

Web Extra Material

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eFigure 1. Correlation of real-time polymerase chain reaction cycle threshold (Ct) values from paired stool specimens and rectal swabs that were positive for a given pathogen with both specimens.

eTable 1. Microbiologic approach to enteric pathogen identification.

CULTURE Organism	Set up (agars specific to organisms)	Isolation Methodology
<i>Campylobacter</i>	Stool/swab is planted to Campylobacter Blood Free Agar and incubated up to 72 hours at 42 +/-2C under microaerophilic conditions.	Plates are read for typical growth, and a gram stain is performed for confirmation. Isolate is speciated by MALDI-TOF.
<i>E. coli O157:H7</i>	Stool/swab is planted to Colorex O157-T, a chromogenic agar containing 2.5mg/L potassium tellurite, and incubated at 35+/-2C for up to 24hrs.	Typical mauve colonies are screened for O157 serology and confirmed E. coli by API/Vitek 2
<i>Salmonella</i>	Stool/swab is planted to MacConkey agar with Crystal Violet, Hektoen agar, as well as Shigella-Salmonella agar after 22-24hr enrichment in mannitol-selenite.	Typical colonies are identified by API 20E/Maldi-TOF and serotyped using Check & Trace Salmonella molecular serotyping (Check-Points, Netherlands).
<i>Shigella</i>	Stool/swab is planted to MacConkey agar with Crystal Violet, Hektoen agar, as well as Shigella-Salmonella agar after 22-24hr enrichment in mannitol-selenite broth.	Typical colonies are identified by API 20E/Vitek 2 as Shigella and serogrouped. Groups B (flexneri) and D (sonnei) are serotyped at PL. Groups A (dysenteriae) and C (boydii) are referred to NML for further serotyping.
<i>Yersinia</i>	Stool/swab is planted to Yersinia Agar (CIN) and incubated up to 24hrs at 35Deg C.	Typical colonies are identified by API20E/Maldi-Tof and speciated using biochemicals.
<i>Aeromonas</i>	Stool/swab is planted to Blood Agar Plate and incubated up to 24hrs at 35Deg C. Aeromonas can also be detected from the CIN plate.	Typical alpha haemolytic colonies are confirmed with oxidase test, identified by API20E/Maldi-Tof and speciated using biochemical.

eTable 2. Pathogen yield of collection approaches – all specimens obtained included.

