Supplemental Information

Performance of forty-one microbial source tracking methods: A twenty-seven laboratory evaluation study

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Table SI-1. Filters used for virus analysis by lab. AdV is adenovirus, EV is enterovirus, NV is norovirus, and PyV is polyomavirus.

Target	Lab Number	Additive
Human AdV	5	0.1 M $MgCl_2$
Human AdV	9	pH 3.5
Human AdV	13	none
Human EV	5, 23	0.1 M $MgCl_2$
Human EV	3	none
Human EV	3, 9	pH 3.5
Human NV	5	0.1 M $MgCl_2$
Human PyV	9	pH 3.5

Table SI-2. Methods for MST assays used in this study. The intended fecal host, assay, arbitrary, blinded lab identifier, DNA/RNA extraction method (DNA/RNA extract. meth.), DNA/RNA quantification method (DNA/RNA quant. meth.), reference for the method protocol (ref), mastermix and probe if applicable, instrument, standard curve (SC), standard material (standard), lower limit of quantification (LLOQ), and the method for reporting values below the LLOQ (BLOQ reported) are provided. N.a. indicates 'not applicable'. For DNA/RNA quantification method, N is Nanodrop (all types of instrument, Thermo-Scientific, Wilmington, DE), Q is Qubit Quantit Fluorometer (Life Technologies, Grand Island, NY) or other fluorometer, and UV is UV spectrometer. For mastermix and probe, the mastermix is provided and if a zen probe is used, it is designated so here. For standard curve, S indicates a single standard curve is used per plate while P indicates that all standard curves were pooled (Sivaganesan et al. 2010). For standard, linear plasmids (lin. plas.), circular plasmids (circ. plas.), genomic DNA (gDNA), fecal DNA, meat DNA, synthesized oligos, genomic RNA (gRNA), virons, or in vitro transcribed (trans.) RNA is indicated. Units of LLOQ are copies per filter. For the final column which indicates the method for reporting values BLOQ, ND means that samples BLOQ were reported as not detected; negative, no Ct, detected, detectable, BDL (below detection limit), and DNQ (detected but not quantified) were also reported by the labs.

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Cow	CowM2	qPCR	1	GeneRite	N,Q	(Shanks et al. 2008)	ABI universal	Biorad CFX	P	lin. plas.	986	DNQ
			2	GeneRite	N	(Shanks et al. 2008)	ABI universal	ABI StepOnePlus	P	lin. plas.	328	DNQ
			3	GeneRite	N,Q	(Shanks et al. 2008)	ABI universal	Biorad CFX	P	lin. plas.	22620	DNQ
			4	GeneRite	N,Q	(Shanks et al. 2008)	ABI universal	ABI StepOnePlus	P	lin. plas.	1957	DNQ
			5	GeneRite	N,Q	(Shanks et al. 2008)	ABI universal	ABI StepOnePlus	P	lin. plas.	1000	DNQ
	CowM3	qPCR	2	GeneRite	N	(Shanks et al. 2008)	ABI universal	ABI StepOnePlus	P	lin. plas.	371	DNQ
Ruminant	BacCow	qPCR	6	GeneRite	N	(Kildare et al. 2007)	ABI environmental	ABI StepOnePlus	S	circ. plas.	145	ND
			1	GeneRite	N,Q	(Kildare et al. 2007)	ABI environmental	Biorad CFX	P	circ. plas.	9570	DNQ
			2	GeneRite	N	(Kildare et al. 2007)	ABI environmental	ABI StepOnePlus	P	circ. plas.	2116	DNQ
			3	GeneRite	N,Q	(Kildare et al. 2007)	ABI environmental	Biorad CFX	P	circ. plas.	6639	DNQ
			5	GeneRite	N,Q	(Kildare et al. 2007)	ABI environmental	ABI StepOnePlus	P	circ. plas.	1000	DNQ

