JCM Accepts, published online ahead of print on 8 September 2010
J. Clin. Microbiol. doi:10.1128/JCM.00947-10
Copyright © 2010, American Society for Microbiology and/or the Listed Authors/Institutions. All Rights Reserved.

JCM Accepts published online ahead of print

1 Multicenter evaluation of BD Max™ GBS Assay for detection of Group B streptococcus
2 in prenatal vaginal/rectal screening swabs from pregnant women
3
4 Jennifer Riedlinger[1], Safedin H. Beqaj[2], Marsha A. Milish[2], Stephen Young[3,4], Rebecca
5 Smith[3], Monique Dodd[3], Rosemary E. Hankerd[5], William D. LeBar[5], and Duane W.
6 Newton[5*]
7
8 [1]BD-HandyLab, Ann Arbor, MI
9 [2]DCL Medical Laboratories, Indianapolis, IN
10 [3]TriCore Reference Laboratories, Albuquerque, NM
11 [4]Department of Pathology, University of New Mexico, Albuquerque, NM
12 [5]Department of Pathology, University of Michigan Health System, Ann Arbor, MI
13
14 Running title: BD Max™ detection of GBS
15
16 Key words: Group B streptococcus, PCR, automated, neonatal sepsis, BD Max
17
18 Word count: 1474
19

JCM Accepts published online ahead of print

1 *Corresponding author:
2 Duane W. Newton, Ph.D., D(ABMM)
3 Department of Pathology
4 University of Michigan Health System
5 1500 E. Medical Center Dr., UH2G332
6 Ann Arbor, MI 48109-5054
7 Phone: 734-936-6847
8 Fax: 734-647-9093
9 Email: dnewton@med.umich.edu

JCM Accepts published online ahead of print

1 ABSTRACT

2

3 A new integrated extraction and real time PCR-based system for detection of Group B
4 streptococci (GBS) in antepartum screening samples enriched in LIM broth was
5 compared to the CDC-recommended culture method. The BD Max™ GBS Assay
6 exhibited acceptable sensitivity (95%) and specificity (96.7%) compared to culture in this
7 multi-site evaluation.

JCM Accepts published online ahead of print

1 Neonatal Group B Streptococcus (GBS) infections have decreased significantly over the
2 past 4 decades, however GBS still remains one of the most common causes of neonatal
3 sepsis in the United States (3). Current management guidelines recommend that all
4 pregnant women be screened for vaginal/rectal GBS colonization at 35-37 weeks
5 gestation, with those found to be colonized receiving intrapartum antibiotic prophylaxis.
6
7 While culture-based methods have historically been the gold standard for demonstrating
8 GBS colonization, several recent studies have demonstrated the utility of PCR-based
9 detection to be a sensitive and specific alternative (1, 2, 4-6, 8, 9). The BD Max™ GBS
10 Assay (BDM) implemented on the BD Max™ System (previously known as the
11 HandyLab® Jaguar system; BD-HandyLab, Ann Arbor, MI) is one such PCR-based
12 alternative. The BD Max™ is a bench-top molecular diagnostic system, which provides
13 fully automated clinical sample preparation, cell lysis, nucleic acid extraction, and mixing
14 of nucleic acid with master mix reagents. With no user intervention, the system then
15 dispenses the sample into a microfluidic chamber where real-time PCR amplification and
16 detection is performed.
17
18 The goal of this 3-site investigational study was to compare the results obtained from
19 BDM to those obtained by the CDC-recommended culture procedure, which served as
20 the reference method (3). This study was designed to generate data necessary for 510(k)
21 submission to the FDA, so the study design, reference method and evaluation criteria
22 were performed as required by the FDA. Performance characteristics of the assay were
23 derived from the results of 601 compliant specimens collected from antepartum women

JCM Accepts published online ahead of print

1 presenting for routine pre-natal screenings at clinical locations within the United States
2 (site 1, University of Michigan Health System, Ann Arbor, MI [UM]: 184 specimens; site
3 2, DCL Medical Laboratories, Indianapolis, IN [DCL]: 198 specimens; site 3, TriCore
4 Reference Laboratories, Albuquerque, NM [TRL]: 219 specimens). The vaginal/rectal
5 swab specimens were inoculated in LIM broth (DCL and UM: ThermoFisher-Remel,
6 Lenexa, KS; TRL, Becton-Dickinson, Sparks, MD) and incubated 18-24 hours (as per
7 protocol) prior to testing by either method. The growth from each LIM broth was
8 subcultured to a sheep blood agar plate and incubated up to 48 hrs. Colonies with
9 morphology and color suggestive of GBS (both hemolytic and non-hemolytic) were
10 Gram stained, tested for catalase production, and confirmed as GBS using latex
11 agglutination (DCL and TRL: PathoDx Strep Grouping reagents, ThermoFisher-Remel;
12 UM: bioMérieux SLIDEX, Durham, NC) and/or CAMP test. BDM (*cfb* gene target,
13 limit of detection 200 CFU GBS/mL of sample preparation reagent, data not shown) was
14 performed on a residual 15 μL aliquot of LIM broth and when possible, an alternate,
15 validated PCR method was performed at each site (DCL: IDI Strep B™ Assay performed
16 on Cepheid SmartCycler®, *cfb* gene target; TRL: Roche analyte-specific reagent
17 performed on Roche LightCycler, *pstI* gene target; UM: Cepheid Smart GBS assay
18 performed on Cepheid SmartCycler®, proprietary GBS-specific target) and used to
19 resolve discrepancies between BDM and culture. This alternate PCR method was only
20 performed on the discrepant specimens. LIM broths were stored at 4°C for up to 7 days
21 prior to BDM testing. Stability studies demonstrated no loss of analytic performance for
22 samples stored in this manner (data not shown). In addition to the alternate PCR assay,
23 "false negative" specimens were re-tested at each site using BDM and "false positive"

