CLINICAL EVALUATION OF THE PORTRAIT GBS ASSAY IN DETECTING *STREPTOCOCCUS AGALACTIAE* IN ENRICHED LIM BROTH CULTURES OF RECTOVAGINAL SWABS FROM PREGNANT WOMEN

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ABSTRACT

Objectives: *Streptococcus agalactiae* (Lancefield group *B Streptococcus*, GBS) is an important cause of neonatal sepsis and meningitis and may be prevented through the screening and treatment of pregnant women carriers. To evaluate the clinical performance of the Portrait GBS assay (Great Basin Scientific, Inc.) for detection of GBS in enriched LIM broth cultures of rectovaginal swabs from pregnant women, Portrait GBS results were compared to broth enriched culture and two FDA cleared molecular assays.

Methods: The Portrait GBS assay utilizes biotinylated primers to amplify a conserved region of the *cfb* gene of *S. agalactiae* which is subsequently hybridized to immobilized probes and detected by an optical change on the chip surface. Rectovaginal swabs submitted for GBS detection at three clinical sites were prospectively enrolled into the study. Swabs were cultured under standard conditions in LIM broth for ≥18 hours for enrichment of GBS. A true positive was defined by standard microbiological identification of GBS by Gram staining, catalase activity, and latex agglutination of subcultured isolates. Additionally, results were compared to two commercially available molecular methods for GBS detection: Cepheid Xpert® GBS LB (2 sites), and BD MAX™ GBS (1 site).

Results: A total of 518 rectovaginal swab LIM broth cultures were enrolled in the study with a GBS prevalence of 21.6% (112/518) based on culture data. Using standard microbiological techniques to define a true positive site 1 tested 120 specimens with a sensitivity of 100% (CI_{95}, 86.3-100.0%) and a specificity of 95.5% (CI_{95}, 87.2-98.4%); site 2 tested 222 specimens with a sensitivity of 95.7% (CI_{95}, 84.3-99.3%) and a specificity of 97.1% (CI_{95}, 93.1-98.9%); site 3 tested 176 specimens with a sensitivity of 100% (CI_{95}, 87.4-100%) and a specificity of 95.1% (CI_{95}, 89.7-97.8%). Overall the Portrait GBS assay had a sensitivity of 98.2% (CI_{95}, 93.1-99.7%) and a specificity of 96.1% (CI_{95}, 93.4-97.6%). This is compared to the Cepheid Xpert® GBS LB sensitivity of 96.2% (CI_{95}, 88.4-99.0%) and specificity of 98.5% (CI_{95}, 95.8-99.5%), and the BD MAX™ GBS sensitivity of 100% (CI_{95}, 87.4-100%) and specificity of 94.4% (CI_{95}, 88.8-97.4%). Of 16 false positive specimens with the Portrait GBS assay, 10 (63%) were confirmed positive for GBS by either the Cepheid Xpert® GBS LB or the BD MAX™ GBS assay.

Conclusions: The Portrait GBS assay is a convenient, walk-away assay for the detection of GBS in enriched LIM broth cultures of rectovaginal swabs from pregnant women. It performed well in this clinical evaluation when compared to standard microbiological techniques with a sensitivity and specificity of 98.2% and 96.1% respectively. Its performance was statistically equivalent to other commercially available GBS molecular detection methods tested.

INTRODUCTION

Streptococcus agalactiae (Lancefield group *B Streptococcus*, GBS) is an important cause of early- and late-onset neonatal sepsis and meningitis, which respectively present on or before the sixth day of life, or later (1). The principal risk factor for developing early-onset neonatal GBS infection is maternal urogenital or gastrointestinal tract carriage of GBS (2, 3). Intrapartum antibiotic treatment of known GBS carriers has been shown to significantly reduce the risk of early-onset neonatal GBS infection (3-5).

Conventional methods for detecting carriage of GBS in pregnant women include enrichment and subculture of rectovaginal swabs collected at 35-37 weeks gestation. Among some of the potential drawbacks of subculture based methods are the inherent subjectivity of culture plate direct examination, inconsistent detection of non-hemolytic GBS isolates, lengthy culture incubation steps, and overgrowth of competing microbiota.

Alternatively, molecular methods for the detection of GBS in enriched LIM broth rectovaginal swab cultures may be employed. These platforms have the potential to realize higher laboratory efficiency and better clinical outcomes due to increased sensitivity and shortened turn-around-times compared to standard microbiological methods (6). The Portrait GBS assay is a fully automated, sample-to-result format that employs hot-start PCR for the specific amplification of the GBS *cfb* gene using biotin-labeled primers with subsequent hybridization and detection steps. All testing is performed in a closed-system, single-use cassette (Figure 1) on the Portrait instrument (Figure 2).

We present data from a multi-center clinical evaluation, which compared results of GBS detection by the Portrait GBS assay to standard microbiological identification and two commercially available, FDA-approved real-time PCR GBS detection methods in enriched LIM broth cultures: Cepheid Xpert® GBS LB (2 sites), and BD MAX™ GBS (1 site).

