

Supplementary Material

STable 1. Primer and probes for the conventional multiplex PCR and real-time RT-PCR

Organisms	Primers	Target	Primer Sequences (5'-3')	Amplicons (bp)	References
DAEC	daaE-F	<i>daaE</i>	GAA CGT TGG TTA ATG TGG GGT AA	542	38
	daaE-R		TAT TCA CCG GTC GGT TAT CAG T		
EAEC	aaiC-F	<i>aaiC</i>	ATT GTC CTC AGG CAT TTC AC	215	39
	aaiC-R		ACG ACA CCC CTG ATA AAC AA		
	aggR-F	<i>aggR</i>	GTA TAC ACA AAA GAA GGA AGC	254	40
	aggR-R		ACA GAA TCG TCA GCA TCA GC		
EIEC	ipaH-F	<i>ipaH</i>	CTC GGC ACG TTT TAA TAG TCT GG	933	38
	ipaH-R		GTG GAG AGC TGA AGT TTC TCT GC		
EPEC	Bfp-F	<i>bfpA</i>	AAT GGT GCT TGC GCT TGC TGC	326	40
	Bfp-R		GCC GCT TTA TCC AAC CTG GTA		
	eae384-F	<i>eae</i>	GAC CCG GCA CAA GCA TAA GC	384	39
	eae384-R		CCA CCT GCA GCA ACA AGA GG		
	eae917-F	<i>eae</i>	CTG AAC GGC GAT TAC GCG AA	917	40
	eae917-R		CGA GAC GAT ACG ATC CAG		
ETEC	LT-F	<i>Elt</i>	CAC ACG GAG CTC CTC AGT C	508	39
	LT-R		CCC CCA GCC TAG CTT AGT TT		
	ST-F	<i>est</i>	GCT AAA CCA GTA G/AGG TCT TCA AAA	147	39
	ST-R		CCC GGT ACA G/AGC AGG ATT ACA ACA		
	LngA1-F	<i>lngA</i>	ATGAGCCTGCTGGAAGTTATCATT	621	This study
	LngA621-R		TTAACGGCTACCTAAAGTAATTGA		
STEC	eae917-F	<i>eae</i>	CTG AAC GGC GAT TAC GCG AA	917	40
	eae917-R		CGA GAC GAT ACG ATC CAG		
	eae384-F	<i>eae</i>	GAC CCG GCA CAA GCA TAA GC	384	39
	eae384-R		CCA CCT GCA GCA ACA AGA GG		
	stx1-F	<i>stx1</i>	ATA AAT CGC CAT TCG TTG ACT AC	180	39
	stx1-R		AGA ACG CCC ACT GAG ATC ATC		
	stx2-F	<i>stx2</i>	GGC ACT GTC TGA AAC TGC TCC	255	39
	stx2-R		TCG CCA GTT ATC TGA CAT TCT G		
<i>E. coli</i>	uidA-F	<i>uidA</i>	GCGTCTGTTGACTGGCAGGTGGTGG	503	18
	uidA-R		GTTGCCCGCTTCGAAACCAATGCCT		
<i>Campylobacter</i>	cadF-F	<i>cadF</i>	CTGCTAAACCATAGAAATAAAATTTCTCAC		16
	cadF-R		CTTTGAAGGTAATTTAGATATGGATAATCG		
Norovirus GI	Cog 1F	ORF1– ORF2 junction (GI)	CGYTGGATGCGNTTYCATGA		41
	Cog 1R		CTTAGACGCCATCATCATTYAC		

Norovirus GII	Cog 2F	ORF1– ORF2 junction (GII)	CARGARBCNATGTTYAGRTGGATGAG	41
	Cog 2R		TCGACGCCATCTTCATTACACA	
MS phage	MS2.F	289–387	TGGCACTACCCCTCTCCGTATTACAG	19
	MS2.R		GTACGGGCGACCCACGATGAC	
Astrovirus	AsFF	ORF1b	GGC CAG ACT CAC AGA AGA GCA	20
	AsFr		GTC CTG TGA CAC CTT GTT TCC TGA	
Sapovirus	SaV124F	ORF1	GAY CAS GCT CTC GCY ACC TAC	42
	SaV1F		TTG GCC CTC GCC ACC TAC	
	SaV5F		TTT GAA CAA GCT GTG GCA TGC TAC	42
	SaV1245R		CCC TCC ATY TCA AAC ACT A	
Adenovirus	JTVFF	Hexon	AAC TTT CTC TCT TAA TAG ACG CC	44
	JTVFR		AGG GGG CTA GAA AAC AAA A	
Organisms	Probes	Target	Probe Sequences (5'-3')	References
Campylobacter	cadFP	cadF	[HEX]-CATTTTGACGATTTTGGCTTGA-[BHQ2]	16
Norovirus GI	Ring 1E	ORF1– ORF2 junction (GI)	[6-FAM]-AGATYGCGRTCYCCTGTCCA-[Tamra]	41
Norovirus GII	Ring 2	ORF1– ORF2 junction (GII)	[CY5]-TGGGAGGGCGATCGCAATCT-[BHQ2]	41
MS phage	MS2.P probe	neucleoti de 289– 387	[5-HEX]- CACATCGATAGATCAAGGTGCCTACAAGC- [BHQ1]	19
Astrovirus	AstZFb	ORF1b	[5-HEX]-CCA TCG CAT TTG GAG GGG AGG ACC AGC GA-[BHQ1]	20
Sapovirus	SaV124TP	ORF1	[6-FAM]-CCR CCT ATR AAC CA-[MGB-NQF]	42
	SaV5TP	ORF1	[6-FAM]-TGC CAC CAA TGT ACC A-[MGB-NQF]	42
Adenovirus	JTVFAP	Hexon	[6-FAM]-CGA AGA GTG CCC GTG TCA GC-[BHQ1]	44