JCM Accepts published online ahead of print

1 specimens were re-subcultured from the original LIM broth at each site. The repeat PCR
2 and culture testing was performed for informational purposes only, and was not used to
3 adjust the observed performance characteristics. This study was approved by
4 Institutional Review Boards as appropriate by the performance sites.
5
6 Table 1 shows a summary of the combined site results obtained using BDM compared to
7 culture. Overall agreement was 96.3%. Thirteen of the 15 BDM-positive, culture-
8 negative specimens ("false positives") were available for testing by the alternate PCR
9 assay, and eight tested positive. Twelve of 15 "false positives" were also re-cultured and
10 10/12 again yielded negative results. The two culture positive specimens in this subset
11 were also positive when tested by the alternate PCR assay. These results are consistent
12 with previous studies that have demonstrated an increased rate of GBS detection from
13 broth-enriched specimens using PCR compared to culture (7), likely reflecting the
14 detection of bacteria present at levels below the limits of detection for culture.
15 Furthermore, there is a low probability that "false positives" were due to non-specific
16 amplification as no significant cross-reactivity was demonstrated against a panel of 127
17 non-target pathogens where 119 viable bacteria, fungi and viruses, and 8 genomic DNA
18 samples were tested (see supplemental data for the complete list of organisms tested).
19 Five of the seven culture-positive, BDM-negative specimens ("false negatives") were
20 retested by the alternate PCR assay, and three tested negative. Four of the seven "false
21 negatives" were also retested using BDM and three yielded a negative result a second
22 time. It is unlikely that these "false-negative" BDM results are the result of sample
23 degradation or overgrowth from competing organisms during storage, as the stability

JCM Accepts published online ahead of print

1 studies mentioned above demonstrated the maintenance of integrity of positive signal for
2 specimens stored under these conditions. The "false-negatives" are most likely a result of
3 sampling error due to low levels of organism in the sample, as evidenced by the
4 fluctuation in results following repeat testing by both culture and PCR. Although the
5 BDM "false-negative" rate might be reduced by increasing the volume of input sample
6 from 15 μL, this volume of sample was found by BD-HandyLab through internal
7 development studies to be optimal for BDM processing in the context of the biological
8 amplification of target from LIM broth enrichment (data not shown).
9
10 Table 2 shows the site-specific and overall performance characteristics of BDM
11 compared to culture. Importantly, the prevalence of disease was very similar among the
12 sites, and there did not appear to be substantial differences in performance of the assay
13 among the sites. Overall, BDM performed well compared to culture, exhibiting 95%
14 sensitivity and 96.7% specificity.
15
16 The screening and detection method currently considered the 'gold standard' to assess
17 GBS colonization status is the culture procedure recommended by the CDC in 2002.
18 This technique includes a variety of confirmatory tests that are to be performed on
19 suspected colonies following inoculation and incubation of vaginal/rectal swab
20 specimens into a selective broth medium (3). One benefit of this method is that the GBS
21 isolate is readily available for susceptibility testing often ordered by health care
22 providers—however, there are also limitations. The culture technique requires hands-on
23 time by trained laboratory personnel; technologists must be qualified to set-up and

JCM Accepts published online ahead of print

1 examine the plates for suspected GBS colonies, which may or may not be present among
2 other bacterial growth. Culture is also slow to yield results – often requiring 48-72 hours
3 for GBS identification (7). Although this standard has recognized limitations, it was
4 necessary to use in this evaluation as it was required by the FDA for the 510(k)
5 submission.
6
7 Although modifications to the CDC-recommended testing method have improved the
8 sensitivity of culture-based methods, molecular test methods utilizing nucleic acid
9 amplification have emerged as an alternative approach to diagnostic testing. Several
10 PCR assays used to determine GBS colonization status in pregnant women have
11 demonstrated substantial improvements in time to detection, without compromising
12 performance characteristics. Studies evaluating the performance of PCR for GBS from
13 direct patient specimens have demonstrated equivalent rates of GBS detection compared
14 to broth-enriched cultures (4, 5, 8, 9). In addition, studies evaluating the performance of
15 PCR from broth-enriched specimens have exhibited variability in detection rates of GBS
16 compared to broth-enriched cultures. Block, et al, showed similar detection rates
17 between PCR and culture (2), while Goodrich and Miller showed that improved
18 detection with PCR depended on the method used (6). However, Rallu et al,
19 demonstrated 1.5 – 2.5 fold enhanced detection by PCR compared to broth-enriched
20 cultures (7).
21
22 Cost differences between conventional and molecular methods can be a barrier for many
23 laboratories desiring to implement molecular testing. Although specific pricing

