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Detection of GBS Directly from ESwab Collected Samples Using the BD MAX™ GBS Assay

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Amended Abstract

The standard culture and molecular-based Group B Streptococcus (GBS) screening method utilizes initial growth of GBS in Lim Broth followed by an acceptable means of identification. The BD MAX™ GBS assay, performed on the BD MAX™ System, can provide results in approximately 2.5 hours after the initial incubation (≥ 18 hours) of vaginal and rectal swabs in Lim Broth culture. The objective of this study was to evaluate the detection of GBS directly from ESwab* collected samples, using the BD MAX™ GBS assay after two different time points: (a) 0h- when the sample arrives in the laboratory; and (b) 24h - after ESwab is held at 25°C for 18-24h. **Methods**: A total of 163 vaginal and rectal ESwab samples were tested using the BD MAX GBS assay in three different ways. First, a 400µL aliquot of the ESwab medium was inoculated directly into the BD MAX GBS Sample Preparation Reagent and PCR was performed. Second, a 200µL aliquot of the ESwab medium was inoculated into Lim Broth and incubated for ≥18 hours at 37°C; PCR was performed using 15µL of the enriched Lim Broth culture. Lastly, PCR was performed using a 200µL aliquot of the remaining ESwab medium after being held for 18-24 hours at room temperature. All Lim Broth enriched cultures were sub-cultured onto TSA II and CNA (100µL) and the plates were incubated at 35-37°C for 48h. Colonies resembling GBS were counted to determine CFU/mL and identified by Gram stain, catalase and the BD BBL™ Streptocard™ Acid Latex Test. GBS results from direct ESwab PCR at 0 & 24 hrs, Lim Broth PCR and culture were analyzed and compared. The sample collection time and the direct (0h) PCR run time were saved for all samples included in this study. **Results**: Out of 163 samples tested, 120 were negative and 35 were positive by all three PCR tests and culture. Eight ESwab samples had discordant results among the tests; discordant samples are described in table 2. An average of 7.9 hours (range between 0.2h to 23h) was calculated between sample collection and direct (0h) ESwab PCR test on receipt in the laboratory. **Conclusion**: The performance of direct ESwab PCR on receipt of samples in the laboratory and after 24 hours was excellent for the detection of GBS using the BD MAX GBS assay on the BD MAX System. Use of ESwab with the BD MAX GBS assay can potentially facilitate earlier detection of GBS in pregnant women by eliminating the overnight Lim Broth enrichment step.

*Use of ESwab samples with the BD MAX GBS assay is outside of package insert claims.

Introduction

Group B Streptococcus (GBS) can cause severe disease in a newborn and is known to be a leading cause of life threatening bacterial infections in newborns. The current standard of care for preventing neonatal GBS disease is screening pregnant women at 35-37 weeks of gestation to determine their GBS colonization status. Most GBS testing is performed by culture and can take up to 48 hours for definitive identification of GBS following the initial ≥ 18 hours incubation of vaginal and rectal swabs in a selective broth medium. The BD MAX GBS Assay, as implemented on the BD MAX System, can provide results from up to 24 specimens in approximately 2.5hrs after the initial ≥ 18 hour incubation/enrichment step.

We believe the combination of ESwab and BD MAX GBS Assay can improve and speed up the detection of GBS in pregnant women by skipping the LIM broth inoculation and the ≥ 18h incubation steps. The objective of this study was to evaluate the detection of GBS directly from ESwab collected samples, using the BD MAX™ GBS assay after two different time points: (a) 0h- when the sample arrives in the laboratory; and (b) 24h - after ESwab is held at 25°C for 18-24h

Methods

A total of 163 clinician-collected rectal-vaginal ESwab specimens submitted for GBS test at TGH were included in this study. Tests were performed as follow:

Standard of Care Procedure:

1. Vaginal and rectal ESwab collected sample was transported to the laboratory
2. In the laboratory, the ESwab was vortexed with the transport medium (Amies Liquid) and an aliquot of 200µL of the medium was inoculated into selective LIM Broth [Todd-Hewitt Broth supplemented with colistin (10µg/mL) and nalidixic acid (15µg/mL)].
3. Inoculated LIM Broth culture was incubated for ≥18 hours at 37°C
4. After incubation, a 15µL aliquot of the broth was mixed with BD MAX GBS Sample Preparation Reagent and processed on the BD MAX System using the BD MAX GBS Assay.
5. 100µL of the LIM Broth culture, after ≥ 18 hours at 35-37°C, on the agar surfaces of inoculated onto TSA II and CNA plates .
6. Plates were incubated at 35-37°C for 48h. Colonies resembling GBS were identified using Gram stain, catalase and the BD BBL Streptocard™ Acid Latex Test (BD, MD).

