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A comparison of flocked swabs and traditional swabs, using multiplex real timePCR for detection of common gastroenteritis pathogens in Botswana

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Abstract

We compared the performance of flocked and matched traditional rectal swabs collected from 236 children admitted with gastroenteritis in Botswana. All samples were tested using real time multiplex-PCR assays for nine enteric pathogens. There was a 20% higher detection of *Shigella* from flocked swabs, but most other pathogens had similar detection rates.

Enteric infections are the second leading cause of death in children under the age of 5 years (1). Botswana has experienced a number of diarrhoea outbreaks which have been associated with significant mortality (2). At outpatient clinic visits, children may not be able to produce stool in the short time interval between clinician assessment and return home; on inpatient wards, health-care providers are regularly in short supply, commonly have competing priorities, and so stool collections may not happen in a timely fashion. For these reasons, swabs have been used as an alternative to bulk stool samples (4). There is demonstrated

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Conflict of Interest

The authors have nothing to disclose and declare no potential conflict of interest.

superior performance of flocked swabs over traditional fibre wrapped swabs for respiratory as well as vaginal specimens (5,6). Flocked swabs have been used for the detection of respiratory viruses and bacteria (7). In a previous study, we found that specifically designed flocked rectal swabs resulted in higher detection of bacterial pathogens using multiplex PCR assays compared to bulk stool samples (8). For this study we compared the performance of flocked rectal swabs to traditional rectal swabs for the molecular detection of enteric pathogens in children with acute gastroenteritis in Botswana.

Two-hundred thirty-six children (109 females) aged ≤5 years presenting with diarrhoea at Princess Marina Hospital (PMH), Gaborone, Botswana were studied. Diarrhoea was defined according to World Health Organization criteria (9). Ethical approval was obtained from the Ministry of Health, PMH, University of Pennsylvania and McMaster University research ethics committees.

Paired flocked rectal swab and traditional rayon fibre swab (Copan Italia, Brescia, Italy) specimens were collected consecutively. The flocked swab was collected first and the swabs were transported chilled in a cooler box and stored at −80°C until testing. The swabs were pre-treated as previously described (8). Total nucleic acid extraction was performed according to the NucliSENS easy MAG instrument (bioMérieux, Marcy l'Étoile, France) Specific A protocol with final elution in 70uL.

Laboratory-developed real time multiplex PCR assays on the ABI 7500 FAST (Life Technologies) were used to detect the nine most prevalent gastrointestinal pathogens (rotavirus, norovirus GI/GII, adenovirus, *Shigella, Salmonella, Campylobacter*, ETEC LT/ST, *Giardia* and *Cryptosporidium*). Not all samples were tested for *Campylobacter* as the reagents for this target were not available for a portion of the study. The primers and probes areas previously described (8). Additional primers and probes for *Giardia, Cryptosporidium*, and ETEC LT/ST are shown in Table 1. Five microlitres of extracted nucleic acid from each swab was added to the primers, probes, and the QuantiTect multiplex no ROX PCR kit reagents for amplification of the bacterial and parasitic pathogens and QuantiTect Virus ROX Vial kit reagents for the viral pathogens (Qiagen, Mississauga, Ontario). Extraction and master-mix negative controls and a positive control were included with each assay. Cycling parameters for bacteria and parasite assays were: 1 min at 60°C, 15 min at 95°C, followed by 45 cycles of 20 sec at 95°C and 1 min 10 sec at 60°C, and a final hold of 1 min at 60°C. Cycling parameters for the viral assay were as follows: 1 min at 50°C, 20 min at 50°C, 5 min at 95°C, for reverse transcription and denaturation followed by 45 cycles of 15 sec at 95°C, 1 min 15 sec at 60°C), and a final 1 min hold at 60°C.