JCM Accepts published online ahead of print

1 information is not yet available for the BD Max GBS assay, reagent costs are expected to
2 be approximately $25 US/test, whereas the list prices for the culture reagents used in this
3 study totaled approximately $9 US/test. While there is a substantial difference in these
4 costs, final costs for the user will be impacted by volume and workload differences for
5 each method. Ultimately, these costs, in addition to reimbursement rates, test
6 performance, and workflow differences, will all need to be considered by the user in
7 making a final decision regarding the appropriate method/platform to utilize for testing.
8
9 Nevertheless, based on the data generated in this multi-center study, the clinical
10 performance of the BD Max™ GBS Assay as implemented on the BD Max™ platform
11 demonstrated acceptable sensitivity (95.0%) and specificity (96.7%), with a slightly
12 increased detection rate with PCR compared to culture (148/601 [25%] vs. 140/601
13 [23%], respectively), which is consistent with the finding of the studies mentioned above.
14 The self-contained workstation is designed to accommodate on-demand and batch
15 workflows. In addition, the level of technical expertise required to operate this system is
16 lower than that required for most other currently available molecular platforms. Finally,
17 it requires minimal laboratory space and can generate up to 24 real-time PCR results in
18 approximately two hours. As new guidelines from the CDC emerge and include
19 molecular testing as an alternative to culture for the detection of GBS (in press), the assay
20 and platform described here could serve as an efficient, sensitive, and specific option for
21 laboratories desiring to utilize a molecular method.
22
23 ACKNOWLEDGEMENTS

JCM Accepts published online ahead of print

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

1. **Bergeron, M. G., D. Ke, C. Menard, F. J. Picard, M. Gagnon, M. Bernier, M. Ouellette, P. H. Roy, S. Marcoux, and W. D. Fraser.** 2000. Rapid detection of group B streptococci in pregnant women at delivery. N Engl J Med **343:**175-9.
2. **Block, T., E. Munson, A. Culver, K. Vaughan, and J. E. Hryciuk.** 2008. Comparison of carrot broth- and selective Todd-Hewitt broth-enhanced PCR protocols for real-time detection of Streptococcus agalactiae in prenatal vaginal/anorectal specimens. J Clin Microbiol **46:**3615-20.
3. **Centers for Disease Control and Prevention.** 2002. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep **51:**1-22.
4. **Davies, H. D., M. A. Miller, S. Faro, D. Gregson, S. C. Kehl, and J. A. Jordan.** 2004. Multicenter study of a rapid molecular-based assay for the diagnosis of group B Streptococcus colonization in pregnant women. Clin Infect Dis **39:**1129-35.
5. **El Helali, N., J. C. Nguyen, A. Ly, Y. Giovangrandi, and L. Trinquart.** 2009. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis **49:**417-23.
6. **Goodrich, J. S., and M. B. Miller.** 2007. Comparison of culture and 2 real-time polymerase chain reaction assays to detect group B Streptococcus during antepartum screening. Diagn Microbiol Infect Dis **59:**17-22.
7. **Rallu, F., P. Barriga, C. Scrivo, V. Martel-Laferriere, and C. Laferriere.** 2006. Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B streptococcus carriage in pregnant women. J Clin Microbiol **44:**725-8.
8. **Uhl, J. R., E. A. Vetter, K. L. Boldt, B. W. Johnston, K. D. Ramin, M. J. Adams, P. Ferrieri, U. Reischl, and F. R. Cockerill, 3rd.** 2005. Use of the Roche LightCycler Strep B assay for detection of group B Streptococcus from vaginal and rectal swabs. J Clin Microbiol **43:**4046-51.

JCM Accepts published online ahead of print

1 9. **Wernecke, M., C. Mullen, V. Sharma, J. Morrison, T. Barry, M. Maher, and**
2 **T. Smith.** 2009. Evaluation of a novel real-time PCR test based on the ssrA gene
3 for the identification of group B streptococci in vaginal swabs. BMC Infect Dis
4 **9:**148.
5

JCM Accepts published online ahead of print

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

2
3

		GBS Culture		
		Positive	Negative	Total
BD Max™ GBS Assay	Positive	133	15	148
	Negative	7	446	453
	Total	140	461	601

4
5 Table 2. Site-specific and overall performance characteristics of BD Max™ GBS Assay compared to
6 culture
7

	Performance parameter, % (95% C.I.):				
	Prevalence[a]	Sensitivity	Specificity	PPV[b]	NPV[c]
Site 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)
Site 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)
Site 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)

8
9 [a]Based on all compliant reference culture results
10 [b]Positive predictive value
11 [c]Negative predictive value
12

13