SATURDAY
Poster #469

Great Basin Stool Bacterial Pathogens Panel for Rapid Identification of *Salmonella*, *Shigella*, Shiga Toxin-Producing *E. coli*, and *Campylobacter* in Symptomatic Patients

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Introduction

Bacterial infections that result in acute diarrhea represent a substantial healthcare burden worldwide. The Foodborne Disease Burden Epidemiology Reference Group, established by the World Health Organization (WHO), reports that diarrheal disease agents due to foodborne disease were responsible for 550 million illnesses and 230,000 deaths every year.[1] Accurate and rapid diagnosis is imperative to benefit patient outcome and disease spread.

Great Basin Scientific has developed a rapid, multiplex, diagnostic assay to simultaneously detect *Salmonella*, *Shigella*, Shiga toxin-producing *E. coli* (*stx1*, *stx2*, & O157 serotype-specific genes), and *Campylobacter* species (*C. coli* and *C. jejuni*). The Stool Bacterial Pathogens Panel (SBPP) is a PCR-based, multiplex assay that detects bacterial agents in preserved stool specimens, from symptomatic patients.

Materials & Methods

- Contrived samples were prepared with bacterial cells spiked into pooled, negative clinical stool.
- Eight (8) bacterial strains representative of all SBPP target analytes were tested under each condition.
- Four (4) simple steps needed to process 250 µl of preserved stool.
- Minimal sample process time (<5 minutes).
- Final results in ~2 hours.

Figure 1. Great Basin Scientific Analyzer

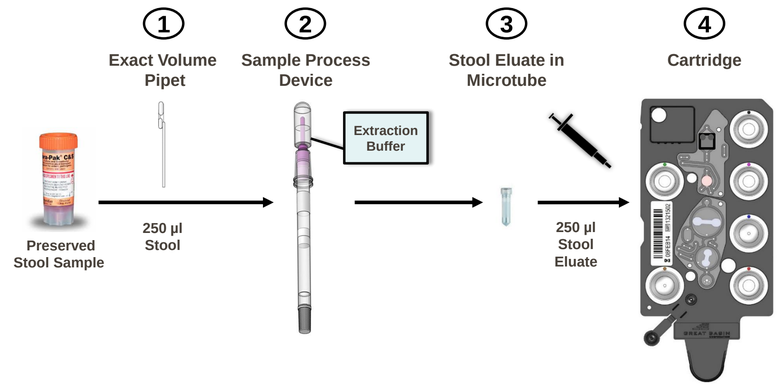
Analytes Detected

- ***Campylobacter coli/jejuni***
- ***Salmonella* spp.**
- **Shiga toxin 1 (*stx1*)**
- **Shiga toxin 2 (stx2)**
- ***E. coli* Serotype O157**
- ***Shigella* spp.**

Conclusions

- Multiplexed, qualitative test detects 7 enteric bacterial pathogens directly, form Cary Blair and C&S Media preserved stool specimens, with results in ~2 hours.
- Demonstrated competitive limits of detection (LoD) for all targets.
- A specific, sensitive and reproducible test contained on a single card with minimal sample processing needed.

SBPP Assay Format



Results

Limit of Detection (LoD)

Strain	ATCC ID	LoD (CFU/mL)
Campylobacter coli	43486	1.8×10^3
Campylobacter jejuni	49943	1.3×10^3
Escherichia coli (*stx1+*)	BAA-2215	5.7×10^3
Escherichia coli (*stx2+*)	51435	4.4×10^3
Escherichia coli (*stx1+/stx2+*)	BAA-2196	1.1×10^4
Escherichia coli (*stx1+/stx2+*/O157+)	43895	1.6×10^4
Salmonella bongori	43975	2.5×10^3
Salmonella enterica	13311	1.9×10^4
Shigella flexneri	25929	5.2×10^3
Shigella sonnei	29930	1.4×10^4

Table 1. Reported Limit of Detection. LoD was assessed by testing 10 bacterial strains representative of all SBPP target analytes.

Interfering Substances Study

Substance	Concentration Tested
Ampicillin	50 mg/mL
Bacitractin Zinc Ointment	50 mg/mL
Benzalkonium Chloride, Ethanol	9.5% v/v
Bovine Mucin	6.25 mg/mL
Calcium Carbonate	200 mg/mL
Cholesterol	5% v/v
Hemoglobin	10% w/v
Human Whole Blood	50% v/v
Hydrocortisone	75 mg/mL
Imodium®	10% v/v
Kaopectate®	10% v/v
Milk of Magnesia	5% v/v
Mineral Oil	50% v/v
Naproxen Sodium	9.5% w/v
Nystatin	5% v/v
Pepto-Bismol®	10% v/v
Pork Mucin	6.25 mg/mL
Sennosides	9.7 mg/mL
Triglycerides	10% v/v

