

Amended Abstract

Background: The BD MAX™ Enteric Bacterial Panel and BD MAX™ Extended Enteric Bacterial Panel (BDM GIP) are multiplex PCR tests that can detect up to eight different GI pathogens. The approved specimen types for these panels are unpreserved or Cary-Blair preserved stools. Copan FecalSwab™ (FS) is a convenient system using Cary-Blair medium for transporting fecal samples. The objectives of this study were to assess the ideal volume of FS stool preserved sample to use with the BDM GIP and to compare the performance of FS to the recommended Meridian Para-Pak® Cary-Blair medium (PP) for the BDM GIP. **Materials/methods:** Negative stool samples were inoculated with ATCC® strains representing each of the eight targets detected by the BDM GIP and transferred to FS and PP mediums. Detection of each enteric pathogen was performed on the BD MAX™ System using 10 µl (BDM GIP package insert recommendation) of the PP preserved specimens and 10 µl, 25 µl and 50 µl of the FS preserved samples. Tests were performed in triplicate. Results from FS tests were analyzed and compared to PP test results. Additionally, 139 unpreserved stool samples submitted for GI testing at Tampa General Hospital laboratory were analyzed. The stool samples were transferred to PP and FS mediums, following manufacturer's recommendations. PP and FS preserved specimens were tested by BDM GIP using 10 µl of PP, as recommended, and 50 µl of FS medium. **Results:** Analysis of Ct values from each input volume tested concluded that 50 µl of FS stool preserved sample was optimal for the BDM GIP. Of 139 clinical samples evaluated using 10 µl of PP and 50 µl of FS medium, 93 were positive for at least one of the BDM GIP targets and 46 were negative. An agreement of 100% was observed between PP and FS results. **Conclusions:** The performance of 50 µl of FS stool preserved sample was equivalent to 10 µl of traditional Cary-Blair PP preserved specimens for the detection of GI pathogens using the BD MAX Enteric Bacterial Panel in conjunction with BD MAX Extended Enteric Bacterial Panel on the BD MAX System.

Introduction

The diagnosis of diarrhoea can be long and complex due to the variety of potential pathogens involved. The use of molecular techniques is becoming a valid diagnostic tool. The BD MAX™ Enteric Bacterial Panel and BD MAX™ Extended Enteric Bacterial Panel are multiplex PCR tests that can detect up to eight different GI pathogens. The approved specimen types for these panels are unpreserved or Cary-Blair preserved stools. Copan FecalSwab™ is a convenient system using Cary-Blair medium for transporting fecal samples. The objectives of this study were to assess the ideal volume of FS stool preserved sample to use with the BD Max GI Panels and to compare the performance of FecalSwab to the recommended Meridian Para-Pak® Cary-Blair medium for the BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel.

Methods

Analytical Testing:

Comparison of Copan FecalSwab preserved stool with Meridian Para-Pak preserved stool:

Strains Tested:

S. typhimurium (ATCC 14028) *S. sonnei* (ATCC 9290)
E. coli (ATCC 43890) *C. jejuni* (ATCC 33291)
P. shigelloides (ATCC 14029) *V. parahaemolyticus* (ATCC 17802)
E. coli (ETEC) (ATCC 35401) *Y. enterocolitica* (ATCC 9610)

- A 0.5 McFarland suspension was prepared in PBS for each strain and diluted 1:20 to make a working dilution.
- A 5 ml volume of the working dilution was used to inoculate 50 ml of negative stool sample.
- Para-Pak and FecalSwab devices were inoculated with stool according to package instructions (6 Para-Pak and 18 FecalSwab per strain tested).
- Inoculated devices were used to inoculate BD MAX Sample Buffer Tubes as follows:
 - 10 µl input volume from all 6 Para-Pak devices
 - 10 µl (n=6), 25 µl (n=6) and 50 µl (n=6) input volume for a total of 18 FecalSwab devices

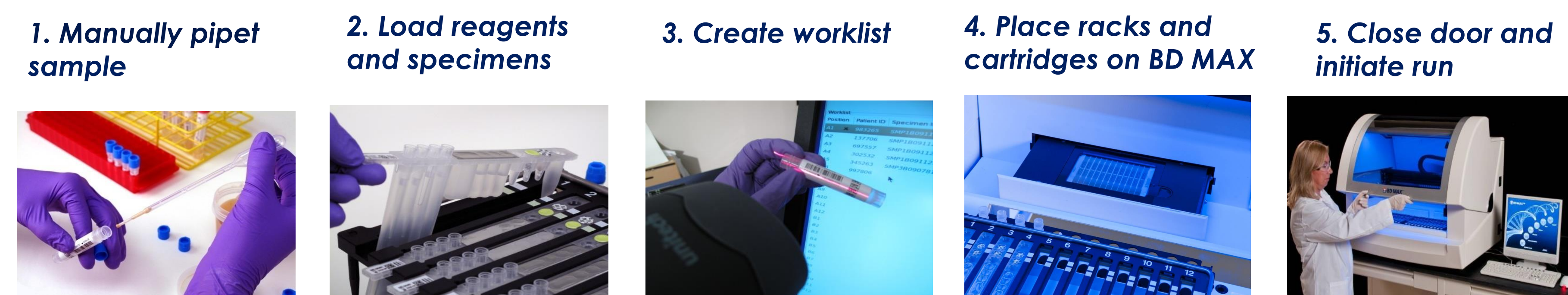
- Tests were performed in triplicate on the BD Max by each of the two BD Max panels: The BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel.
- Additionally, to verify the time and temperature of storage of pooled stool sample, the same 24 tubes of pooled stool preserved samples (6 tubes Meridian Para-Pak® and 18 tubes of Copan FecalSwab™), tested at time 0 h were stored at room temperature (20-24°C) for 24 h (T1) and at refrigerated temperature (2-8°C) for 5 days (T2). The samples were tested by the two BD MAX Enteric panels at the T1 and T2 time points.