	Either Stool or Swab Positive N; (%)	Rectal Swab Positive N; (%)	Stool Positive N; (%)
Any Test[†]			
	N=1519	N=1514	N=1147
Any Pathogen	1121 (73·8)	1024 (67·6)	871 (75·9)
‘in-house’ Viral Panel (GEV)[¶]			
	N=1518	N=1512	N=1144
Any GEV Target	1021 (67·3)	937 (62·0)	799 (69·8)
Adenovirus	241 (15·9)	180 (11·9)	195 (17·1)
Astrovirus	40 (2·6)	34 (2·3)	34 (3·0)
Norovirus	373 (24·6)	324 (21·4)	289 (25·3)
Rotavirus	395 (26·0)	376 (24·9)	315 (27·5)
Sapovirus	126 (8·3)	116 (7·7)	96 (8·4)
Luminex GPP[§]			
	N=1518	N=1512	N=1143
Any GPP Viral Target	822 (54·2)	743 (49·1)	649 (56·8)
Adenovirus 40/41	107 (7·1)	97 (6·4)	87 (7·6)
Norovirus GI/GII	340 (22·4)	288 (19·1)	264 (23·1)
Rotavirus	395 (26·0)	371 (24·5)	314 (27·5)
Any GPP Bacterial Target	230 (15·2)	190 (12·6)	166 (14·5)
<i>Campylobacter</i>	10 (0·7)	9 (0·6)	5 (0·4)
<i>C. difficile tcdA/B</i>	174 (11·5)	148 (9·8)	121 (10·6)
<i>E. coli</i> O157:H7	7 (0·5)	6 (0·4)	6 (0·5)
ETEC LT/ST	4 (0·3)	2 (0·13)	3 (0·26)
<i>Salmonella</i>	21 (1·4)	14 (0·9)	17 (1·5)
<i>stx1/stx 2</i>	19 (1·3)	13 (0·9)	16 (1·4)
<i>Shigella</i>	5 (0·3)	5 (0·33)	4 (0·35)
<i>Vibrio cholerae</i>	0	0	0
<i>Yersinia enterocolitica</i>	1 (0·1)	0	1 (0·09)
Any GPP Parasite Target	7 (0·5)	1 (0·07)	6 (0·5)
<i>Cryptosporidium</i>	0	0	0
<i>Entamoeba histolytica</i>	3 (0·2)	0	3 (0·3)
<i>Giardia</i>	4 (0·3)	1 (0·07)	3 (0·3)
Bacterial Culture[*]			
	N=1501	N=1468	N=998
Any Bacterial Culture Target	50 (3·3)	43 (2·9)	35 (3·5)
<i>Aeromonas</i> spp.	16 (1·1)	9 (0·6)	7 (0·7)
<i>Campylobacter</i> spp.	9 (0·6)	7 (0·5)	6 (0·6)
<i>E. coli</i> O157:H7	3 (0·2)	3 (0·2)	1 (0·1)
<i>Salmonella</i> spp.	22 (1·5)	18 (1·2)	14 (1·4)
<i>Shigella</i> spp.	4 (0·3)	4 (0·27)	3 (0·3)
<i>Yersinia</i> spp.	4 (0·3)	2 (0·14)	3 (0·3)
Other	1 (0·07)	0	1 (0·1)

† Dry rectal swabs were tested on ‘in-house’ gastroenteritis virus panel and Luminex GPP but were not tested on bacterial culture; rectal swab in gel were tested on bacterial culture but were not tested on ‘in-house’ gastroenteritis virus panel or Luminex GPP; stool specimens were tested on ‘in-house’ gastroenteritis virus panel, Luminex GPP and on bacterial culture. There were 1519 children had a rectal swab and/or a stool specimen tested of which 1512 children had a dry rectal swab tested, 1468 had a rectal swab in gel tested and 1147 had a stool specimen tested.

¶ 1512 children had dry rectal swabs obtained – all were tested on the ‘in-house’ gastroenteritis virus panel and Luminex GPP

§ 1468 children had rectal swab in gel obtained – all were tested on bacterial culture.

* 1147 stool specimens were obtained; 3 stool specimens were unable to be tested employing the ‘in-house’ gastroenteritis virus panel. 4 stool specimens were unable to be tested employing the Luminex GPP. 149 stool specimens deemed not suitable for standard enteric bacterial culture (128 insufficient quantity, 15 laboratory linked record could not be identified, 5 requisition incomplete, 1 sample leakage).

eTable 3. Comparative yields in relation to specimen type at a pathogen level.

