Multicenter evaluation of BD Max™ GBS Assay for detection of Group B streptococcus in prenatal vaginal/rectal screening swabs from pregnant women

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ABSTRACT

A new integrated extraction and real time PCR-based system for detection of Group B streptococci (GBS) in antepartum screening samples enriched in LIM broth was compared to the CDC-recommended culture method. The BD Max™ GBS Assay exhibited acceptable sensitivity (95%) and specificity (96.7%) compared to culture in this multi-site evaluation.
Neonatal Group B Streptococcus (GBS) infections have decreased significantly over the past 4 decades, however GBS still remains one of the most common causes of neonatal sepsis in the United States (3). Current management guidelines recommend that all pregnant women be screened for vaginal/rectal GBS colonization at 35-37 weeks gestation, with those found to be colonized receiving intrapartum antibiotic prophylaxis.

While culture-based methods have historically been the gold standard for demonstrating GBS colonization, several recent studies have demonstrated the utility of PCR-based detection to be a sensitive and specific alternative (1, 2, 4-6, 8, 9). The BD Max™ GBS Assay (BDM) implemented on the BD Max™ System (previously known as the HandyLab® Jaguar system; BD-HandyLab, Ann Arbor, MI) is one such PCR-based alternative. The BD Max™ is a bench-top molecular diagnostic system, which provides fully automated clinical sample preparation, cell lysis, nucleic acid extraction, and mixing of nucleic acid with master mix reagents. With no user intervention, the system then dispenses the sample into a microfluidic chamber where real-time PCR amplification and detection is performed.

The goal of this 3-site investigational study was to compare the results obtained from BDM to those obtained by the CDC-recommended culture procedure, which served as the reference method (3). This study was designed to generate data necessary for 510(k) submission to the FDA, so the study design, reference method and evaluation criteria were performed as required by the FDA. Performance characteristics of the assay were derived from the results of 601 compliant specimens collected from antepartum women.
presenting for routine pre-natal screenings at clinical locations within the United States
(site 1, University of Michigan Health System, Ann Arbor, MI [UM]: 184 specimens; site
2, DCL Medical Laboratories, Indianapolis, IN [DCL]: 198 specimens; site 3, TriCore
Reference Laboratories, Albuquerque, NM [TRL]: 219 specimens). The vaginal/rectal
swab specimens were inoculated in LIM broth (DCL and UM: ThermoFisher-Remel,
Lenexa, KS; TRL, Becton-Dickinson, Sparks, MD) and incubated 18-24 hours (as per
protocol) prior to testing by either method. The growth from each LIM broth was
subcultured to a sheep blood agar plate and incubated up to 48 hrs. Colonies with
morphology and color suggestive of GBS (both hemolytic and non-hemolytic) were
Gram stained, tested for catalase production, and confirmed as GBS using latex
agglutination (DCL and TRL: PathoDx Strep Grouping reagents, ThermoFisher-Remel;
UM: bioMérieux SLIDEX, Durham, NC) and/or CAMP test. BDM (cfb gene target,
limit of detection 200 CFU GBS/mL of sample preparation reagent, data not shown) was
performed on a residual 15 µL aliquot of LIM broth and when possible, an alternate,
validated PCR method was performed at each site (DCL: IDI Strep B™ Assay performed
on Cepheid SmartCycler®, cfb gene target; TRL: Roche analyte-specific reagent
performed on Roche LightCycler, pstI gene target; UM: Cepheid Smart GBS assay
performed on Cepheid SmartCycler®, proprietary GBS-specific target) and used to
resolve discrepancies between BDM and culture. This alternate PCR method was only
performed on the discrepant specimens. LIM broths were stored at 4°C for up to 7 days
prior to BDM testing. Stability studies demonstrated no loss of analytic performance for
samples stored in this manner (data not shown). In addition to the alternate PCR assay,
“false negative” specimens were re-tested at each site using BDM and “false positive”
specimens were re-subcultured from the original LIM broth at each site. The repeat PCR and culture testing was performed for informational purposes only, and was not used to adjust the observed performance characteristics. This study was approved by Institutional Review Boards as appropriate by the performance sites.

Table 1 shows a summary of the combined site results obtained using BDM compared to culture. Overall agreement was 96.3%. Thirteen of the 15 BDM-positive, culture-negative specimens (“false positives”) were available for testing by the alternate PCR assay, and eight tested positive. Twelve of 15 “false positives” were also re-cultured and 10/12 again yielded negative results. The two culture positive specimens in this subset were also positive when tested by the alternate PCR assay. These results are consistent with previous studies that have demonstrated an increased rate of GBS detection from broth-enriched specimens using PCR compared to culture (7), likely reflecting the detection of bacteria present at levels below the limits of detection for culture. Furthermore, there is a low probability that “false positives” were due to non-specific amplification as no significant cross-reactivity was demonstrated against a panel of 127 non-target pathogens where 119 viable bacteria, fungi and viruses, and 8 genomic DNA samples were tested (see supplemental data for the complete list of organisms tested). Five of the seven culture-positive, BDM-negative specimens (“false negatives”) were retested by the alternate PCR assay, and three tested negative. Four of the seven “false negatives” were also retested using BDM and three yielded a negative result a second time. It is unlikely that these “false-negative” BDM results are the result of sample degradation or overgrowth from competing organisms during storage, as the stability
studies mentioned above demonstrated the maintenance of integrity of positive signal for specimens stored under these conditions. The “false-negatives” are most likely a result of sampling error due to low levels of organism in the sample, as evidenced by the fluctuation in results following repeat testing by both culture and PCR. Although the BDM “false-negative” rate might be reduced by increasing the volume of input sample from 15 µL, this volume of sample was found by BD-HandyLab through internal development studies to be optimal for BDM processing in the context of the biological amplification of target from LIM broth enrichment (data not shown).

