Evaluation of the Copan FecalSwab™ for collection, storage and molecular detection of diarrheagenic bacteria

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St. Joseph’s Healthcare Hamilton
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• Comprehensive laboratory testing for Hamilton Health Sciences and St. Joseph’s Healthcare Hamilton

• Laboratory services:
  ▫ Clinical chemistry and immunology
  ▫ Genetics
  ▫ Haematology and transfusion medicine
  ▫ Microbiology
  ▫ Anatomic Pathology
Microbiology at HRLMP

Microbiology

- Bacteriology
- Mycology

- Virology
- Molecular

Hamilton General Hospital

St. Joseph’s Healthcare Hamilton, Charlton Campus
Welcome to the Virology/Molecular Lab!

- 10 FTE MLT, 1.7 FTE MLA, 1 TS, 2 PhD Scientists

- Equipment:
  - Genie II (Optigene-ProLab Diagnostics) x2
  - BD Max (Becton Dickinson) x2
  - Rotorgene (Qiagen) x 8
  - easyMag (bioMérieux) x 3
Bacterial and Viral Enteric Panels

- **In-house assay on the BD Max**
  - Bacterial panel: June 4\(^{th}\), 2018 (n=2795)
    - Salmonella, Shigella, C. jejuni, C. coli, E. coli 0157 Stx 1&2, Yersinia
  - Viral panel: January 1\(^{st}\), 2018 (n=1564)
    - Rotavirus, Adenovirus, Norovirus

- **Current workflow:**

Para-Paks → BD Max Prep → Sample processing on BD Max
The Stool Sample Incident

Ugh! Look at this!

Yuck! Get that thing away from me!
Copan FecalSwab™

Advantages over Para-Pak:

- No excess
- Easy to use
- Easy to transport
- Improve workflow
  ▫ Sample suitable for automatic specimen processor
- Cost-effective
- Rectal swab?
Despite the apparent pre-analytic benefits of the FecalSwab...

1. Can the FecalSwab be adapted to, and achieve equal detection on the BD Max as the Para-Pak for use with our in-house bacterial enteric assay?

2. How well does the FecalSwab perform as a storage system at various temperatures over 7 days?
   - Molecular detection stability (BD Max)
   - Bacterial viability for drug susceptibility testing
Methods - Adapting the FecalSwab to our LDT on the BD Max

Para-Pak

5 mL stool in 15 mL Cary Blair

Dilution = 1/4

5 µl

FecalSwab

150 µl stool in 2 mL Cary Blair

Dilution = 1/13

Negative stool spiked with:
E. coli O157 stx-1/2 - S. enteritiditis – S. sonnei - C. coli - C. jejuni – Y. entercolitica

Experimental
10 µl
20 µl
30 µl
50 µl
Optimal Fecal Swab Volume for BD Max - RT-PCR Plots -

- E. coli O157
- Shiga toxin 1/2
- S. sonnei
- Y. entercolitica

RFU vs. Cycle Number

Not all data shown, results are representative of two independent experiments.
Summary - FecalSwab Specimen Volume for BD Max

• No difference in Ct values when 20, 30, or 50 ul were loaded on the BD max

• Able to adapt the FecalSwab to our BD Max bacterial enteric LDT using 20 ul of stool sample
- To do list -

1. Can the FecalSwab be adapted to, and achieve equal detection on the BD Max as the Para-Pak for use with our in-house BD Max bacterial enteric assay?

2. How well does the FecalSwab perform as a storage system at various temperatures over 7 days?

   A) Effect of time and temperature on molecular detection stability of enteropathogenic bacteria stored in the FecalSwab
Methods - Molecular Detection Stability

Salmonella or Campylobacter spp. or Yersinia or Shigella or E. coli or

0.5 McFarland → 1:10 → 1:2 → ~150 µl → 20 µl

Saline → Neg. stool mix 7.5 x 10^6 CFU/mL →

4°C 1 2 3

22°C 1 2 3

35°C 1 2 3

Storage Time: Baseline
1 day
2 days
7 days

Samples run same day as collected on BD Max
Molecular detection of enteric bacteria over a one week period at various temperatures

**C. jejuni**

![Graph for C. jejuni](image)

**S. sonnei**

![Graph for S. sonnei](image)

**C. coli**

![Graph for C. coli](image)

**Y. entercolitica**

![Graph for Y. entercolitica](image)

Mean ± S.D. of three independent experiments; *p < 0.05, ***p < 0.0001
Molecular detection of enteric bacteria over a one week period at various temperatures

**E. coli O157**

**Shiga toxin 1/2**

**S. enteritidis**

Mean ± S.D. of three independent experiments
Summary - Molecular Detection by the BD Max

• Fecal Swabs stored at 4°C or room temperature show no loss in detection for all targets for at least one week

• Ability of the Fecal Swab to maintain the stability of molecular detection for at least 48 h at 35°C suggests this system may suitable for short-term storage or transportation in warmer climates
- To do list -

1. Can the FecalSwab be adapted for use with our in-house BD Max bacterial enteric assay?

2. How well does the FecalSwab perform as a storage system under various conditions over 7 days?
   B) Maintain bacterial viability for future drug susceptibility testing
Methods - Bacterial Viability

Salmonella
Campylobacter spp.
Yersinia
Shigella
E. coli

0.5 McFarland
Saline
Saline

7.5 x 10^6 CFU/mL

~150 µl

Storage Time:
Baseline
1 day
2 days
7 days

Blood Agar

Dilutions plated in duplicate

10^{-1}

100 µl

10^{-2}

100 µl

10^{-3}

100 µl
Recovery of Enteropathogenic Bacteria from Fecal Swab

**C. jejuni**

- 4°C
- 22°C
- 35°C

**C. coli**

- 4°C
- 22°C
- 35°C

**S. sonnei**

- 4°C
- 22°C
- 35°C

**S. enteritidis**

- 4°C
- 22°C
- 35°C

*Error bars* represent the mean ± S.D. of three independent experiments.
Recovery of Enteropathogenic Bacteria from Fecal Swab

**E. coli O157 stx-1/2**

- 4°C
- 22°C
- 35°C

**Y. entercolitica**

- 4°C
- 22°C
- 35°C

Error bars represent the means ± S.D. of three independent experiments.
Summary - Recovery of Isolates

• Storage of Fecal Swab at 4°C is optimal
  ▫ Prevents bacterial overgrowth
  ▫ Recovery of *Campylobacter* spp.

• If *Campylobacter* spp. culture is not of interest, non-refrigerated Fecal Swab specimens can still be used to recover other pathogens
Take Home Message

• Copan FecalSwab can be adapted to our BD Max LDT for bacterial enterics

• FecalSwab appears to be a well-suited swab device for the transport and storage of enteric bacteria
  ▫ Maintains bacterial viability
  ▫ Molecular detection stability

• FecalSwab -> molecular assays -> antimicrobial susceptibility testing
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