

## Supplementary materials

**Table S1. TAC pathogen targets, primer-probe sequences and CT cut-offs for diarrhea associated pathogens**

Pathogen	Target	Primers & Probe sequences (Forward primer-F, Reverse primer-R, Probe-P)	CT for diarrhea associated*	Ref
Adenovirus 40/41	Fiber gene	F: AACTTTCTCTCTTAATAGACGCC R: AGGGGGCTAGAAAACAAAA P: CTGACACGGGCACTCT	35.0	[1]
Astrovirus	Capsid	F: CAGTTGCTTGCTGCGTTCA R: CTTGCTAGCCATCACACTTCT P: CACAGAAGAGCAACTCCATCGC	25.5	[1]
Norovirus GI	ORF1-2	F: CGYTGGATGCGNTTYCATGA R:CTTAGACGCCATCATCATTYAC P:TGGACAGGAGATCGC		[1]
Norovirus GII	ORF1-2	F: AGGATCCATTGCAAGAGGG R: CTACATCAAGCGTGATGGC P: TGGTCAGTTGGTACCGGAG	27.6	[1]
Rotavirus	<i>NSP3</i>	F:ACCATCTWCACRTRACCCTCTATGAG R: GGTCACATAACGCCCCCTATAGC P:AGTAAAAAGCTAACACTGTCAAA	35.0	[1]
Sapovirus	<i>RdRp</i>	F: GAYCASGCTCTCGCYACCTAC F: TTGGCCCTCGCCACCTAC R: CCCTCCATYTCAAACACTA P: CCRCCTATRAACCA	31.6	[1]
EAEC	<i>aaiC</i>	F: ATTGTCCTCAGGCATTTTAC R:ACGACACCCCTGATAAACAA P: TAGTGCATACTCATCATTTAAG		[1]
EAEC	<i>aatA</i>	F: CTGGCGAAAGACTGTATCAT R: TTTTGCTTCATAAGCCGATAGA P:TGGTTCTCATCTATTACAGACAGC		[1]
EPEC	<i>eae</i>	F: CATTGATCAGGATTTTTCTGGTGATA R: CTCATGCGGAAATAGCCGTTA P: ATACTGGCGAGACTATTTCAA		[1]
EPEC	<i>bfpA</i>	F: TGGTGCTTGCCTTGCT R: CGTTGCGCTCATTACTTCTG P: CAGTCTGCGTCTGATTCCAA	19.5	[1]
ETEC	<i>LT</i>	F: TTCCACCGGATCACCAA R: CAACCTTGTTGGTGCATGATGA P: CTTGGAGAGAAGAACCCT		[1]
ETEC	<i>STh</i>  <i>STp</i>	F: GCTAAACCAGYAGRGTTCTTCAAAA F: CCCGGTACARGCAGGATTACAACA R: TGGTCCTGAAAGCATGAA	26.2	[1]

		R: TGAATCACTTGACTCTTCAAAA P: GGCAGGATTACAACAAAGTT P: TGAACAACACATTTTACTGCT		
STEC	<i>stx1</i>	F: ACTTCTCGACTGCAAAGACGTATG R: ACAAATTATCCCCTGWGCCACTATC P: CTCTGCAATAGGTACTCCA		[1]
STEC	<i>Stx2</i>	F: CCACATCGGTGTCTGTTATTAACC R: GGTCAAAACGCGCCTGATAG P: TTGCTGTGGATATACGAGG		[1]
Aeromonas	<i>Aerolysin</i>	F: TYCGYTACCAGTGGGACAAG R: CCRGCAAACCTGGCTCTCG P: CAGTTCCAGTCCCACCACTT		[1]
<i>Campylobacter</i>	<i>cpn60</i>	F: AAAGTIGGMAAAGATGGTGTAT F: AAAGTIGGWAAAGACGGYGTTAT R: TCAAATTGCATACCYTCAAC P: TTTGCCTCTTCMACAGT P: TTTGCTTCTTCWACAGT		[1]
<i>Campylobacter jejuni</i>	<i>hipO</i>	F: CTTGCGGTCATGATGGACATAC R: AGCACCACCCAAACCCTCTTCA P: TGCTTGCTGCAAAGTATT	19.7	[1]
<i>Campylobacter coli</i>	<i>GlyA</i>	F: AAACCAAAGCTTATCGTGTGC R: AGTGCAGCAATGTGTGCAAT P: TAAGCTCCAACCTTCATCCG		[1]
<i>Clostridium difficile</i>	<i>tcdA</i>	F: TTCAAGCAGAAATAGAGCACTC R: TATCAGCCCATTGTTTTATGTATTC P: CACTGACTTCTCCACCTATCCA		[1]
	<i>tcdB</i>	F: GGTATTACCTAATGCTCCAAATAG R: TTTGTGCCATCATTTTCTAAGC P: CCTGGTGTCCATCCTGTTTC		[1]
<i>Cryptosporidium</i>	18S rRNA	F: GGGTTGTATTTATTAGATAAAGAACCA R: AGGCCAATACCCTACCGTCT P: TGACATATCATTCAAGTTTCTGAC	29.1	[1]
<i>Cyclospora cayetanensis</i>	18S rRNA	F: AAAAGCTCGTAGTTGGATTTCTG R: AACACCAACGCACGCAGC P: AAGGCCGGATGACCACGA		[1]
<i>Helicobacter pylori</i>	<i>ureC</i>	F: GACACCAGAAAAAGCGGCTA R: AGCGCATGTCTTCGGTTAAA P: TCACTAAAGCGTTTTCTACC	30.8	[1]
<i>Cystoisospora belli</i>	18S rRNA	F: ATATTCCCTGCAGCATGTCTGTTT R: CCACACGCGTATTCCAGAGA P: CAAGTTCTGCTCACGCGCTTCTGG		[1]
<i>Mycobacterium tuberculosis</i>	IS6110	F: GGGTAGCAGACCTCACCTATG R: AGCGTAGGCGTCGGTGA P: TCGCCTACGTGGCCTTT		[1]
<i>Salmonella enterica</i>	<i>ttr</i>	F: CTCACCAGGAGATTACAACATGG R: AGCTCAGACCAAAAGTGACCATC P: CACCGACGGCGAGACCGACTTT	32.4	[1]