Clinical Stool Sample Testing:

Evaluation of stool samples preserved in Copan FecalSwab and Meridian Para-Pak:

- A total of 139 unpreserved clinical stool samples were tested.
- At the lab, raw stool samples were transferred to Copan FecalSwab and Meridian Para-Pak transport medium according to manufacturer's recommendations.
- Para-Pak preserved stool clinical samples were tested by the BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel using 10 µl of the medium, as recommended.
- Analysis of the PCR cycle threshold (Ct) values from each input volume tested on the Analytical Testing concluded that 50 µl of FecalSwab stool preserved sample was optimal for the BD MAX GI Panels testing.
- FecalSwab preserved stool clinical samples were tested by the BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel using 50 µl of the medium.

BD Max Operation:



This study was supported by Copan Diagnostics and BD Diagnostic Systems.

Results

Analytical Testing

Table 1: Average of Ct values using different FecalSwab volumes, incubation temperatures and times.

	<i>S. typhimurium</i> (ATCC 14028)			<i>S. sonnei</i> (ATCC 9290)			<i>E. coli</i> (ATCC 43890)			<i>C. jejuni</i> (ATCC 33291)		
	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)
FS_10µl	34.4	34.6	33.88	30.01	30.51	30.94	35.47	35.57	34.88	28.7	29.37	30.5
FS_25µl	32.3	31.29	32.07	28.76	29	28.54	33.67	32.17	32.66	27.2	27.28	27.93
FS_50µl	32.65	30.52	30.66	28.47	28.08	28.2	32.67	31.36	31.85	27.22	27.13	26.84
PP_10µl	33	31.04	32.37	29.51	29.24	28.89	32.93	30.56	31.72	27.72	27.8	26.76

	<i>P. shigelloides</i> (ATCC 14029)			<i>V. parahaemolyticus</i> (ATCC 17802)			<i>E. coli</i> ETEC (ATCC 35401)			<i>Y. enterocolitica</i> (ATCC 9610)		
	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)	0 HRS	24 HRS RT	5 DAYS (2-8°C)
FS_10µl	30.64	31.41	34.44	32.01	35.73	33.04	31.52	32.27	31.73	32.79	33.1	32.97
FS_25µl	29.51	31.2	29.18	29.37	32.68	30.66	29.44	29.44	29.2	31.08	31.25	31.18
FS_50µl	30.28	29	28.77	28.36	31.56	29.33	28.1	28.45	28.02	30.73	30.14	29.84
PP_10µl	30.6	30	30.32	28.56	32.29	31.08	28.52	32.11	30.23	30.1	30.77	29.98

Legend: PP: Para-Pak; FS: FecalSwab; RT: Room temperature (20-24°C).

Clinical Testing

Table 2: Result agreement between Fecal Swab and Para-Pak for 139 clinical samples tested.

	<i>Salmonella</i>				<i>Shigella/EIEC</i>				<i>STEC</i>				<i>Campylobacter</i>			
	PP Results		FS Results		PP Results		FS Results		PP Results		FS Results		PP Results		FS Results	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
FS Results	39	0	39	0	14	0	14	0	8	0	8	0	29	0	29	0
Results	0	100	0	100	0	125	0	125	0	131	0	131	0	110	0	110

	<i>Plesiomonas</i>				<i>Vibrio</i>				<i>ETEC</i>				<i>Yersinia</i>			
	PP Results		FS Results		PP Results		FS Results		PP Results		FS Results		PP Results		FS Results	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
FS Results	3	0	3	0	0	0	0	0	1	0	1	0	3	0	3	0
Results	0	136	0	136	0	139	0	139	0	138	0	138	0	136	0	136

Legend: PP: Para-Pak; FS: FecalSwab; RT: Room temperature (20-24°C).



Fig1.
 A) Copan FecalSwab
 B) Meridian Para-Pak

Conclusions

- Evaluation of Ct values observed with 10 µl, 25 µl, and 50 µl input volume of FecalSwab preserved stool samples indicated that 50 µl is the optimal input volume for testing FecalSwab by the BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel.
- Clinical stool samples preserved with Copan FecalSwab and Meridian Para-Pak presented an overall agreement of 100% when tested on the BD MAX System, using the BD MAX Enteric Bacterial Panel and BD MAX Extended Enteric Bacterial Panel.
- The performance of 50 µl of FecalSwab stool preserved sample is equivalent to 10 µl of the Meridian Para-Pak preserved specimens for the detection of GI pathogens using the BD MAX Enteric Bacterial Panel in conjunction with BD MAX Extended Enteric Bacterial Panel on the BD MAX System.