Sensitivities were calculated using an expanded reference standard of either flocked swab or traditional swab positive (consensus standard), and the results were compared using the McNemar exact test for paired samples. Mean threshold cycle (Ct) values were calculated and mean differences (95% CI) for matching swabs determined. Samples were considered positive with a Ct value of less than 45. A paired sample *t* test was used to compare the Ct values in the matched positive/concordant samples. A p value < 0.05 was considered significant.

A total of 236 matched swab pairs were tested for *Shigella spp., Salmonella spp* and ETEC LT/ST; 189 matched swab pairs were tested for *Campylobacter jejuni/coli*; 210 matched swab pairs were tested for rotavirus, adenovirus and norovirus GI/GII; and 213 matched swab pairs were tested for *Giardia* and *Cryptosporidium*. The median age of the study participants that were sampled was 9.1 months (25–75% ile 4.5–13.9 months).

Sensitivities for flocked swabs across all pathogens ranged from 80%–96%, whereas for traditional swabs, sensitivities ranged from 70%–96%, Table 2. For viruses and parasites, no differences were observed between the two swabs, except for adenovirus, for which the detection rate was marginally higher in flocked compared to the traditional swab but the difference was not statistically significant, 96% vs 80%, p=0.070. For bacterial pathogens, the sensitivity for the flocked swab was significantly higher for *Shigella spp* compared to the traditional swab, 91% vs 70%, p=0.016. Overall, 25% of the samples were positive for *Shigella* using flocked swabs as compared to 19% using traditional swabs (yield difference 5.5%, 95% CI 1.0–10.0%). No statistically significant differences were observed for the other bacterial pathogens. Table 3 compares Ct values for reactive concordant samples. The mean Ct values for flocked swabs ranged from 23–34, whereas for traditional swabs they ranged from 22–35. When positive samples only were examined, detection of *Shigella, Campylobacter, Salmonella*, and *Cryptosporidium* occurred at statistically lower Ct using flocked swabs. Detection of rotavirus using flocked swabs occurred at a statistically higher Ct (p=0.002) compared to the traditional swabs, however the sensitivity for rotavirus using flocked swabs remained acceptable at 96.1%. There was no significant difference between Ct values for flocked and traditional swabs for the detection of norovirus GI/GII, ETEC LT/ST and *Giardia* detection. We also looked at the Ct trends of multiple pathogens within the same patient sample, and in 30 samples with *Campylobacter* and *Shigella*, 19/30 (63%) flocked swabs had lower Ct values for both pathogens compared to 3/30 (10%) for traditional swabs.

Our findings suggest that the anatomically designed flocked rectal swabs provide a 20% increase in sensitivity for *Shigella* detection when compared with matched traditional swab samples. Given that *Shigella* is a common and treatable cause of severe diarrhoea in children and its detection has been associated with increased risk of mortality, our results have important ramifications for those seeking to optimize paediatric care in resource-limited settings (15–17).

The main limitation of our study is that the flocked swab samples were collected just prior to traditional swab samples and this may have potentially favoured the flocked swab samples. We had however collected 38 pairs of flocked rectal swab samples as part of our initial validation and PCR testing on both matched swabs revealed very close correlation for bacterial and viral targets and identical results for *Shigella* detection (10 positive and 28 negative, data not shown). Also given that for some pathogens (e.g. rotavirus) the mean nucleic acid yield was actually higher with the traditional swabs, this would suggest that sequence of sample collection did not always disadvantage the second swab. Several paired swab studies of other mucosal surfaces have shown that sequence of swab collection did not significantly affect sensitivity for pathogen detection (18, 19). One possible reason that the flocked rectal swab outperformed the traditional swab for *Shigella* detection is that it has a "stopper" at 3.2cm mark and this might aid in ensuring adequate sampling at the rectal mucosa (where *Shigella* causes pathology). Another possible reason is the "flocked" nature of the swab, which as mentioned has been shown to improve pathogen and cellular yield at other mucosal surfaces when compared with traditional swabs (5,20).

Overall, our findings suggest that these rectal flocked swabs may offer improved molecular detection of *Shigella* when compared with traditional swabs.