<i>Shigella</i> /enteroinvasive <i>E. coli</i> (EIEC)	<i>ipaH</i>	F: CCTTTTCCGCGTTCCTTGA R: CGGAATCCGGAGGTATTGC P: CGCCTTTCCGATACCGTCTCTGCA	33.1	[1]
<i>Shigella flexneri</i>	Putative periplasmic protein	F: TGGGTGCATCCTGACCTGT R: GACAAACAATAACGAGCTACCGAT P: ACCACGGAATAATCCCGCAG		[2]
	O-antigen#	F: CTCCTATCCGTGATTATAGTGCA R: GCACACACAACTCACTGTATTT P: TCCTTCTCACGATTAAAATC		[2]
	Type 3 restriction enzyme#	F: CTTTCAACGCACGAATATCAAC R: GAACCTGATCCAGACGGAGA P: TTCTTCAGAACCGGGTTTTG		[2]
<i>Shigella sonnei</i>	Putative methylase	F: TGCCGCTAAAATCCTTCTGT R: GCGTACGACGAAAGGAAAAA P: GAAGTTATTGATTCCGCCC		[2]
<i>Plesiomonas shigelloides</i>	<i>gyrB</i>	F: CCGCCGTGAAGGCAAAG R: GCTACCGGCTCACCCAGAT P: CACACCCAAGAATAC		[1]
<i>Vibrio cholerae</i>	<i>hlyA</i>	F: ATCGTCAGTTTGGAGCCAGT R: TCGATGCGTTAAACACGAAG P: ACCGATGCGATTGCCCAA	34.9	[1]
<i>Enterocytozoon bieneusi</i>	ITS	F: CACCAGGTTGATTCTGCCTGAC R: CTAGTTAGGCCATTACCTAACTACCA P: CTATCACTGAGCCGTCC		[1]
<i>Encephalitozoon intestinalis</i>	SSU rRNA	F: TGTGTAGGCGTGAGAGTGTATCTG R: CATCCAACCATCACGTACCAATC P: CACTGCACCCACATCCCTCACCTT		[1]
<i>Entamoeba histolytica</i>	18S rRNA	F: ATTGTCGTGGCCTCCTAACTCA R: GCGGACGGCTCATTATAACA P: TCATTGAATGAATTGGCCATTT		[1]
<i>Giardia lamblia</i>	18SrRNA	F: GACGGCTCAGGACAACGGTT R: TTGCCAGCGGTGTCCG P: CCCGCGGCGGTCCCTGCTAG		[1]
<i>Ancylostoma duodenale</i>	ITS2	F: GAATGACAGCAAACCTCGTTGTTG R: ATACTAGCCACTGCCGAAACGT P: ATCGTTTACCGACTTTAG		[1]
<i>Ascaris lumbricoides</i>	ITS1	F: GCCACATAGTAAATTGCACACAAAT R: GCCTTTCTAACAAGCCCAACAT P: TTGGCGGACAATTGCATGCGAT		[1]
<i>Necator americanus</i>	ITS2	F: CTGTTTGTGCAACGGTACTTGC R: ATAACAGCGTGCACATGTTGC P: CTGTACTACGCATTGTATAC		[1]
<i>Strongyloides stercoralis</i>	Dispersed repetitive sequence	F: TCCAGAAAAGTCTTCACTCTCCAG R: TGC GTTAGAATTTAGATATTATTGTTGCT P: TCAGCTCCAGTTGAACAACAGCCTCCAA		[1]
<i>Trichuris trichiura</i>	18S rRNA	F: TTGAAACGACTTGCTCATCAACTT R: CTGATTCTCCGTAAACCGTTGTC		[1]