	Either Stool or Swab Positive N; (%)	Rectal Swab Positive N; (%)	Stool Positive N; (%)	*P Value
Any Test N=1142				
Any Enteropathogen N (%)	885 (77.5)	793 (69.4)	866 (75.8)	< 0.0001‡
'in-house' Viral Panel (GEV); N=1138				
Any GEV Target N (%)	803 (70.6)	725 (63.7)	793 (69.7)	< 0.0001‡
Adenovirus N (%)	196 (17.2)	137 (12.0)	193 (17.0)	< 0.0001‡
Astrovirus N (%)	33 (2.9)	28 (2.5)	33 (2.9)	0.063
Norovirus GI/GII N (%)	288 (25.3)	241 (21.2)	287 (25.2)	< 0.0001‡
Rotavirus N (%)	319 (28.0)	302 (26.5)	313 (27.5)	0.035
Sapovirus N (%)	99 (8.7)	89 (7.8)	96 (8.4)	0.092
Luminex GPP; N=1137				
Any GPP Viral Target N (%)	653 (57.4)	580 (51.0)	643 (56.6)	< 0.0001‡
Adenovirus 40/41 N (%)	85 (7.5)	77 (6.8)	85 (7.5)	0.0078
Norovirus GI/GII N (%)	264 (23.2)	214 (18.8)	262 (23.0)	< 0.0001‡
Rotavirus N (%)	320 (28.1)	298 (26.2)	312 (27.4)	0.016
Any GPP Bacterial Target N (%)	194 (17.1)	155 (13.6)	165 (14.5)	0.275
<i>Campylobacter</i> sp. N (%)	5 (0.4)	4 (0.4)	5 (0.4)	>0.999
<i>C. difficile</i> tcdA/B N (%)	147 (12.9)	122 (10.7)	120 (10.6)	0.890
<i>E. coli</i> O157:H7 N (%)	6 (0.5)	5 (0.4)	6 (0.5)	>0.999
ETEC LT/ST, N (%)	3 (0.26)	1 (0.09)	3 (0.26)	0.500
<i>Salmonella</i> sp. N (%)	20 (1.8)	13 (1.1)	17 (1.5)	0.344
<i>stx1/stx2</i> N (%)	18 (1.6)	12 (1.1)	16 (1.4)	0.289
<i>Shigella</i> sp. N (%)	5 (0.4)	5 (0.4)	4 (0.4)	>0.999
<i>Vibrio cholerae</i> N (%)	0	0	0	-
<i>Yersinia enterocolitica</i> N (%)	1 (0.09)	0	1 (0.09)	-
Any GPP Parasite Target N (%)	6 (0.5)	0	6 (0.5)	-
<i>Cryptosporidium</i> N (%)	0	0	0	-
<i>Entamoeba histolytica</i> N (%)	3 (0.3)	0	3 (0.3)	-
<i>Giardia</i> N (%)	3 (0.3)	0	3 (0.3)	-
Bacterial Culture; N=965				
Any Bacterial Culture Target N (%)	43 (4.6)	27(2.8)	35 (3.6)	0.152
<i>Aeromonas</i> spp. N (%)	11 (1.1)	4 (0.4)	7 (0.7)	0.549
<i>Campylobacter</i> spp. N (%)	6 (0.6)	4 (0.4)	6 (0.6)	0.500
<i>E. coli</i> O157:H7 N (%)	1 (0.1)	1 (0.1)	1 (0.1)	>0.999
<i>E. coli</i> O26:H11 N (%)	1 (0.1)	0	1 (0.1)	-
<i>Salmonella</i> spp. N (%)	16 (1.7)	12 (1.2)	14 (1.5)	0.687
<i>Shigella</i> spp. N (%)	4 (0.4)	4 (0.4)	3 (0.3)	>0.999
<i>Yersinia</i> spp. N (%)	4 (0.4)	2 (0.2)	3 (0.3)	>0.999

*P value for McNemar test. P value for summary measures (any pathogen; any GEV target; any GPP viral target; any GPP bacterial target; any GPP parasite target; and any bacterial culture target) adjusted using Benjamini-Hochberg procedure (N=5) and significance was determined separately from those of the individual pathogen targets (N=27).

‡Indicates statistically significant after correction via to Benjamini-Hochberg procedure for multiple comparisons.

eTable 4. Agreement between collection approaches, submitted paired samples (i.e. rectal swab and stool) only.