METHODS

Specimen selection: Excess enriched LIM broth cultures of rectovaginal swabs submitted for GBS screening of pregnant women at 35-37 weeks gestation were prospectively enrolled following routine processing per the standard-of-care at each of three test sites (herein referred to as sites-1, -2, and -3). All LIM broth cultures were inoculated with rectovaginal swabs and incubated at 35°C (+/-2°C) for ≥18 hours.

All participating clinical sites de-identified the clinical specimens and no patient information, other than patient age was collected or made available to the study sponsor. This study was conducted as per the April 25th, 2006, FDA guidance document "Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable." Each site was granted approval to conduct the study through their respective Institutional Review Boards (IRB).

Standard-of-care screening for GBS in rectovaginal swabs: Depending on the clinical site, enriched LIM broth cultures were tested using the Cepheid Xpert® GBS LB platform (Cepheid, Sunnyvale, CA) (sites-1 and -2) or the BD MAX™ GBS assay (Becton Dickinson, Franklin Lakes, NJ, USA) (site-3).

Portrait GBS assay detection of GBS in rectovaginal swabs: A 50µL volume of enriched LIM broth was pipetted into the Portrait GBS assay test cartridge (Great Basin Scientific, Inc., Salt Lake City, UT, USA) followed by additional procedures as stated per the manufacturer's instructions (sites-1, -2, and -3).

Standard microbiological identification of GBS: Within 30 minutes of performing the Portrait GBS assay, the enriched LIM broth was subcultured onto 5% sheep blood agar (SBA) and incubated for 24 hours at 35°C (+/-2°C) with 5% CO2. Colonies of catalase negative, Gram positive cocci were tested for Lancefield Group B antigen by SLIDEX® Strepto Plus latex agglutination (bioMerieux, Marcy-l'Etoile, France) to confirm GBS. If no growth was observed after 24 hours the plate was re-incubated, inspected at 48 hours, at which point was reported as no growth if none was seen (sites-1, -2, and -3).

Statistics: Standard microbiological testing was considered the gold standard for defining true positive and negative specimens when calculating sensitivity, specificity, positive and negative predictive values. Ninety-five percent confidence intervals (CI_{95}) were calculated by the efficient-score method. Cohen's Kappa coefficient was calculated for inter-rater agreement between the Portrait GBS assay versus both the Cepheid Xpert® GBS LB and the BD MAX™. Specimens with "invalid" test results were re-run on the respective platform until a valid result was obtained, which was used for statistical analysis.

Figure 1: The single-use Portrait assay cassette.

Figure 2: The fully automated Portrait assay instrument.

Test Site	No. Tested	Sensitivity (CI_{95})	Specificity (CI_{95})	PPV (CI_{95})	NPV (CI_{95})
1	120	100% (86.3-100%)	95.5% (87.2-98.4%)	88.6% (72.3-96.3%)	100% (94.1-100%)
2	222	95.7% (84.3-99.3%)	97.1% (93.1-98.9%)	90.0% (77.4-96.3%)	98.8% (95.4-99.8%)
3	176	100% (87.4-100%)	95.1% (89.7-97.8%)	82.9% (67.4-92.3%)	100% (96.6-100%)
Overall	**518**	**98.2% (93.1-99.7%)**	**96.1% (93.4-97.6%)**	**87.3% (79.9-92.3%)**	**99.5% (97.9-99.9%)**

Table 1: Enriched LIM broth cultures of rectovaginal swabs from pregnant women were tested for group *B Streptococcus* (GBS) by the Portrait GBS assay and compared to traditional microbiological techniques. Results of statistical analyses are listed. Abbreviations: 95% confidence interval (CI_{95}), positive predictive value (PPV), negative predictive value (NPV).

Test Site	No. Tested	Sensitivity (CI_{95})	Specificity (CI_{95})	PPV (CI_{95})	NPV (CI_{95})
1 (Cepheid)	120	90.3% (73.1-97.5%)	100% (94.4-100%)	100% (85.0-100%)	96.7% (89.2-99.1%)
2 (Cepheid)	222	100% (90.6-100%)	97.7% (93.9-99.3%)	92.2% (80.3-97.5%)	100% (97.3-100%)
Cepheid Overall	**342**	**96.2% (88.4-99.0%)**	**98.5% (95.8-99.5%)**	**94.9% (86.9-98.4%)**	**98.9% (96.3-99.7%)**
3 (BD MAX)	**176**	**100% (87.4-100%)**	**94.4% (88.8-97.4%)**	**81.0% (65.4-90.9%)**	**100% (96.5-100%)**

Table 2: Enriched LIM broth cultures of rectovaginal swabs from pregnant women were tested for group *B streptococcus* (GBS) by the standard-of-care assay at the test site, either the Cepheid Xpert® GBS LB or the BD MAX™ GBS assay, and compared to traditional microbiological techniques. Results of statistical analyses are listed. Abbreviations: 95% confidence interval (CI_{95}), positive predictive value (PPV), negative predictive value (NPV).