		P: CGATGGTACGCTACGTGCTTACCATGG		
MS2	<i>MS2gl</i>	F: TGGCACTACCCCTCTCCGTATTAC R: GTACGGGCGACCCACGATGAC P: CACATCGATAGATCAAGGTGCCTACAAGC		[1]
PhHV	<i>gB</i>	F: GGGCGAATCACAGATTGAATC R: GCGGTTCCAAACGTACCAA P: TATGTGTCCGCCACCATCT		[1]

\*This assay detects most *S. flexneri* serotypes except for serotype 6.

#The combination of these two assays identifies *S. flexneri* serotype 6 when both are positive (Cq≤35).

## References

1. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, et al. Optimization of quantitative PCR methods for enteropathogen detection. PLoS One. 2016;11: 1–11. doi:10.1371/journal.pone.0158199
2. Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet. 2016;388: 1291–1301. doi:10.1016/S0140-6736(16)31529-X

## Effect of co-infection on the clinical presentation of under five years children admitted with diarrhea

The univariate analysis comparing the effect of co-infection on the clinical presentation of the ill children at admission shows that those with co-infection were two times more likely to present with flatulence compared to those with no or mono-infection IRR; 95%CI = 2.42 (1.11-5.42). After adjusting for other clinical symptoms, co-infection was significantly associated with flatulence and loss of appetite as compared to those with no or mono-infection IRR; 95%CI = 2.24 (1.09-4.58) and 1.81 (1.06 – 3.08) respectively. Further, those with running nose were less likely to have co-infection IRR; 95%CI = 0.50 (0.28-0.89). No statistical significance was observed in other clinical presentations. More details are found in the table below

Table S2. Univariate and multivariable analysis of effect of co-infection by diarrhea associated pathogens on the clinical presentation of hospitalized children (N=146).

Clinical Symptoms	Co-infection		Crude IRR	Adjusted IRR (95% CI)	P-value
	No (N=108)	Yes (N=38)			
No: -_stools in 24hr					
3-5	62 (75.61)	20 (24.39)	Ref		
≥6	46 (71.88)	18 (28.13)	1.15 (0.67-1.99)	-	
Nausea					
No	104 (74.29)	36 (25.71)	Ref	-	
Yes	4 (66.67)	2 (33.33)	1.29 (0.40-4.18)	-	
Vomiting					
No	13 (76.47)	4 (23.53)	Ref		
Yes	95 (73.64)	34 (26.36)	1.12 (0.45 – 2.77)	-	

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Abdominal						
Pain						
No	103 (73.57)	37 (26.43)	Ref			
Yes	5 (83.33)	1 (16.67)	0.63 (0.10– 3.88)	-		
Fever						
No	43 (74.14)	15 (25.86)	Ref			
Yes	65 (73.86)	23 (26.14)	1.00 (0.74 – 1.36)	-		
Fatigue						
No	101 (75.37)	33 (24.63)	Ref			
Yes	7 (58.33)	5 (41.67)	1.69 (0.81 – 3.53)	-		
Flatulence						
No	106 (75.18)	35 (24.82)		Ref		
Yes	2 (40.00)	3 (60.00)	2.42 (1.11 – 5.24)	2.24 (1.09-4.58)	0.028	
Running nose						
No	54 (67.50)	26 (32.50)	Ref	Ref		
Yes	54 (81.82)	12 (18.18)	0.56 (0.31- 1.02)	0.5 (0.28-0.89)	0.019	
Coughing						
No	56 (70.00)	24 (30.00)	Ref			
Yes	52 (78.79)	14 (21.21)	0.71 (0.39-1.25)	-		
Loss of appetite						
No	63 (79.75)	16 (20.25)	Ref	Ref		
Yes	45 (67.16)	22 (32.84)	1.62 (0.93-2.83)	1.81 (1.06-3.08)	0.030	
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In this study, we found that co-infections were significantly high among children with flatulence and loss of appetite as compared to no or mono-infection, and lower in those with running nose. After adjusting for other clinical features, flatulence, running nose and loss of appetite remained statistically significant associated with confections. However, there were no further evaluations to compare the diarrhea severity among the cases, hence cannot be considered as a criterion for existence of co-infection.