Table 3. Potential Interfering Substances. 19 different substances commonly present as stool contaminants were tested at the concentrations listed in stool with representative target organisms at ≤3X LoD**. No interference in the SBPP was observed for any of the substances at the concentration listed.**

Analytical Reactivity (Inclusivity)

Organism/Serotype	Total Strains Tested
Campylobacter coli	5
Campylobacter jejuni	6
Salmonella enterica subsp. enterica: Typhi, Newport, Choleraesius, Stanley, Heidelberg, Muenchen, Paratyphi B, Bareilly, Kentucky, Saint Paul, Tennessee, Paratyphi A, Typhimurium, arizonae, Dublin, houtenae, Newport, diarizonae, Newington, Virchow, Agona, Bristol, Montevideo, Infantis, and *Mississippi. Salmonella enterica subsp.: salamae, diarizonae, houtenae, indica,* and *salamae. Salmonella bongori*	33
Shigella boydii (Serotypes 1, 2, 3, 8), *Shigella flexneri* (Serotypes 1a, 2a, 2b, 5, 6) *Shigella sonnei*, and *Shigella dysenteriae* (Serotypes 1, 2, 3, 12, 13)	20
Shiga-toxin producing *E. coli* (Serotypes O111:H8, O26:H11, O21:H19, O111, O45:H2, O123:H25, O103:H2, O103:H11, O91:H21, O113:H21, O145:H25, O145:H28, O121:H19, O104:H4, O157:H7, O157:NM, O157:H7:K	24
Shiga-toxin producing *Shigella dysenteriae* Type1	3

Table 2. Organisms Tested for Inclusivity. Analytical reactivity was assessed by testing 91 well characterized bacterial strains representing the organisms detected in the SBPP, at ≤3X LoD, in stool. **All organisms tested were correctly identified by the SBPP.**

Reproducibility

Analyte	Concentration	% Agreement
Campylobacter coli/jejuni	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Salmonella	1.5X LoD	96.7% (87/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Shiga toxin 1	1.5X LoD	97.8% (90/92)
	3X LoD	100% (90/90)
	Negative	100% (540/540)
Shiga toxin 2	1.5X LoD	95.7% (88/92)
	3X LoD	100% (90/90)
	Negative	100% (540/540)
E. coli Serotype O157	1.5X LoD	97.8% (90/92)
	3X LoD	100% (90/90)
	Negative	100% (540/540)
Shigella	1.5X LoD	100% (90/90)
	3X LoD	100% (90/90)
	Negative	99.8% (540/541)
Negative	Negative	100% (540/540)

Table 4. Reproducibility Study. Conducted at Great Basin Scientific and 2 external sites, the study consisted of 6 different operators, 70 different Analyzers, and 10 different cartridge lots.

Results

Analytical Reactivity (Exclusivity)

Bacteria			
Genus	**Species**	**Genus**	**Species**
Abiotrophia	*defectiva*	*Prevotella*	*melaninogenicus*
Acinetobacter	*baumannii*	*Proteus*	*mirabilis*
Aeromonas	*hydrophilia*		*penneri*
Anaerococcus	*tetradius*		*vulgaris*
Bacillus	*cereus*	*Providencia*	*alcalifaciens*
Bacteroides	*fragilis*		*rettgeri*
	vulgatus		*stuartii*
Bifidobacterium	*adolescentis*	*Pseudomonas*	*aeruginosa*
	bifidum		*putida*
	longum	*Ruminococcus*	*bromii*
Campylobacter	*curvus*	*Serratia*	*liquefaciens*
	fetus fetus		*marcescens*
	fetus venerealis	*Staphylococcus*	*aureus*
	hyointestinalis		*epidermidis*
	lari (5 strains)	*Stenotrophomonas*	*maltophilia*
	upsaliensis	*Streptococcus*	*agalactiae*
Citrobacter	*amalonaticus*		*dysgalactiae*
	freundii		*intermedius*
Clostridium	*difficile*		*pyogenes*
	histolyticum		*uberis*
	perfringens	*Trabulsiella*	*guamensis*
	sordellii	*Veillonella*	*parvula*
Enterobacter	*aerogenes*	*Vibrio*	*cholera*
	cloacae		*parahaemolyticus*
Enterococcus	*cecorum*		*vulnificus*
	faecalis	*Yersinia*	*bercovieri*
	faecium		*enterocolitica*
EAEC *Escherchia*	*coli*		*pseudotuberculosis*
EIEC *Escherichia*	*coli*		*rohdei*
ETEC *Escherichia*	*coli*	**Fungi**	
Escherichia	*coli*	*Candida*	*albicans*
	fergusonnii		*catenulata*
	hermannii	*Saccharomyces*	*boulardii*
Fusobacterium	*varium*	**Viruses & Parasites**	
Gardnerella	*vaginalis*	Adenovirus Type 2	
Helicobacter	*pylori*	Adenovirus Type 40, strain Dugan	
Klebsiella	*pneumoniae*	Adenovirus Type 41, strain Tak	
	oxytoca	Coxsackie B4	
Lactobacillus	*acidophilus*	*Cryptosporidium parvum*	
	casei	*Entamoeba histolytica*	
Leminorella	*grimonti*	Enterovirus	
Listeria	*grayi*	*Giardia intestinalis*	
	innocua	Norovirus GI	
	monocytogenes	Norovirus GII	
Morganella	*morganii*	Rotavirus	
Peptostreptococcus	*anaerobius*	Rotavirus A	
Pleisomonas	*shigelloides*		
Porphyromonas	*asaccharolytica*	Human genomic DNA	