Table 2 shows the site-specific and overall performance characteristics of BDM compared to culture. Importantly, the prevalence of disease was very similar among the sites, and there did not appear to be substantial differences in performance of the assay among the sites. Overall, BDM performed well compared to culture, exhibiting 95% sensitivity and 96.7% specificity.

The screening and detection method currently considered the ‘gold standard’ to assess GBS colonization status is the culture procedure recommended by the CDC in 2002. This technique includes a variety of confirmatory tests that are to be performed on suspected colonies following inoculation and incubation of vaginal/rectal swab specimens into a selective broth medium (3). One benefit of this method is that the GBS isolate is readily available for susceptibility testing often ordered by health care providers—however, there are also limitations. The culture technique requires hands-on time by trained laboratory personnel; technologists must be qualified to set-up and
examine the plates for suspected GBS colonies, which may or may not be present among
other bacterial growth. Culture is also slow to yield results – often requiring 48-72 hours
for GBS identification (7). Although this standard has recognized limitations, it was
necessary to use in this evaluation as it was required by the FDA for the 510(k)
submission.

Although modifications to the CDC-recommended testing method have improved the
sensitivity of culture-based methods, molecular test methods utilizing nucleic acid
amplification have emerged as an alternative approach to diagnostic testing. Several
PCR assays used to determine GBS colonization status in pregnant women have
demonstrated substantial improvements in time to detection, without compromising
performance characteristics. Studies evaluating the performance of PCR for GBS from
direct patient specimens have demonstrated equivalent rates of GBS detection compared
to broth-enriched cultures (4, 5, 8, 9). In addition, studies evaluating the performance of
PCR from broth-enriched specimens have exhibited variability in detection rates of GBS
compared to broth-enriched cultures. Block, et al, showed similar detection rates
between PCR and culture (2), while Goodrich and Miller showed that improved
detection with PCR depended on the method used (6). However, Rallu et al,
demonstrated 1.5 – 2.5 fold enhanced detection by PCR compared to broth-enriched
cultures (7).

Cost differences between conventional and molecular methods can be a barrier for many
laboratories desiring to implement molecular testing. Although specific pricing
information is not yet available for the BD Max GBS assay, reagent costs are expected to be approximately $25 US/test, whereas the list prices for the culture reagents used in this study totaled approximately $9 US/test. While there is a substantial difference in these costs, final costs for the user will be impacted by volume and workload differences for each method. Ultimately, these costs, in addition to reimbursement rates, test performance, and workflow differences, will all need to be considered by the user in making a final decision regarding the appropriate method/platform to utilize for testing.

Nevertheless, based on the data generated in this multi-center study, the clinical performance of the BD Max™ GBS Assay as implemented on the BD Max™ platform demonstrated acceptable sensitivity (95.0%) and specificity (96.7%), with a slightly increased detection rate with PCR compared to culture (148/601 [25%] vs. 140/601 [23%], respectively), which is consistent with the finding of the studies mentioned above. The self-contained workstation is designed to accommodate on-demand and batch workflows. In addition, the level of technical expertise required to operate this system is lower than that required for most other currently available molecular platforms. Finally, it requires minimal laboratory space and can generate up to 24 real-time PCR results in approximately two hours. As new guidelines from the CDC emerge and include molecular testing as an alternative to culture for the detection of GBS (in press), the assay and platform described here could serve as an efficient, sensitive, and specific option for laboratories desiring to utilize a molecular method.

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The authors would like to thank Judy Stempien, Ben Berg and Jessica Soper for their expert technical assistance in the performance of this study. In support of this evaluation, BD-HandyLab provided reagents, supplies, instrumentation, and reimbursement for labor and travel expenses for each site to attend a meeting. The BD Max™ GBS Assay received FDA 510(k) clearance 05/2010. Conflicts of interest: J.R. is an employee of BD-HandyLab.

REFERENCES

Table 1. Summary comparison of BD Max™ GBS Assay to culture from all performance sites

<table>
<thead>
<tr>
<th>GBS Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Max™ GBS Assay</td>
<td>Positive</td>
<td>133</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7</td>
<td>446</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>140</td>
<td>461</td>
</tr>
</tbody>
</table>

Table 2. Site-specific and overall performance characteristics of BD Max™ GBS Assay compared to culture

<table>
<thead>
<tr>
<th>Performance parameter, % (95% C.I.):</th>
<th>Prevalence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NPV&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>20.0</td>
<td>97.4 (86.2 – 99.9)</td>
<td>96.6 (92.2 – 98.9)</td>
<td>86.4 (80.2 – 90.8)</td>
<td>98.4 (97.2 – 99.1)</td>
</tr>
<tr>
<td>Site 2</td>
<td>25.1</td>
<td>92.0 (80.8 – 97.8)</td>
<td>95.9 (91.4 – 98.5)</td>
<td>89.5 (84.5 – 93.0)</td>
<td>97.9 (96.3 – 98.8)</td>
</tr>
<tr>
<td>Site 3</td>
<td>23.6</td>
<td>96.2 (86.8 – 99.5)</td>
<td>97.6 (94.0 – 99.3)</td>
<td>88.7 (83.4 – 92.4)</td>
<td>98.0 (96.6 – 98.9)</td>
</tr>
<tr>
<td>Overall</td>
<td>23.0</td>
<td>95.0 (90.0 – 98.0)</td>
<td>96.7 (94.7 – 98.2)</td>
<td>88.3 (82.9 – 92.2)</td>
<td>98.1 (96.7 – 98.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on all compliant reference culture results
<sup>b</sup>Positive predictive value
<sup>c</sup>Negative predictive value