Multicenter Evaluation of the BD Max GBS Assay for Detection of Group B Streptococci in Prenatal Vaginal and Rectal Screening Swab Specimens from Pregnant Women^{▽†}

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Received 11 May 2010/Returned for modification 14 June 2010/Accepted 26 August 2010

A new integrated extraction and real-time PCR-based system for the detection of group B streptococci 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 those of the culture method in this multisite evaluation.

The number of neonatal group B streptococcus (GBS) infections has 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 to 37 weeks of 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 as a sensitive and specific alternative (1, 2, 4–6, 8, 9). The BD Max GBS assay (BDM) implemented using 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 system is a benchtop 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 are performed.

The goal of this three-site investigational study was to compare the results obtained by BDM to those obtained by the CDC-recommended culture procedure, which served as the reference method (3). This study was designed to generate the 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 prenatal screenings at clinical locations within the United States (site 1, University of Michigan Health System [UM], Ann Arbor, MI, collected 184 specimens; site 2, DCL Medical Laboratories [DCL], Indianapolis, IN, collected 198 specimens; and site 3, TriCore Reference Laboratories [TRL], Albuquerque, NM, collected 219 specimens). The vaginal/rectal swab specimens were inoculated in Lim broth (DCL and UM obtained broth from Thermo Fisher-Remel, Lenexa, KS; TRL obtained broth from Becton-Dickinson, Sparks, MD) and incubated for 18 to 24 h (as per protocol) prior to testing by either method. The growth obtained with each Lim broth was subcultured onto a sheep blood agar plate and incubated for up to 48 h. Colonies with morphology and color suggestive of GBS (both hemolytic and nonhemolytic) were Gram stained, tested for catalase production, and confirmed as GBS using latex agglutination (DCL and TRL used PathoDX Strep Grouping reagents, Thermo Fisher-Remel; UM used Slidex, bioMérieux, Durham, NC) and/or CAMP testing. BDM (*cfb* gene target and limit of detection of 200 CFU of GBS/ml of sample preparation reagent) (data not shown) was performed using a residual 15- μ l aliquot of Lim broth, and when possible, an alternate, validated PCR method was performed at each site (at DCL, the IDI-Strep B assay was performed on the Cepheid SmartCycler system with a *cfb* gene target; at TRL, the Roche analyte-specific reagent was used on the Roche LightCycler system with a *pstI* gene target; and at UM, the Cepheid Smart GBS assay was performed on the Cepheid SmartCycler system, with a proprietary GBS-specific target) and used to resolve discrepancies between BDM and the culture method. This alternate PCR method was performed only using 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 in samples stored in this manner (data not shown). In addition to the alternate PCR assay, “false-negative” specimens were retested at each site using BDM, and “false-positive” specimens were resubcultured 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

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† Supplemental material for this article may be found at <http://jcm.asm.org/>.

[▽] Published ahead of print on 8 September 2010.

TABLE 1. Summary comparison of BDM and culture from all performance sites

Result of BDM	No. of GBS cultures with indicated result		Total
	Positive	Negative	
Positive	133	15	148
Negative	7	446	453
Total	140	461	601

characteristics. This study was approved by Institutional Review Boards, as appropriate, of the performance sites.

Table 1 shows a summary of the combined site results obtained using BDM and the culture-based method. Overall agreement was 96.3%. A total of 13 of the 15 BDM-positive, culture-negative specimens ("false positives") were available for testing by the alternate PCR assay, and 8 tested positive. A total of 12 of the 15 "false positives" were also recultured, and 10 of the 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 those from previous studies that have demonstrated an increased rate of GBS detection with broth-enriched specimens using PCR compared to that with 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 nonspecific amplification, as no significant cross-reactivity was demonstrated against a panel of 127 nontarget pathogens in which 119 viable bacteria, fungi, and viruses and 8 genomic DNA samples were tested (see the supplemental material for a 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 for a second time. It is unlikely that these "false-negative" BDM results are the result of sample degradation or overgrowth of competing organisms during storage, as the stability studies mentioned above demonstrated the maintenance of integrity of positive signals for specimens stored under these conditions. The "false negatives" are most likely a result of sampling error due to low levels of the 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 the input sample from 15 μ l, 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 the target from Lim broth enrichment (data not shown).

Table 2 shows the site-specific and overall performance characteristics of BDM compared to those of the culture-based method. Importantly, the prevalences of disease were 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 for assessing 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 the 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 to 72 h 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 testing methods utilizing nucleic acid amplification have emerged as alternative approaches 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 detection using direct patient specimens have demonstrated rates of GBS detection equivalent to those of broth-enriched cultures (4, 5, 8, 9). In addition, studies evaluating the performance of PCR using broth-enriched specimens have exhibited variability in the detection rates of GBS compared to broth-enriched cultures. Block et al. showed similar detection rates between PCR and culture (2), while Goodrich

TABLE 2. Site-specific and overall performance characteristics of BDM compared to those of culture

Site	% performance parameter (95% CI) ^d				
	Prevalence ^a	Sensitivity	Specificity	PPV ^b	NPV ^c
1	20.0	97.4 (86.2–99.9)	96.6 (92.2–98.9)	86.4 (80.2–90.8)	98.4 (97.2–99.1)
2	25.1	92.0 (80.8–97.8)	95.9 (91.4–98.5)	89.5 (84.5–93.0)	97.9 (96.3–98.8)
3	23.6	96.2 (86.8–99.5)	97.6 (94.0–99.3)	88.7 (83.4–92.4)	98.0 (96.6–98.9)
Overall	23.0	95.0 (90.0–98.0)	96.7 (94.7–98.2)	88.3 (82.9–92.2)	98.1 (96.7–98.9)

^a Based on all compliant reference culture results.

^b Positive predictive value.

^c Negative predictive value.

^d CI, confidence interval.

and Miller showed that improved detection with PCR depended on the method used (6). However, Rallu et al. demonstrated 1.5- to 2.5-fold enhanced detection by PCR compared to that by the broth-enriched culture method (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/test, whereas the list prices for the culture reagents used in this study totaled approximately \$9/test. While there is a substantial difference in these costs, the final cost to the user will be impacted by the 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 multicenter 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 that of culture (148/601 [25%] compared to 140/601 [23%], respectively), which is consistent with the findings 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 those 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 2 h. As new guidelines from the CDC emerge and include molecular testing as an alternative to culture for the detection of GBS, the assay and platform described here could serve as an efficient, sensitive, and specific option for laboratories desiring to utilize a molecular method.

We 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 in May 2010.

J.R. is an employee of BD-HandyLab.

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