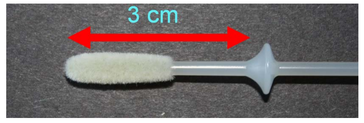
COPAN FLOCKED RECTAL SWAB SAMPLES OUTPERFORM BULK STOOL FOR THE DETECTION OF DIARRHEAL PATHOGENS IN CHILDREN WHEN USING THE xTAG GASTROINTESTINAL PATHOGEN PANEL (GPP ™) ASSAY

David M Goldfarb[1,4], Andrew P Steenhoff[2,4], Jeffrey M Pernica[1], Santina Castriciano[3], Sylvia Chong[1], Kathy Luinstra[1], Gerard AJ Morris[4], Margaret Mokomane[5], Irene Goercke[4], James Mahony[1], and Marek Smieja[1]
[1]McMaster University, Hamilton, Canada; [2]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States, [3]Copan Italia SpA, Brescia, Italy,
[4]Botswana UPenn Partnership, Gaborone, Botswana, and [5]Botswana National Health Laboratory, Gaborone, Botswana

BACKGROUND

• Acute gastroenteritis is a leading cause of morbidity and mortality in children in much of the world. It is often difficult to obtain bulk stool specimens in a timely fashion making identification of enteric pathogens challenging.
• We developed a FLOQ™ (Copan Italia, Brescia, Italy) swab that was anatomically designed to specifically sample the rectal mucosa in young children.



OBJECTIVE

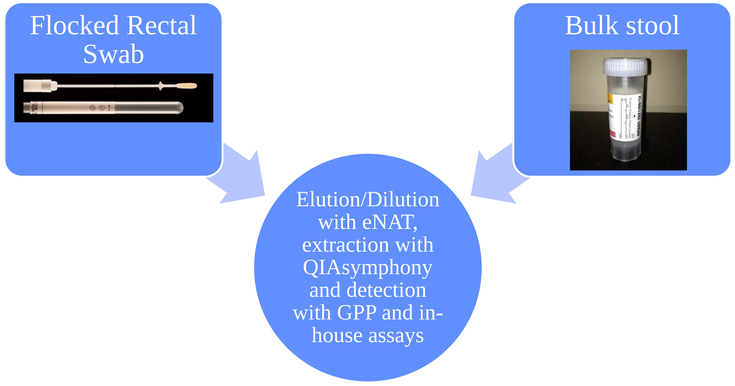
• To assess the ability of a 15 target multiplex PCR to detect stool borne pathogens in children admitted to hospital in Botswana with acute gastroenteritis using both flocked swab rectal samples and bulk stool samples.

METHODS

• Children < 13 years of age admitted to hospital with a diagnosis of acute gastroenteritis were enrolled prospectively from Princess Marina Hospital, the largest referral hospital in Botswana.
•Clinical data was collected and both an anatomically designed pediatric flocked rectal swab and bulk stool samples were obtained from each child as soon as possible after enrolment.
•Specimens were stored at -80 C prior to shipment to McMaster University, Canada for testing. Both the swabs and the stools were eluted in 1ml of eNAT (Copan Italia).
•Specimens were extracted via QIAsymphony (Qiagen, Germantown MD) using the DSP Virus/Pathogen Mini-Kit.

METHODS (cont'd)

•Reverse transcription, amplification and detection of 15 pathogen targets (3 viruses, 3 parasites and 9 bacteria) were performed using the Gastrointesintal Pathogen Panel (GPP) assay (Luminex Molecular Diagnostics, Toronto, Canada) on the MAGPIX ® system.
•Samples were also tested by in-house viral (rotavirus A, norovirus GI/II, adenovirus) and bacterial (*Salmonella* spp., *Shigella* spp., *Campylobacter jejuni/coli*) multiplex PCR assays adapted from published assays (Logan C, 2006; Malorny 2004; Vu Dinh Thiem 2004; Josefsen 2004)
• . McNemar's test for paired samples used to assess assay performance.



RESULTS

•Total of 195 flocked rectal swab specimens were collected of which 164 (84%) also had matched bulk stool collected
•One child did not have rectal swab collected as had imperforate anus
•Specimens were collected from Sept 6, 2012 until Jan 29, 2013
•Mortality for children with any specimen collected was 4.6% (9/195)
•Using the GPP assay swabs detected 9.5% more pathogen targets (211 vs. 191)

RESULTS (cont'd)

•The majority of the difference seen between the performance of each specimen type was due to higher yield of bacterial targets

Table 1. Detection of any bacterial targets using GPP assay (p < 0.001)

	Bulk stool (+)	Bulk stool (-)
Rectal swab (+)	65	22
Rectal swab (-)	5	65

Table 2. Detection of any bacterial targets with in-house bacterial multiplex assay (p < 0.001)

	Bulk stool (+)	Bulk stool (-)
Rectal swab (+)	69	25
Rectal swab (-)	4	66

Table 3. Detection of any viral targets using GPP assay (p = 0.450)

	Bulk stool (+)	Bulk stool (-)
Rectal swab (+)	55	2
Rectal swab (-)	5	101

Table 4. Detection of any viral targets using in-house viral multiplex assay (p=0.114)

	Bulk stool (+)	Bulk stool (-)
Rectal swab (+)	64	2
Rectal swab (-)	8	90

• Flocked swab collection was deemed acceptable or slightly acceptable when compared with bulk stool collection by 98% of parents/guardians

CONCLUSIONS

• Flocked rectal swab samples collected from children with severe gastroenteritis tested with the GPP assay and other molecular assays performed as well or better than bulk stool when tested with the same assays.
•The improved performance was primarily due to increased identification of bacterial pathogens that reside in the large bowel which may be better sampled with this method. Flocked rectal swab specimens may greatly facilitate the rapid molecular diagnosis of diarrheal disease.