Broth™ ; 6 by standard lab culture; and 5 by the IDI-Strep B™ SmartCycler® assay. For 3 specimens, GBS was not able to be isolated by intense examination of Carrot Broth subculture plates and were designated as false positives There were two false positives with the SmartCycler® in which no GBS was able to be identified in culture. There was one false positive by the clinical laboratory (LIM broth culture) that was negative by the other two methods in which GBS was not able to be identified by culture of the Carrot broth specimen or by repeat inoculation in LIM broth from original frozen buffer sample. Of the 5 specimens not detected by direct real-time PCR all 5 were positive by real-time PCR after incubation in Carrot Broth™. Real-time PCR using the IDI-Strep B™ SmartCycler® assay of specimens that had incubated in Carrot Broth™ for 24-48 hours resulted in 20 false positive GBS results from 89 samples (22.5 %) in which GBS was not isolated by vigorous examination of broth subculture plates. There was a single (1/31 = 3.2%) non-hemolytic GBS isolated in this study. This specimen was reported as positive by the IDI-Strep B™ SmartCycler® assay and the standard LIM broth culture by the laboratory staff, however, this specimen did not produce a color change in the Carrot Broth™ assay.

TABLE 1 n=120	Direct PCR	Carrot Broth™	LIM Broth Culture
TP	26	29	27
FP	2	0	1
TN	87	89	88
FN	5	2	4
Sensitivity (%)	83.9	93.5	87.1
Specificity (%)	97.8	100	98.9
PPV (%)	92.9	100	96.4
NPV (%)	94.6	97.8	95.7
Accuracy (%)	94.2	98.3	95.8

RESULTS

TABLE 2 Spec. No.	DIRECT PCR	CARROT BROTH™ COLOR	LIM CULTURE	CARROT BROTH™ CULTURE
41	POS	POS	NEG	POS
18	POS	POS	NEG	POS
112	POS	POS	NEG	POS
45	NEG	POS	POS	POS
69	NEG	POS	POS	POS
74	NEG	POS	POS	POS
21	NEG	POS	POS	POS
118	NEG	POS	NEG	POS
84	POS	NEG	POS	POS
63	POS	NEG	POS	POS
13	POS	NEG	NEG	NEG
5	POS	NEG	NEG	NEG
91	NEG	NEG	POS	NEG

CONCLUSIONS

- Carrot Broth™ was the best performing antepartum assay and was superior to standard culture for detection of GBS at 35-37 weeks gestation.
- The IDI-Strep B™ SmartCycler® assay was not as sensitive as the Carrot Broth™ assay and would therefore have limited use in the antepartum setting without a reliable method of retesting negative results which may make this approach cost prohibitive
- Real-time PCR using the IDI-Strep B™ SmartCycler® assay may be useful for intrapartum detection of GBS with sensitivities comparable to LIM Broth culture and results available in 2 hrs.