EVALUATION OF COPAN FECALSWAB™ FOR MOLECULAR DETECTION OF PATHOGENS USING THE BD MAX™ ENTERIC BACTERIAL PANEL

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REVISED ABSTRACT

Background: The Copan FecalSwab™ (FS) is an FDA-cleared collection device consisting of a flocked swab in modified Cary-Blair (CB) medium for transporting and preserving enteric stool pathogens for culture. Collection of a rectal swab specimen at the time the patient presents addresses the unmet clinical need of consistently obtaining a specimen for analysis. No GI molecular assays are FDA-cleared for use with the FS. The objective of this study was to evaluate and validate the FS for detection of GI pathogens using the BD MAX™ Enteric Bacterial Panel (EBP).

Methods: Optimal specimen input volume, limit of detection (LoD) and clinical performance for the FS with the EBP assay were assessed. Input volume for EBP was determined by testing volumes between 50-500 µL in triplicate of pooled negative stool in the FS medium and analytical sensitivity (LoD) by spiking negative FS medium matrix with concentrations (1.5×10^6 – 1.5×10^1 CFU/mL, 3 replicates/dilution) of strains bearing the assay targets. LoD was set as the lowest concentration that was positive for 3/3 replicates with matching corresponding bacterial concentration in CFU/mL. Clinical performance was determined by FS EBP results compared to FS culture results. Discrepant analysis included EBP and culture from CB stool (CBS) and alternate PCR.

Results: Input volume of 500 µL FS medium was the highest volume found to be non-inhibitory for EBP based on Ct value of the Sample Processing Control (SPC) compared to the control specimens. The LoD values were 571 CFU/mL Sample Buffer Tube (SBT) for *Salmonella*, 100 CFU/mL SBT for *Shigella*, 1261 CFU/mL SBT for STEC and 20 CFU/mL SBT for *Campylobacter*. Clinical performance among 101 FS samples tested showed 8 samples positive for 9 pathogens (8%): *Salmonella* (4), *Shigella* (1), *Campylobacter* (2) and STEC (2). Paired CB stool were concordant with FS results after discrepant analysis.

Conclusions: Detection of enteric pathogens using the FS collection system and EBP assay was equivalent to culture and EBP results from stool collected in CB. LoD values were similar to those reported in the EBP package insert. The FecalSwab™ provides convenient collection, preservation and testing by multiple methods, including culture and the BD MAX™ Enteric Bacterial Panel.

BACKGROUND

The Copan FecalSwab™ (FS) is an FDA-cleared device consisting of a flocked swab in 2 mL modified Cary-Blair (CB) medium tube. It is intended as a specimen collection device, which is then placed into the CB tube allowing preservation of the sample on transport to the lab for culture of enteric stool pathogens and for ease of use with automated processing instruments. However, a patient is often unable to produce a stool specimen at the time of evaluation; whereas, a rectal swab may be a suitable alternative specimen.

The BD MAX™ Enteric Bacterial Panel (BD Diagnostic Systems, Quebec, Canada) is a nucleic acid amplification based assay for the detection of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. (*C. jejuni* & *C. coli*), and Shiga-like toxin genes (*stx1* and/or *stx2*) in stool specimens using the fully automated BD MAX™ System (BD Diagnostic Systems, Sparks, MD, USA).

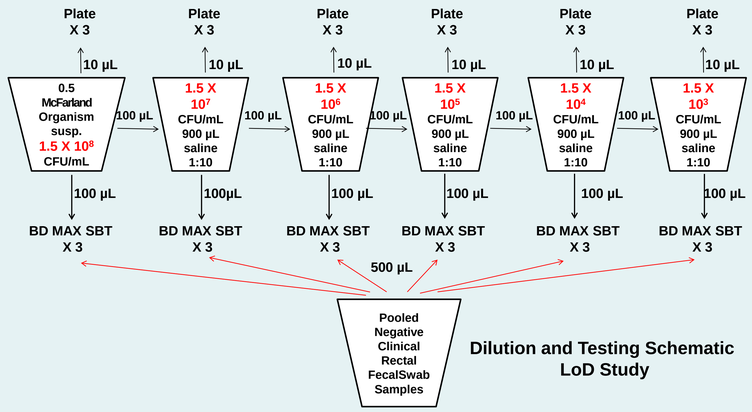
The purpose of this study was to validate use of FS for collection of rectal specimens and detection of GI pathogens using the BD MAX™ Enteric Bacterial Panel (EBP). Rectal swabs and the Copan FecalSwab media are both not FDA-cleared for clinical testing with the EBP.