RESULTS

Portrait GBS assay versus standard microbiological and other commercial molecular methods: A total of 518 enriched rectovaginal cultures were prospectively enrolled and demonstrated a GBS prevalence of 21.6% (112/518) based on culture data. Using standard microbiological techniques to define true positives and negatives, site-1 tested 120 specimens using the Portrait GBS assay with a sensitivity of 100% (CI_{95}, 86.3-100.0%) and a specificity of 95.5% (CI95, 87.2-98.4%); site-2 tested 222 specimens with a sensitivity of 95.7% (CI_{95}, 84.3-99.3%) and a specificity of 97.1% (CI_{95}, 93.1-98.9%); and site-3 tested 176 specimens with a sensitivity of 100% (CI_{95}, 87.4-100%) and a specificity of 95.1% (CI_{95}, 89.7-97.8%). Overall, including all three test sites, the Portrait GBS assay demonstrated a sensitivity of 98.2% (CI_{95}, 93.1-99.7%) and a specificity of 96.1% (CI_{95}, 93.4-97.6%) (Table 1).

In addition to the Portrait GBS assay and standard microbiological techniques, each enriched rectovaginal culture was also tested by either Cepheid Xpert® GBS LB (sites-1 and -2) or BD MAX ™ GBS (site-3), as dictated by the standard-of-care for the test site. When compared to the Portrait GBS results, calculated Kappa coefficients for the Cepheid Xpert® GBS LB and BD MAX ™ GBS were 0.90 (CI_{95}, 0.85-0.96) and 0.98 (CI_{95}, 0.95-1) respectively.

Of the discrepant results between the Portrait GBS and standard microbiological techniques, 16 false positive specimens were identified. Ten (63%) of which were confirmed positive for GBS by either the Cepheid Xpert® GBS LB (*n*=3) or the BD MAX™ GBS (*n*=7) assay. Two false negative specimens were identified that tested positive by the Cepheid Xpert® GBS LB assay.

Cepheid Xpert® GBS LB versus standard microbiological methods: A total of 342 enriched rectovaginal cultures were tested by the Cepheid Xpert® GBS LB assay from sites-1 and -2. Again using standard microbiological techniques to define true positives and negatives, the Cepheid assay at site-1 demonstrated a sensitivity of 90.3% (CI_{95}, 73.1-97.5%) and a specificity of 100% (CI_{95}, 94.4-100%), and at site-2 a sensitivity of 100% (CI_{95}, 90.6-100%) and a specificity of 97.7% (CI_{95}, 93.9-99.3%). An overall sensitivity of 96.2% (CI_{95}, 88.4-99.0%) and a specificity of 98.5% (CI_{95}, 95.8-99.5%) were observed with the Cepheid Xpert® GBS LB assay (Table 2). Of the discrepant results between the Cepheid Xpert® GBS LB assay and standard microbiological techniques at site-2, 4 false positive were encountered; 3 of which also tested positive by the Portrait GBS assay. Site-1 did not observe any false positive results with the Cepheid assay. Three false negatives were observed at site-1 with the Cepheid Xpert® GBS LB assay, all three of which tested positive by the Portrait GBS assay.

BD MAX™ GBS versus standard microbiological methods: A total of 176 enriched rectovaginal cultures were tested by the BD MAX™ GBS assay at site-3. Using standard microbiological techniques to define true positives and negatives, the BD MAX™ GBS assay demonstrated a sensitivity of 100% (CI_{95}, 87.4-100%) and a specificity of 94.4% (CI_{95}, 88.8-97.4%) (Table 2). Of the discrepant results between the BD MAX™ GBS assay and standard microbiological techniques, 8 false positive were encountered; 7 of which also tested positive by the Portrait GBS assay. No False negatives were observed with the BD MAX™ GBS assay.

Invalid rate of the Portrait GBS assay: An overall initial invalid rate of 1.7% was observed during the study. Site-1 experienced the highest rate of invalid results at 6.7% (8/120), with sites-2 and -3 having experienced 0.45% (1/222) and 0% (0/176) respectively. Repeat testing of all specimens with an initial invalid test result ultimately agreed with standard microbiological techniques.

CONCLUSIONS

When compared to conventional microbiological methods the Portrait GBS assay demonstrated a sensitivity of 98.2% (CI95, 93.1-99.7%) and a specificity of 96.1% (CI_{95}, 93.4-97.6%), similar to published reports of other FDA-cleared molecular methods(7, 8).

Of 16 false positive Portrait GBS assay results, 10 (63%) were confirmed positive for GBS by either the Cepheid Xpert® GBS LB (*n*=3) or the BD MAX™ GBS (*n*=7) assay. Two false negative specimens were identified that tested positive by the Cepheid Xpert® GBS LB assay.

Results from the Portrait GBS assay demonstrated no statistical differences when compared to the Cepheid Xpert® GBS LB and the BD MAX™ GBS assays.

The Portrait GBS assay is a convenient, walk-away assay for the detection of GBS in enriched LIM broth cultures of rectovaginal swabs from pregnant women. It performed well when compared with standard microbiological and two FDA-approved commercially available platforms. Its use in the clinical laboratory is likely to be helpful in the detection of GBS in rectovaginal swabs from pregnant women to mitigate the risk of early-onset neonatal GBS infections.

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