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Highlights of the research

- Sensitivities for flocked swabs across all pathogens ranged from 80%–96%, whereas for traditional swabs, sensitivities ranged from 70%–96%
- Our findings suggest that the anatomically designed flocked rectal swabs provide a 20% increase in sensitivity for *Shigella* detection when compared with matched traditional swab samples
- Our results have important ramifications for those seeking to optimize pediatric care in resource-limited settings

Table 1

Pathogen primers and probes used (for other assay targets see reference 8)

Pathogen target	Forward primer	Reverse Primer	Probe sequence	Target	Adapted from
Giardia	GACGGCT CAGGACA ACGGTT	TTGCCAG CGGTGTC CG	CCCGCGGCGG TCCCTGCTAG	SSU RNA gene	10
Cryptosporidium	CAAATTG ATACCGT TTGTCCT TCTG	GGCATGT CGATTCT AATTCAG CT	TGCCATACATT GTTGTCCTGAC AAATTGAA	COWP gene	11
E. coli ST	TTCACCT TTCGCTC AGGATG	ACCCGGT ACAAGCA GGATTA	CTGAAAGCAT GAATAGTAGC AATTACTG	Stable toxin (ST) gene	12
E. coli LT	AACAGGG AATATAG AGACCG	CAACCTT GTGGTGC ATGATG	AGAGGATGGT TACAGATTAG CAGGT	Labile toxin (LT) gene	13,14

Table 2

Comparison of the sensitivities of the flocked swabs and the traditional swabs. Sensitivities were calculated using either sample positive as the reference test.

Pathogen target (number of paired samples tested)	Result (number of samples for the pathogen)					Sensitivity of Flocked*	Sensitivity of Traditional*	McNemar exact test p-value
	Either positive	Both positive	Flocked swab only positive	Traditional swab only positive	Both negative			
Viruses								
Rotavirus (210)	51	47	2	2	159	96.1	96.1	0.617
Adenovirus (210)	46	35	9	2	164	95.7	80.4	0.070
Norovirus (210)	22	19	2	1	188	95.5	90.9	1.000
All viruses	119	101	13	5	511	95.8	89.1	0.099
Bacteria								
Shigella (236)	64	39	18	6	173	90.5	71.4	0.025
Campylobacter (189)	121	85	23	16	65	87.1	81.4	0.34
Salmonella (236)	24	17	4	2	213	91.3	82.6	0.69
ETEC LT/ST (236)	54	39	4	11	182	79.6	92.6	0.121
All bacteria	263	180	49	35	633	86.7	81.4	0.16
Parasites								
Giardia (213)	18	13	2	3	195	83.3	88.9	1.000
Cryptosporidium (213)	39	33	4	2	174	94.9	89.7	0.683
All parasites	57	46	6	5	369	91.2	89.5	1.000

Table 3

Comparison of mean cycle threshold (Ct) values according to swab type and Ct value differences for matched concordant samples, mean (95% CI).

Pathogen target	Mean Flocked Ct	Mean Traditional Ct	Mean difference of Ct values (95% CI)	p value
Rotavirus	23.39	22.29	1.10 (0.44 to 1.76)	0.002
Adenovirus	22.61	24.03	−1.42 (−2.16 to −0.67)	<0.001
Norovirus	24.43	25.27	−0.85 (−2.19 to 0.50)	0.201
Shigella	24.52	25.87	−1.35 (−2.02 to −0.69)	<0.001
Campylobacter	33.18	34.26	−1.08 (1.64 to −0.53)	<0.001
Salmonella	32.49	33.89	−1.40 (−2.72 to −0.09)	0.038
ETEC LT/ST	29.69	29.44	0.25 (−1.09 to 1.58)	0.708
Giardia	34.47	35.01	−0.54 (−1.96 to 0.88)	0.424
Cryptosporidium	32.75	34.11	−1.36 (2.19 to −0.52	<0.001