METHODS

A. Optimal FecalSwab Input Volume for EBP

- A pool of negative stool specimens previously confirmed by EBP and culture, was prepared in the FS medium.
- 50 - 500 µL of the negative stool/FS was tested in triplicate by EBP
- The highest volume showing no inhibition based on Ct values of the Sample Processing Control (SPC) was considered the optimum input volume.

B. Limit of Detection/Analytical Sensitivity



Dilution and Testing Schematic LoD Study

- The assay LoD for each target was defined as the lowest concentration at which 3/3 replicates were positive.
- The corresponding bacterial concentration in CFU/mL (in the SBT) was determined as the LOD.

C. Clinical performance of EBP with rectal specimens

- Pediatric ER patients were prospectively enrolled as part of an acute gastroenteritis study and consented to providing paired Cary-Blair stool (CBS) & rectal FS specimens.
- Rectal FS specimens were collected by providers and placed directly into the FS transport tube according to the manufacturer's instructions.
- In the laboratory, the rectal FS specimens were tested as follows:
  - 500 µL by EBP
  - Reference method: Bacterial culture and identification by standard stool culture methods and Shiga toxin testing by EIA (Meridian Bioscience, OH) from Gram negative (GN) broth.
- Discrepant analysis was done as follows:
  - EBP on CB stool (CBS)
  - Culture and EIA of CB stool
  - Alternate PCR assay (FilmArray™GI Panel, BioFire, Salt Lake City, UT)

RESULTS

- **Optimal input volume of FecalSwab**
  - The highest volume of FS medium determined as non-inhibitory based on SPC Ct values was 500 µL.

- **LoD of EBP with bacterial GI pathogens in FecalSwab**
  - Ranged from 20 to 1261 CFU/mL in SBT for the strains tested.
  - Consistent with values reported in the EBP package insert for CB stool specimens.

Table 1. Analytical sensitivity (LoD) of EBP assay with FecalSwab

Target	LoD (CFU/mL in SBT)
Salmonella spp. (lab strain)	571
Shigella sonnei (lab strain)	100
Campylobacter jejunil ATCC 29428	20
E. coli O157:H7 ATCC 43890	1261

- **Clinical performance of EBP with rectal FS specimens**
  - Of 141 patients enrolled, 101 provided a rectal FS specimen and only 77 provided paired FS and CB stool specimens. All FS specimens were tested by EBP.
  - Three non-paired FS samples were culture negative for four analytes and classified as EBP false positive (FP) before discrepant analysis.

Table 2. Clinical performance of EBP with rectal FS specimens compared to culture/toxin EIA testing methods

Target	TP	FN	TN	FP
Salmonella	3	0	97	1
Shigella/EIEC	1	0	100	0
Campylobacter	1	0	99	1
STEC	0	0	99	2

Table 3. Discrepant Analysis

Pathogen	BD MAX		Culture/ GN broth		Alternate PCR	
	FS	CBS	FS	CBS	FS	CBS
Campylobacter	+	NC	-	NC	+	NC
Campylobacter	+	+	+	+	+	+
Salmonella	+	+	+	+	+	+
Salmonella	+	+	+	+	+	+
Salmonella	+	+	+	+	+	+
Salmonella	+	NC	-	NC	+	NC
Shigella	+	NC	+	NC	+	NC
STEC	+	+	NT	+	+	+
STEC	+	NC	-	NC	+	NC

NC:Not collected; STEC:Shiga-toxin producing *E. coli;* NT: not tested

CONCLUSION

- BD MAX™ Enteric Bacterial Panel showed high correlation with culture/EIA for public health reportable bacterial GI pathogens using a rectal swab specimen after discrepant analysis.
- FecalSwab™ is a convenient collection, preservation and testing system for use with the BD MAX™ Enteric Bacterial Panel.

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