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Comparative Evaluation of a PCR Amplification and Array Detection Stool Bacterial Pathogens Panel with Conventional Methods for Detecting Common Bacterial Enteric Pathogens

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Background

The Great Basin Stool Bacterial Pathogens Panel (SBPP) (Great Basin Scientific, Salt Lake City, UT) performed in the PA500 Portrait Analyzer uses an automated, hot-start PCR technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface. The entire assay is performed in a self-contained, disposable cartridge. The purpose of this study was to compare the performance of the SBPP with conventional cultural and EIA methods for the detection of common bacterial enteric pathogens in diarrheal stool specimens. Discordant results were arbitrated by using two different, FDA-cleared, nucleic acid amplification tests (NAATs).

Methods

A total of 1,479 fresh stool specimens were evaluated in this study. Specimens were collected from patients symptomatic for diarrheal disease at four geographically distinct U.S. locations. Specimens were transported to each of the four respective clinical laboratory study sites in modified Cary-Blair medium where the SBPP was performed according to the manufacturer's instructions. Basically, using a disposable 250 µl pipette, the preserved stool was loaded into a sample preparation device (SPD) and the eluate was collected into a tube provided with the SBPP kit. The stool eluate (250 µl) was transferred into an SBPP cartridge and the cartridge was then placed into the Portrait Analyzer for analysis. The SBPP screens for the specific DNA sequences that target *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni/Campylobacter coli*, Shiga toxin 1, Shiga toxin 2, and *Escherichia coli* O157.

Conventional culture methods were used for the recovery and identification of the bacterial enteric pathogens using established standard of care procedures. An EIA test (Meridian Biosciences, Cincinnati, OH) was used for the detection of Shiga toxin 1 and Shiga toxin 2. Discordant SBPP and culture/ EIA results were arbitrated using two different FDA-cleared assays: the BioFire GI Syndromic Panel (BioFire, Salt Lake City, UT) and the Verigene EnteroPathogen (EP) assay (Luminex, Austin, TX).

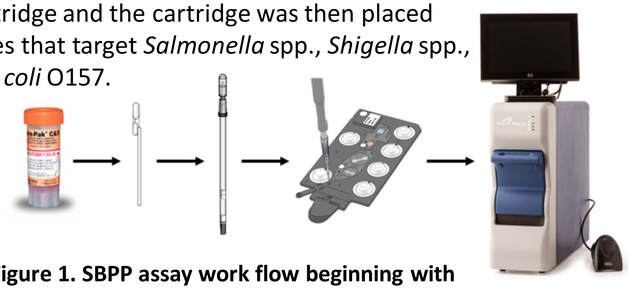

Figure 1. SBPP assay work flow beginning with preserved stool through testing on Portrait Analyzer includes an exact volume pipette, a sample preparation device (SPD), an eluate collection tube and the SBPP cartridge.

Results

Table 1 shows the percent agreement between SBPP and conventional culture/EIA for the 1,479 stool specimens evaluated in this study. Overall, the SBPP compared favorably with culture. However, 3 false-negative and 51 false-positive SBPP results were observed compared to culture and EIA. Discordant analyses of these discrepant specimens were performed using the BioFire and Nanosphere NAATs. As shown in Table 2, 49 of the 51 false-positive SBPP results were, in fact, resolved as true positives following NAATs arbitration with culture and EIA yielding false-negative results for these 49 specimens. Only 3 of 1,479 specimens (0.002%) produced false-negative SBPP results – two specimens for *Salmonella* spp. and one for *C. jejuni/C. coli*. Two false-positive SBPP results (0.001%) were observed in the entire study and both were for *C. jejuni/C. coli.* As noted in Table 1, only 16 specimens were tested for the presence of *E. coli O157*. This is because the SBPP assay only reports the presence or absence of *E. coli O157* if the assay is positive for Shiga toxin 1 and/or Shiga toxin 2.

Conclusions

After NAAT arbitration of the discordant specimens, the overall results of this study showed that the SBPP performed considerably better than conventional culture and EIA. The SBPP detected 99 of 102 (97.1%) of the targeted organisms whereas culture/EIA detected only 51 (50%) of these organisms. The Great Basin SBPP benchtop instrument has a small footprint and the moderately complex assay can be easily performed by most technologists with a time-to-result of two hours.

Table 1. Comparison of SBPP with Culture and EIA

Organism	n	% Agreement (95% CI)	
		Positive	Negative
C. jejuni/C. coli	1,479	96.4% (82.3 - 99.4) 27/28	99.2% (98.6 - 99.5) 1439/1451
Salmonella spp.	1,479	83.3% (55.2 - 95.3) 10/12	99.6% (99.1 - 99.8) 1461/1467
Shigella spp.	1,479	100% (56.6 - 100.0) 5/5	99.1% (98.4 - 99.4) 1460/1474
Shiga Toxin 1	1,479	100% (20.7 - 100.0) 1/1	99.5% (99.0 - 99.8) 1471/1478
Shiga Toxin 2	1,479	100% (20.7 - 100.0) 1/1	99.4% (98.8 - 99.7) 1469/1478
E. coli O157	16	100% (51.0 - 100.0) 4/4	75% (46.8 - 91.1) 9/12

Table 2. NAAT Resolution of Discordant SBPP and Culture/EIA Results

Analyte	False Negatives Resolved by NAAT Reference Method	False Positives Resolved by NAAT Reference Method
C. jejuni/C. coli	1/1	10/12
Salmonella	0/2	6/6
stx1	N/A	7/7[a]
stx2	N/A	9/9[b]
E. coli O157	N/A	3/3
Shigella	N/A	14/14

[A]One false positive was not concordant with Verigene EP (negative for stx1 in the Verigene® EP) but was positive for stx1/2 in the BioFire GI Panel.

[B]One false positive was not concordant with Verigene EP (negative for stx2 in the Verigene® EP) but was positive for stx1/2 in the BioFire GI Panel.