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Clinical Performance of the BD MAX™ Enteric Bacterial Panel for Rapid Detection of *Salmonella* spp., *Shigella* spp., *Campylobacter (coli and jejuni)*, and Shiga Toxin-Producing *E. coli*

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REVISED ABSTRACT *

According to the WHO, diarrheal disease is a major health concern in both industrialized and developing countries, and ranks as the third leading cause of infectious disease deaths worldwide (1). The CDC lists *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and Shiga toxin-producing *E. coli* (STEC) as the top four causes of acute bacterial diarrheal disease (2). Current clinical methods for the detection of bacterial enteric pathogens are time-consuming and require significant skill. BD Diagnostics has developed four molecular-based assays contained in a single Enteric Bacterial Panel (EBP) for the rapid detection of *Campylobacter (coli* and *jejuni), Salmonella* spp., *Shigella* spp., and STEC *(*containing *stx1* and/or *stx2* genes*)* in clinical stool specimens. The panel is for use on the fully automated BD MAX™ System, which incorporates sample lysis, extraction, amplification, and detection all in one system. To evaluate the clinical performance characteristics of the EBP, a total of 283 fresh and archived clinical stool specimens were enrolled in the study. Enrollment criteria included a clinical culture result along with a matching alternative PCR result. The alternative PCR result was used to rule out target stability issues associated with archival clinical specimens. Results obtained with the EBP were compared against the reference method culture results. The overall Percent Positive Agreement (PPA) for all 4 assays was 98.2% (95% CI: 0.9484, 0.9963). Individually, STEC and *Shigella* achieved a PPA of 100% (21/21 and 32/32 respectively), *Campylobacter* achieved a PPA of 97.96% (48/49), and *Salmonella* achieved a PPA of 96.92% (63/65). Percent Negative Agreement for each assay was greater than 98%. The BD MAX™ Enteric Bacterial Panel offers a sensitive and time saving IVD alternative to traditional culture-based methods for diagnosing acute bacterial diarrheal disease caused by *Campylobacter (coli* and *jejuni), Salmonella* spp., *Shigella* spp., or Shiga toxin-producing *E. coli*. Moreover, the BD MAX™ Enteric Bacterial panel will allow laboratories to implement the 2009 CDC guidelines and the 2013 Joint Commissions requirment (Standard QSA.04.06,01) for the screening of patients for Shiga toxin-producing *E. coli strains* (3).

* Correction made regarding total enrolled specimens.

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2) **Centers for Disease Control and Prevention**. Major Pathogens, Expanded Tables. *CDC Estimates of Foodborne Illness in the United States*. [Online] 2011. http://www.cdc.gov/foodborneburden/PDFs/11_228412_Pitts_factsheet_tables_remediated.pdf;

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INTRODUCTION AND PURPOSE

Diarrheal disease results in an estimated 2 million deaths per year and remains as a leading cause of infectious disease deaths worldwide (4). Current clinical methods for the detection of enteric bacterial pathogens involve stool culture testing which is time consuming and requires significant skill. The majority (>90%) of cultures are negative and take at least 2 days before results are reported. In addition to creating a large burden for the laboratory, this greatly complicates patient management since specific causative agents are not identified when a patient is first seen by a physician (5).

To alleviate this burden, BD has developed a multiplexed, molecular-based Enteric Bacterial Panel containing four assays for the rapid detection of DNA from *Campylobacter (coli* and *jejuni), Salmonella* spp., Shigellosis disease *(Shigella* spp. and EIEC), and Shiga toxin-producing *E. coli* (containing *stx1* and/or *stx2* genes) in clinical stool specimens.

METHODS

The BD MAX™ Enteric Bacterial Panel (EBP): Testing of the EBP was carried out on the fully automated BD MAX™ System. The EBP kit includes Sample Buffer Tubes (SBT) containing sample diluent formulated to combat the inhibition associated with stool matrices, and Unitized Reagent Strips (URS) containing the reagents necessary for nucleic acid extraction and PCR amplification. Extraction occurs within the URS and the PCR reaction is carried out within a microfluidic cartridge.

Specimen Collection and Enrollment: To evaluate the clinical performance characteristics of the Enteric Bacterial Panel, fresh and archived unpreserved and Cary-Blair preserved clinical stool specimens were collected.

All specimens were initially characterized by routine culture-based methods at the collection site. This initial characterization was used as the reference method (RM) result.

The inclusion of frozen archived specimens in the study was necessary in order to obtain a sufficient number of positive samples. However, for many of these specimens the time from collection to frozen storage and the constancy of frozen storage was unknown. Due to the unknown pedigree of these specimens, enrollment was contingent upon qualification. For the qualification screening, archived specimens were tested using an alternate extraction and PCR assay reference method (6, 7). Archived specimens with concordant reference method results (culture and alternate PCR) were enrolled in the study, while specimens with discordant results were excluded. Unlike the frozen archived specimens, fresh (never frozen) specimens were not subject to qualification screening.

