A multicenter evaluation of the BD MAX Enteric Bacterial Panel (EBP) for the detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and Shiga-toxin-producing *Escherichia coli*

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Background

The World Health Organization reports that worldwide, there are nearly 1.7 billion cases of diarrheal disease every year and that diarrheal disease is the second leading cause of death in children under five years old. Each year diarrhea kills around 760,000 children under the age of five years and most importantly; it is preventable and treatable. Diarrhea is also a leading cause of malnutrition in this same age group. Most of this disease is related to unsafe drinking-water and inadequate sanitation and poor hygiene. Identifying the cause of diarrhea is important for the treatment of individual patients and for public health intervention through outbreak management. Conventional microbiological cultures remain the gold standard for identification, despite their limited sensitivity. The application of nucleic acid amplification methods to the identification of the cause of diarrhea could have a significant impact on diagnosis and treatment as well as understanding the epidemiology of this disease. This multicenter study evaluated the EBP, a nucleic acid amplification based assay for the detection of *Salmonella* spp., *Campylobacter jejuni* and *coli*, *Shigella* spp./Enteroinvasive *E. coli* (EIEC) and Shiga toxin 1 (*stx*1) / Shiga toxin 2 (*stx*2) genes (found in Shiga toxin-producing E. coli [STEC]) as well as *Shigella dysenteriae* in stool specimens using the BDMAX system (BD Diagn, Baltimore, MD, USA).

Collaborators

1. Cincinnati Children's Hospital, Cincinnati, OH, USA
2. Cleveland Clinic Main Campus, Cleveland, OH, USA
3. Medical College of Wisconsin, Milwaukee, WI, USA
4. Children's Medical Center of Dallas, Dallas TX, USA
5. Scott & White Memorial Hospital, Temple, TX, USA
6. Massachusetts General Hospital, Boston, MA, USA
7. Univ. of Calgary Diagnostic & Scientific Centre, Calgary, Alberta, Canada

Results

Campylobacter species

	BD MAX	Reference Method: Pos	Reference Method: Neg	Total
Overall:	Pos	173	53[b]	226
	Neg	5[a]	3169	3174
	Total	178	3222	3400
Prospective:	Pos	44	51[b]	95
	Neg	1[a]	2797	2798
	Total	45	2848	2893
Retrospective:	Pos	129	2	131
	Neg	4	372	376
	Total	133	374	507

a. One prospective specimen was negative by alternate PCR.
b. 22/51 prospective specimens were positive by alternate PCR.
PPA: 97.2% (93.6, 98.8), NPA: 98.4% (97.9, 98.7)
Post-discrepant analysis: PPA: 98.0% (94.9, 99.2) NPA: 99.0% (98.6, 99.3)

Salmonella species

	BD MAX	Reference Method: Pos	Reference Method: Neg	Total
Overall:	Pos	200	27[d]	227
	Neg	6[c]	3307	3313
	Total	206	3334	3540
Prospective:	Pos	34	26[d]	60
	Neg	5[c]	2857	2862
	Total	39	2883	2922
Retrospective:	Pos	166	1	167
	Neg	1	450	451
	Total	167	451	618

c. Five prospective specimens were negative by alternate PCR.
d. 19/ 26 prospective specimens were positive by alternate PCR.
PPA: 97.1% (93.8, 98.7), NPA: 99.2% (98.8, 99.4)
Post-discrepant analysis: PPA: 99.5% (97.5, 99.9) NPA: 99.8% (99.5, 99.9)

Shigella species

	BD MAX	Reference Method: Pos	Reference Method: Neg	Total
Overall:	Pos	115	10[e]	125
	Neg	1	3340	3341
	Total	116	3350	3466
Prospective:	Pos	24	10[e]	34
	Neg	0	2889	2889
	Total	24	2899	2923
Retrospective:	Pos	91	0	91
	Neg	1	451	452
	Total	92	451	543

e. 9/10 prospective specimens were positive by alternate PCR.
PPA: 99.1% (95.3, 99.8), NPA: 99.7% (99.5, 99.8)
Post-discrepant analysis: PPA: 99.2% (95.6, 99.9) NPA: 100% (99.8, 100)

Shiga-toxin

	BD MAX	Reference Method: Pos	Reference Method: Neg	Total
Overall:	Pos	74	17[g]	91
	Neg	2[f]	2437	2439
	Total	76	2454	2530
Prospective:	Pos	8	17[g]	25
	Neg	2[f]	2347	2349
	Total	10	2364	2374
Retrospective:	Pos	66	0	66
	Neg	0	90	90
	Total	66	90	156

f. Two prospective specimens were negative by alternate PCR.
g. Nine of 17 prospective specimens gave a positive result by alternate PCR.
PPA: 97.4% (90.9, 99.3), NPA: 99.3% (98.9, 99.6)
Post-discrepant analysis: PPA: 100% (95.6, 100) NPA: 99.7% (99.4, 99.8)