Any Pathogen (N=1142)		
	Stool Positive \geq 1 Pathogen	Stool Negative for All Pathogens
Rectal Swab Positive \geq 1 pathogen	774	19
Rectal Swab Negative for All Pathogens	92	257
Observed Kappa, κ (95% CI)	0.76 (0.71, 0.80)	
Viruses (N=1138)		
	Stool Positive \geq 1 Virus	Stool Negative for Viruses
Rectal Swab Positive \geq 1 Virus	717	11
Rectal Swab Negative for All Viruses	79	331
Observed Kappa, κ (95% CI)	0.82 (0.79, 0.86)	
Bacteria (N=1142)		
	Stool Positive \geq 1 Bacteria	Stool Negative for All Bacteria
Rectal Swab Positive \geq 1 Bacteria	134	32
Rectal Swab Negative for All Bacteria	45	931
Observed Kappa, κ (95% CI)	0.74 (0.68, 0.80)	
Parasites (N=1137)		
	Stool Positive \geq 1 Parasite	Stool Negative for All Parasites
Rectal Swab Positive \geq 1 Parasite	0	0
Rectal Swab Negative for All Parasites	6	1131
Observed Kappa, κ (95% CI)	-	

eTable 5. Individual pathogen level agreement between collection approaches, submitted paired samples (i.e. rectal swab and stool) only.

Viruses		
Astrovirus	Stool Positive	Stool Negative
Rectal Swabs Positive	28	0
Rectal Swabs Negative	5	1105
Observed Kappa, κ (95% CI)	0.92 (0.84, 0.99)	
Adenovirus	Stool Positive	Stool Negative
Rectal Swabs Positive	134	3
Rectal Swabs Negative	59	942
Observed Kappa, κ (95% CI)	0.78 (0.73, 0.83)	
Norovirus GI/GII	Stool Positive	Stool Negative
Rectal Swabs Positive	240	2
Rectal Swabs Negative	47	849
Observed Kappa, κ (95% CI)	0.88 (0.85, 0.91)	
Rotavirus	Stool Positive	Stool Negative
Rectal Swabs Positive	300	6
Rectal Swabs Negative	18	814
Observed Kappa, κ (95% CI)	0.95 (0.926, 0.968)	
Sapovirus	Stool Positive	Stool Negative
Rectal Swabs Positive	86	3
Rectal Swabs Negative	10	1039
Observed Kappa, κ (95% CI)	0.92 (0.88, 0.96)	
Bacteria		
<i>Clostridium difficile tcdA/B</i>	Stool Positive	Stool Negative
Rectal Swabs Positive	95	27
Rectal Swabs Negative	25	990
Observed Kappa, κ (95% CI)	0.76 (0.70, 0.82)	

eTable 6. Comparison of Ct values of specimen test results on ‘in-house’ gastroenteritis virus panel.

	Ct Values Concordant Positive				Ct Values Stool Positive Swab Negative			Ct Values Swab Positive Stool Negative		
	N	Stool Median (IQR)	Swab Median (IQR)	P Value*	N	Stool Median (IQR)	P Value‡	N	Swab Median (IQR)	P Value‡‡
Astrovirus	28	19 (18, 24)	23 (20, 28)	<0.0001	5	31 (19, 33)	0.088	0	-	-
Adenovirus	134	17 (13, 25)	23 (19, 30)	<0.0001	59	34 (31, 36)	< 0.0001	3	36 (36,37)	0.0019
Norovirus GI	10	22 (19, 26)	25 (20, 30)	0.022	2	19 (16, 22)	0.606	0	-	-
Norovirus GII	231	17 (15, 21)	22 (19, 27)	<0.0001	45	22 (16, 32)	0.00049	1	30-0	0.284
Rotavirus	296	18 (16, 22)	20 (18, 24)	<0.0001	17	22 (17, 27)	0.059	6	29 (20, 32)	0.031
Sapovirus	86	18 (16, 25)	25 (21, 30)	<0.0001	10	34 (22, 35)	0.0021	3	36 (21, 38)	0.177

* P value reflects comparison of paired positive stool and swab specimens, evaluated using Wilcoxon Signed Rank Test.

‡ P value reflects comparison to stool Ct value of concordant positive specimens, evaluated using Mann-Whitney U Test (un-paired specimens).