A total of 283 clinical stool specimens were enrolled in the study. These consisted of 135 fresh specimens and 148 frozen archived specimens. Of these 283 specimens, 169 were characterized by the RM as positive for one the EBP targets and the remaining 114 were characterized by the RM as negative for all of the EBP targets (Table 1).

Testing of the EBP on the BD MAX™ System: For each stool specimen enrolled in the study, a sample was obtained from the specimen and expressed into a SBT using a 10 µL inoculum loop. The SBTs, URSs, and microfluidic cartridges were loaded into the instrument for sample processing. Total run time of the EBP from the start of samp le processing on the BD MAX™ System until the completion of PCR and result reporting is 2 hours and 35 minutes.

Table 1. Specimen Enrollment Totals

	Positive Specimens**				Negative Specimens**
	Salmonella	*Shigella*	*Campylobacter*	STEC	
Fresh	10	1	9	1	114
Archived	56	31	41	20	NA
Total	**66**	**32**	**50**	**21**	**114**

** As characterized by the reference method culture results

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6) *Three-Hour Molecular Detection of* Campylobacter, Salmonella, Yersinia*, and* Shigella *Species in Feces with Accuracy as High as That of Culture.* **Cunningham S. A., et al**. 8, August 2010, JCM, Vol. 48, pp. 2929-2933;

7) *Rapid and sensitive detection of Shiga toxin-producing* Escherichia coli *from nonenriched stool specimens by real-time PCR in comparison to enzyme immunoassay and culture.* **Grys T. E., et al**. 7, July 2009, JCM, Vol. 47, pp. 2008-2012.

RESULTS

The multiplex design of the BD MAX™ Enteric Bacterial Panel allows samples to be screened simultaneously for all of the EBP targets. Target-specific results obtained with the EBP were compared against the RM culture results. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated for each assay and can be found in Figure 1 below.

Figure 1. Results obtained with the EBP as compared to the RM results for *Salmonella* spp. (A), *Shigella* spp. (B), *Campylobacter jejuni* and *coli* (C), and Shiga toxin-producing *E. coli* (D). The values given for Positive and Negative Percent Agreement represent the point-estimate (bolded) and 95% confidence-interval.

A)

***Salmonella* spp.**		**Culture**	
		Pos	Neg
BD MAX	Pos	63	2
	Neg	2	214
Positive Percent Agreement		**96.9%** (89.46, 99.15)	
Negative Percent Agreement		**99.1%** (96.69, 99.75)	

B)

***Shigella* spp.**		**Culture**	
		Pos	Neg
BD MAX	Pos	32	0
	Neg	0	248
Positive Percent Agreement		**100%** (89.28, 100)	
Negative Percent Agreement		**100%** (98.47, 100)	

C)

***Campylobacter* (*jejuni* and *coli*)**		**Culture**	
		Pos	Neg
BD MAX	Pos	48	4
	Neg	1	227
Positive Percent Agreement		**97.9%** (89.31, 99.64)	
Negative Percent Agreement		**98.3%** (95.63, 99.32)	

D)

Shiga toxin-producing *E. coli*		**Culture**	
		Pos	Neg
BD MAX	Pos	21	2
	Neg	0	257
Positive Percent Agreement		**100%** (84.54, 100)	
Negative Percent Agreement		**99.2%** (97.23, 99.79)	

Two specimens tested with the EBP produced unresolved results for all four assays. One specimen tested with the EBP produced a reportable result for only the *Salmonella* spp. assay, but was unresolved for the other three assays. An unresolved result was deemed non-reportable and characterized as a failure (Table 2).

Table 2. Failure Rates of Each Enteric Bacterial Panel Assay

	Unresolved Results	Sample Size	Failure Rate
Salmonella	2	283	0.71%
Shigella	3	283	1.1%
Campylobacter	3	283	1.1%
STEC	3	283	1.1%

CONCLUSION

The BD MAX™ Enteric Bacterial Panel offers a simple and time-saving IVD alternative to traditional culture-based methods for diagnosing acute bacterial diarrheal disease caused by *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., or Shiga toxin-producing *E. coli*. using the fully automated BD MAX™ instrument. With an overall PPA for all 4 assays of 98.2% (95% CI: 0.9484, 0.9963) and an overall NPA for all 4 assays of 99.2% (95% CI: 0.9835, 0.9963), the EBP is both highly sensitive and specific. Moreover, the BD MAX™ Enteric Bacterial panel will allow laboratories to more effectively implement the 2009 CDC guidelines and the 2013 Joint Commissions requirement (Standard QSA.04.06.01) for the screening of patients for Shiga toxin-producing *E. coli* strains (3).

Disclaimer: The BD MAX™ Enteric Bacterial Panel is not approved for sale in the U.S.