ESwab 0h Procedure:

1. A 400µL aliquot of the ESwab was inoculated direct into the BD MAX GBS Sample Preparation Reagent. Sample was processed on the BD MAX System using the BD MAX GBS Assay.
2. Sample collection time and direct (0h) PCR run time were saved for all samples included in this study. In the end, an average of time between sample collection and direct (0h) PCR run was calculated.

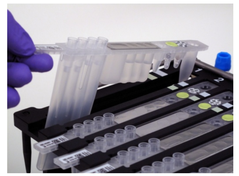
ESwab 24h Procedure:

1. The remaining ESwab medium was held for 24 hours at room temperature (25°C)
2. After that, a 200µL aliquot of the ESwab medium was mixed with BD MAX GBS Sample Preparation Reagent and processed on the BD MAX System using the BD MAX GBS Assay.

Figure 1. BD MAX™ Instrument Operation

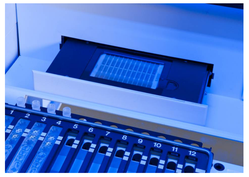
1. Pipetting step: from ESwab or Lim Broth to SBT

2. Load reagents and specimens

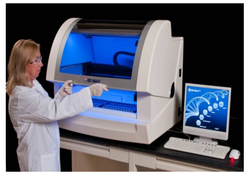


3. Create worklist

4. Place racks and cartridges on BD MAX™



5. Close door to initiate run



Results

Table 1. GBS Results for the four GBS test performed:

Results	LIM Broth PCR	0h* ESwab PCR	24h ESwab PCR	GBS Culture
Positive	42	38	40	36
Negative	121	125	123	127

*An average of 7.9 hours (range between 0.2h to 23h) was calculated between sample collection and direct (0h) ESwab PCR test on receipt in the laboratory.

Table 2. Threshold (Ct*) Values of Discrepant Samples:

Sample	LIM Broth PCR	0h ESwab PCR	24h ESwab PCR	GBS Culture
GBS 096	Neg.	Neg.	Ct=33	Neg.
GBS 052	Ct=25	Neg.	Ct=20	Neg.
GBS 124	Ct=25	Ct=20	Ct=21	Neg.
GBS 131	Ct=30	Ct=30	Ct=25	Neg.
GBS 137	Ct=40	Ct=25	Ct=25	Neg.
GBS 146	Ct=35	Neg.	Neg.	Neg.
GBS 166	Ct=30	Neg.	Neg.	Neg.
GBS 163	Ct=16	Neg.	Neg.	Pos.

* Ct is the number of cycles required for the fluorescent signal to cross the threshold in a real time PCR reaction. Ct levels are inversely proportional to the amount of target nucleic acid in the sample; the lower the Ct level the greater the amount of bacteria concentration, and vice-versa.

Table 3. LIM Broth PCR x ESwab 0h PCR Results:

LIM Broth PCR	0h* ESwab PCR Results	
	Positive	Negative
Positive	38	4
Negative	0	121

*An average of 7.9 hours (range between 0.2h to 23h) was calculated between sample collection and direct (0h) ESwab PCR test on receipt in the laboratory.

This study was supported by Copan and BD Diagnostics.

Table 4. LIM Broth PCR x ESwab 24h PCR Results:

LIM Broth PCR	24h ESwab PCR Results	
	Positive	Negative
Positive	39	3
Negative	1	120

CONCLUSIONS

1. This study was able to compare the performance of GBS detection in pregnant women ESwab collected samples using 4 different approaches: LIM Broth inoculated PCR; Direct ESwab 0h PCR, Direct ESwab 24h PCR and culture. All PCRs were performed on the BD MAX System, using the BD MAX GBS assay.

2. LIM Broth PCR (standard of care) and Direct ESwab PCRs (0h and 24h) displayed an agreement of 97.5%.

3. Eight samples had discrepant results among the tests. Out of them, 7 presented negative cultures and only 1, 5 and 3 of them presented LIM broth, 0h and 24h ESwab PCR results, respectively, negative for GBS.

4. These results made us suspect that discrepant samples may have been due to a very low number of bacteria present in these 7 samples.

5. One sample, however, had LIM broth PCR and culture results positive, and ESwab 0h and 24h results negative. Further studies will be performed on this sample in order to understand this discrepancy.

6. Overall, the performance of direct ESwab PCR on receipt of samples in the laboratory and after 24 hours was excellent for the detection of GBS using the BD MAX GBS assay on the BD MAX System.

7. The use of ESwab with the BD MAX GBS assay can potentially facilitate earlier detection of GBS in pregnant women by eliminating the overnight LIM Broth enrichment step.