‡‡ P value reflects comparison to swab Ct value of concordant positive specimens, evaluated using Mann-Whitney U Test (un-paired specimens).

eTable 7. Comparison of Ct values of specimen test results on ‘in-house’ gastroenteritis virus panel comparing values between children with and without diarrhoea.

	Stool Ct value Median (IQR)					Rectal Swabs Ct value Median (IQR)				
	N	Diarrhea present	N	Diarrhea absent	P Value‡	N	Diarrhea present	N	Diarrhea absent	P Value‡
Astrovirus	28	19.40 (17.93, 23.95)	5	25.90 (18.87, 32.55)	0.157	30	22.00 (19.88, 25.38)	3	34.50 (28.01, -)	0.0040
Adenovirus	153	23.30 (13.80, 32.80)	42	23.30 (16.33, 32.78)	0.589	137	23.00 (18.95, 28.95)	43	26.18 (19.30, 32.40)	0.209
Norovirus GI	6	22.40 (18.66, 26.38)	5	22.10 (15.35, 28.17)	0.662	7	26.10 (22.43, 29.37)	3	20.70 (17.10, -)	0.667
Norovirus GII	175	17.70 (15.80, 22.10)	102	17.25 (14.50, 21.68)	0.181	202	23.25 (19.76, 27.60)	111	23.40 (18.90, 27.83)	0.751
Rotavirus	336	19.40 (17.93, 23.95)	39	20.90 (17.10, 24.50)	0.0099	275	20.22 (17.70, 23.50)	40	21.66 (19.99, 25.83)	0.0043
Sapovirus	71	18.90 (16.50, 27.70)	25	18.10 (14.45, 22.30)	0.227	78	25.39 (21.72, 29.95)	38	24.55 (19.10, 30.82)	0.476

‡ P Mann-Whitney U Test (un-paired specimens).

eTable 8. Comparative and overall yields in relation to specimen type, with *C. difficile* positive specimens considered as negative in participants < 2 years of age.

	No. (%)		Odds Ratios (95% CI)‡
	Rectal Swab	Stool	
Comparative Yield - Unadjusted ≥ 1 Pathogen	1142 770 (67.4)	1142 841(73.6)	1.35 (1.24, 1.47)
Adjusted for Interaction with Diarrhea without Diarrhea			1.23 (1.11, 1.36) 1.71 (1.43, 2.04)
Overall Yield - Unadjusted ≥ 1 Pathogen	1519 991 (65.2)	1519 846 (55.7)	0.67 (0.61, 0.74)
Adjusted for Interaction ED with Diarrhea ED without Diarrhea Home with Diarrhea Home without Diarrhea			0.52 (0.45, 0.60) 0.73 (0.60, 0.88) 0.94 (0.77, 1.15) 1.32 (1.01, 1.72)

‡ORs represent stool relative to rectal swab.

eTable 9. Comparative yield in relation to specimen type with analysis restricted to paired samples collected within 24 hours of each other.

	No. (%)		Odds Ratios (95% CI)‡
	Rectal Swab	Stool	
Comparative Yield - Unadjusted ≥ 1 Pathogen	883 634 (71.8)	883 686 (77.7)	1.37 (1.24, 1.51)
Adjusted for Interaction			
with Diarrhoea			1.25 (1.11, 1.40)
without Diarrhoea			1.75 (1.40, 2.17)

‡ORs represent stool relative to rectal swab.

eFigure 1. Correlation of real-time polymerase chain reaction cycle threshold (Ct) values from paired stool specimens and rectal swabs that were positive for a given pathogen with both specimens.

Figures A – E reflect Real-time polymerase chain reaction Ct values that can be used as a semi-quantitative measure of the amount of nucleic acid of the detected target present in each sample. The value is inversely proportional to the amount of viral nucleic acid in the specimen; lower Ct values correlate with larger amounts of deoxyribonucleic acid/ribonucleic acid of the target.