Conclusions and Comments

- Overall agreement between the BD MAX EBP panel and reference method was high for each target.
- The prevalence: Campylobacter species: 45/2893 = 1.5%; Salmonella: 39/2922 = 1.2%; Shigella: 24/2923 = 0.8%; Shiga toxin 10/2374 = 0.4% in this multi-centered study was low, as might be expected for a study conducted largely in developed countries.
- Most of the "false positive" prospective specimens determined by the BD MAX were confirmed by alternate PCR. These included 22/51 (43%) *Campylobacter* spp., 19/26 (73%) *Salmonella* spp., 9/10 (90%) *Shigella* spp., and 9/17 (53%) Shiga-toxin positive samples. Most PCR positive, but culture negative samples will represent true positive results.
- Of retrospective samples, the BD MAX EBP assay did not detect 4 samples positive by culture and alternate PCR for *Campylobacter* spp., 1 for *Salmonella* spp., and 1 for *Shigella* spp. This may be due to target at the limit of detection or inhibitors in the freeze-thawed samples that were not removed by the BD MAX extraction.
- Among prospective samples, neither the alternate PCR nor the BD MAX EBP PCR assay detected 1 *Campylobacter* spp. and 5 *Salmonella* spp., suggesting either substances inhibitory to PCR were not removed, or a very low amount of target was present in the specimen.
- Two prospective samples positive for Shiga-toxin, but negative by the BD MAX EBP PCR and alternate sequencing may represent false positive EIA results or PCR inhibition.

Materials and Methods

A total of 4,242 diarrheal stool samples from 13 centers in the USA, Canada, and Mexico were enrolled in the study.

Prospective specimens: 3457 (81.5%) stool specimens collected between Dec. 2012 and Sept. 2013. Between 72-89% of specimens were compliant for all study criteria, depending on the target. Cary Blair preserved: 2112 specimens (61.1%). Unpreserved: 1345 specimens (38.9%)

Retrospective specimens: 785 (18.5%) stool specimens. Samples positive for pathogens by culture or Shiga-toxin EIA were frozen at -20°C or lower and were not thawed prior to testing. When possible, positive samples were paired with one or more specimens testing negative during the same time period.

Reference methods

Prospective specimens: Culture and Shiga-toxin EIA were the reference methods for prospective specimens. For preserved specimens the reference method was performed within 96 h of collection and for unpreserved specimens upon receipt in the enrolling lab.

Culture: Specimens were planted on primary culture media: MacConkey and selective Campylobacter agar. Xylose-Lysine-Desoxycholate or Hektoen agar, Trypticase Soy blood agar, Salmonella/Shigella agar, broth enrichment, or filtration culture for Campylobacter were included, but varied among sites. After overnight incubation, colonies suspicious for enteric bacterial pathogens were assessed using biochemical tests (manual or automated) and MALDI-TOF.

Shiga-toxin EIA was performed using enrichment broth and the Immunocard STAT! EHEC or Premier EHEC test method according to manufacturer's instructions. (Meridian Bioscience, Cincinnati, OH, USA)

Retrospective Specimens

Alternate PCR and Bidirectional Sequencing: For retrospective specimens the historical reference (culture or Shiga-toxin) result was confirmed by alternate PCR and bi-directional amplicon sequencing. Only specimens for which the historical result was confirmed were included in the performance calculations.

Enteric Bacterial Panel (EBP)

The EBP kit includes Sample Buffer Tubes (SBT) containing sample diluent formulated to minimize inhibition associated with stool matrices and Unitized Reagent Strips (URS) containing the reagents necessary for nucleic acid extraction and PCR amplification. Testing of the EBP was carried out on the fully automated BD MAX™ System.

BD MAX™ Automated PCR: Stool samples were transferred into an SBT using a 10 µl loop. The SBTs, URSs, and microfluidic cartridges were loaded into the BD MAX. Automated extraction was conducted within the URS. The PCR reaction is carried out within the microfluidic cartridge. Total run time of the EBP including sample processing, PCR, and result reporting is 2 h 35 min.

Analysis

Positive and Negative Percent Agreement were calculated with 95% confidence intervals for all samples compliant for both BD MAX and reference methods. Discordant results for prospective specimens were resolved by alternate PCR and sequencing. Prevalence was calculated as the number positive by standard culture or EIA methods divided by the total number of compliant specimens.

• BD Diagnostics, Baltimore, MD, USA, provided supplies and financial support for this study.