Table 5. Organisms Tested for Exclusivity. 100 organisms phylogenetically related to targeted organisms, as well as other bacteria, fungi/yeast, parasites, viruses, and human genomic DNA were tested at ≥1 x 10^6 CFU/mL, ≥1 µg/mL, ≥1 x 10^6 copies/mL, or ≥1 x 10^5 $TCID_{50}$/mL. **All of the organisms shown gave the expected 'NOT DETECTED' result, indicating there was no cross-reactivity in the SBPP**.

Competitive Inhibition

Organism at Low Titer (2X) LoD:	Organisms at High Titer: ≥10^6 CFU/mL							
	C. coli **(ATCC 43486)**	***C. jejuni*** **(ATCC 49943)**	***E. coli*** **(stx1+/stx2+/ non-O157) (ATCC BAA-2196)**	***E. coli*** **(stx1+/stx2+/ O157+) (ATCC 43895)**	***S. bongori*** **(ATCC 43975)**	***S. enterica*** **(ATCC 13311)**	***S. flexneri*** **(ATCC 25929)**	***S. sonnei*** **(ATCC 29930)**
Campylobacter coli **(ATCC 43486)**	--	--	3/3	3/3	3/3	3/3	3/3	3/3
Campylobacter jejuni **(ATCC 49943)**	--	--	3/3	3/3	3/3	3/3	3/3	3/3
Escherichia coli **(stx1+/stx2+/O157-) (ATCC BAA-2196)**	3/3	3/3	--	--	3/3	3/3	3/3	3/3
Escherichia coli **(stx1+/stx2+/O157+) (ATCC 43895)**	3/3	3/3	--	--	3/3	3/3	3/3	3/3
Salmonella bongori **(ATCC 43975)**	3/3	3/3	3/3	3/3	--	--	3/3	5/6[a]
Salmonella enterica **(ATCC 13311)**	7/9[b]	3/3	3/3	14/19[c] 6/6[d]	--	--	3/3	3/3
Shigella flexneri **(ATCC 25929)**	3/3	3/3	3/3	3/3	3/3	3/3	--	--
Shigella sonnei **(ATCC 29930)**	3/3	3/3	5/6[e]	3/3	3/3	3/3	--	--

[a] In 1/3 replicates, 'high titer' *Shigella sonnei* was not detected and contamination with a *Campylobacter* sp. was noted. An additional 3 replicates were tested and the expected result was obtained for both analytes, in all replicates.
[b] For a 'low titer' *Salmonella enterica* and 'high titer' *Campylobacter coli* sample, the SBPP did not detect *Salmonella* in 2/3 replicates, although *Campylobacter* was correctly identified in all cases. An additional 6 replicates were tested, and the expected result was obtained for both analytes, in all replicates.
[c] For a 'low titer' *Salmonella enterica* and 'high titer' *Escherichia coli* (ATCC 43895, ≥10^6 CFU/mL) sample, the SBPP did not detect *Salmonella* in 1/3 replicates. An additional 16 replicates were tested and 12/16 detected 'low titer' *Salmonella.*
[d] We decreased the concentration of the 'high titer' *E. coli* to 1 x 10^5 CFU/mL in combination with 'low titer' *Salmonella* and tested 6 replicates. The expected result was obtained for both analytes, in all replicates.
[e] In 1/3 replicates, 'low titer' *Shigella sonnei* was not detected, although Shiga Toxin 1 & 2 was detected in all cases. An additional 3 replicates were tested, and the expected result obtained for both analytes, in all replicates.

Table 6. Organisms Tested for Microbial Interference. Combinations of 8 SBPP organisms,, representative of potential dual infections, were tested. The panels were designed so that one organism of each bacterial species was present at a low titer (2X LoD) with the second organism present at a high titer (> 10^6 CFU/mL). **Competitive inhibition was only observed for *Salmonella* when *E. coli* (*stx1+/stx2+*/O157+) was present at concentrations ≥1 x 10^6 CFU/mL.**