DAEC = Diffusely adherent *E. coli*; EAEC = Enteroaggregative *E. coli*; EIEC = Enteroinvasive *E. coli*; EPEC = Enteropathogenic *E. coli*; ETEC = Enterotoxigenic *E. coli*; and STEC = Shiga toxin-producing *E. coli*

STable 2. Reaction Mix for norovirus, astrovirus, sapovirus and Campylobacter PCR

Reaction Mix for GI/GII Norovirus Multiplex RT-qPCR Assay		
Component	Volume per reaction (µl)	Final concentration
2X RT-PCR buffer*	12.5	1X
Nuclease-free water*	1.08	n/a
Detection Enhancer*	1.67	n/a
Cog1F (10 µM)	1	400 nM
Cog1R (10 µM)	1	400 nM
Ring 1E (10 µM)	0.5	200 nM
Cog2F (10 µM)	1	400 nM
Cog2R (10 µM)	1	400 nM
Ring 2 (10 µM)	0.5	200 nM
MS2.F (10 µM)	0.25	100 nM
MS2.R (10 µM)	0.25	100 nM
MS2.P (10 µM)	0.25	100 nM
25X RT-PCR enzyme*	1	1x
RNA sample	3	

Reaction Mix Astrovirus/Sapovirus Duplex RT-qPCR Assay		
Component	Volume per reaction (µl)	Final concentration
2X RT-PCR buffer*	12.5	1X
Nuclease-free water*	2.5	n/a
AsFF (10 µM)	0.6	250 nM
AsFR (10 µM)	0.6	250 nM
AstZFB (10 µM)	0.25	100 nM
SaV 124F (10 µM)	1	400 nM
SaV 1F (10 µM)	1	400 nM
SaV 5F (10 µM)	1	400 nM
SaV 1245R (10 µM)	1	400 nM
SaV124TP (10 µM)	0.25	100 nM
SaV 5TP (10 µM)	0.25	100 nM
25X RT-PCR enzyme*	1	1x
RNA sample	3	

Reaction Mix for adenovirus RT-qPCR Assay		
Component	Volume per reaction (µl)	Final concentration
2X RT-PCR buffer*	12.5	1X
Nuclease-free water*	4.33	n/a
Detection Enhancer*	1.67	n/a
Cog1F (10 µM)	1	400 nM
Cog1R (10 µM)	1	400 nM
Ring 1E (10 µM)	0.5	200 nM
25X RT-PCR enzyme*	1	1x
RNA sample	3	

Reaction Mix for Campylobacter qPCR Assay

Component	Volume per reaction (μl)	Final concentration
TaqMan Environmental Master Mix 2.0	12.5	1X
Nuclease-free water	9	n/a
cadF-F (10 μM)	1	400 nM
cadF-R (10 μM)	1	400 nM
cadF-P (10 μM)	0.5	200 nM
DNA sample	1	

* included in the Ag-Path Kit

STable 3. Socio-economic risk factors associated with acute diarrheal disease in cases and healthy controls

Risk factors	Cases		Controls		<i>P</i>
	No.	%	No.	%	
Annual income*					
Unknown	6	13.3	7	15.6	0.74
≤1	25	55.6	26	57.8	0.83
1-2	10	22.2	10	22.2	0.92
2-6	4	8.9	2	4.4	0.58
Housing Levels					
Unknown	1	2.2	4	8.9	0.36
1	13	28.9	19	42.2	0.17
2	12	26.7	13	28.9	0.94
3	15	33.3	8	17.8	0.14
4	4	8.9	1	2.2	0.36
5	0	0	0	0.0	N.A.
Health System Insurance					
Contributive	31	68.9	22	48.9	0.086
Subsidized	9	20.0	21	46.7	0.02
Private	3	6.7	0	0.0	0.24
Non-insured	1	2.2	2	4.4	1
Unknown	1	2.2	0	0.0	1
Education of caregivers					
Unknown	1	2.2	0	0.0	0.4944
None	0	0.0	2	4.4	
Elementary	9	20.0	10	22.2	
Secondary	18	40.0	19	42.2	
Technological school	11	24.4	10	22.2	0.76
University	6	13.3	4	8.9	0.74

*: Income level 1 = One or less than one minimum salary per household per month (Colombian pesos \$589,500)

N.A.=not applicable

STable 4. Epidemiological risk factors associated with acute diarrheal disease in cases and healthy controls

Risk factors	Cases		Controls		<i>P</i>
	No.	%	No.	%	
Animals exposure	23	51.1	25	55.6	0.83
AGE Contacts outside home	2	4.4	2	4.4	1
Water sources	45	100.0	45	100.0	1
Garbage collection	45	100.0	45	100.0	1
Sewerage	44	97.8	45	100.0	1