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Ruminant (continued)	BacR	qPCR	7	PC	N	(Reischer et al. 2006)	iQ Supermix	Eppendorf Mastercycler	S	circ. plas.	230	ND
			3	GeneRite	N,Q	(Reischer et al. 2006)	ABI universal	Biorad CFX	P	circ. plas.	3374	DNQ
	Rum2Bac	qPCR	8	QIAamp Dm	N	(Mieszkin et al. 2010)	Agilent TaqMan Brilliant II	Biorad CFX	S	lin. plas.	250	detected
	CF128	PCR	21	Qiagen D-BT	N	(Bernhard and Field 2000a, Bernhard and Field 2000b)	GoTaq Flexi (Promega)	Eppendorf Mastercycler	n.a	lin. plas.	n.a	n.a
			22	Qiagen D-BT	n.a.	(Bernhard and Field 2000a)	GoTaq Flexi (Promega)	Thermo Hybaid	n.a	lin. plas.	n.a	n.a
	CF193	PCR	4	GeneRite	N,Q	(Bernhard and Field 2000a)	TaKaRa Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a
Gull	Gull2SYBR	qPCR	8	QIAamp Dm	N	(Lu et al. 2008)	Agilent Brilliant SYBR	Biorad CFX	S	lin. plas.	250	detected
			6	GeneRite	N	(Lu et al. 2008)	Fermentas Maxima SYBR	ABI StepOnePlus	S	circ. plas.	50	ND
			27	MoBio PS	Q	(Lu et al. 2008)	Bio-Rad iTaq SYBR	ABI 7500	S	circ. plas.	100	ND
			10	MoBio PS	Q	(Lu et al. 2008)	ABI Power SYBR Green	ABI 7900	S	circ. plas.	1000	BDL
	Gull2Taqman	qPCR	10	MoBio PS	Q	(Shibata et al. 2010)	ABI universal	ABI 7900	S	circ. plas.	100000	BDL
			11	GeneRite	Q	(Sinigalliano et al. 2012)	Qiagen QuaniTect Probe, ZEN probe	ABI StepOnePlus	S	circ. plas.	n.d.	n.a
			1	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen QuaniTect Probe, FAM-BHQ probe	Biorad CFX	P	circ. plas.	965	DNQ
			3	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen QuaniTect Probe, FAM-BHQ probe	Biorad CFX	P	circ. plas.	114	DNQ

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Gull (continued)			4	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen QuaniTect Probe, FAM-BHQ probe	ABI StepOnePlus	P	circ. plas.	6496	DNQ
			5	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen QuaniTect Probe, FAM-BHQ probe	ABI StepOnePlus	P	circ. plas.	1000	DNQ
	LeeSeagullSpecific	qPCR	12	GeneRite	N	(Lee et al. 2012)	ABI universal	ABI StepOnePlus	P	circ. plas.	5120	ND
	Gull2Endpt	PCR	20	Fast DNA	UV	(Lu et al. 2008)	Promega GoTaq Flexi	Thermo Hybaid	n.a	gull feces DNA	n.a	n.a
			10	MoBio PS	PG	(Lu et al. 2008)	Takara Ex Taq	ABI 7900	n.a	gDNA	n.a	n.a
			4	GeneRite	N,Q	(Lu et al. 2008)	Takara Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a
			3	GeneRite	N,Q	(Lu et al. 2008)	Takara Ex Taq	Biorad CFX	n.a	circ. plas.	n.a	n.a
Pig	Pig2Bac	qPCR	8	QIAamp Dm	N	(Mieszkin et al. 2009)	Invitrogen Platinum qPCR Super Mix-UDG	Biorad CFX	S	lin. plas.	250	detected
			1	GeneRite	N,Q	(Mieszkin et al. 2009)	ABI universal	Biorad CFX	P	lin. plas.	1004	DNQ
			2	GeneRite	N	(Mieszkin et al. 2009)	ABI universal	ABI StepOnePlus	P	lin. plas.	311	DNQ
			3	GeneRite	N,Q	(Mieszkin et al. 2009)	ABI universal	Biorad CFX	P	lin. plas.	22585	DNQ
			5	GeneRite	N,Q	(Mieszkin et al. 2009)	ABI universal	ABI StepOnePlus	P	lin. plas.	1000	DNQ
	PF163	PCR	1	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	Thermo Hybaid	n.a	circ. plas.	n.a	n.a
			5	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a
			4	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a
			3	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	Biorad CFX	n.a	circ. plas.	n.a	n.a
	PigmtDNA	PCR	21	Qiagen D-BT	N	(Martellini et al. 2005)	GoTaq Flexi (Promega)	Eppendorf Mastercycler	n.a	pig meat DNA	n.a	n.a

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Dog	DogBact	qPCR	11	GeneRite	Q	(Shibata et al. 2010)	Qiagen QuaniTect, ZEN probe	ABI StepOnePlus	S	circ. plas.	n.d.	n.a
			19	GeneRite	N	(Shibata et al. 2010)	ABI Universal	Stratagene Mx3000P	S	circ. plas.	16000	No Ct
			1	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen Quantitect	Biorad CFX	P	circ. plas.	837	DNQ
			2	GeneRite	N	(Shibata et al. 2010)	Qiagen Quantitect	ABI StepOnePlus	P	circ. plas.	4178	DNQ
			4	GeneRite	N,Q	(Shibata et al. 2010)	Qiagen Quantitect	Biorad CFX	P	circ. plas.	3997	DNQ
	BacCan	qPCR	6	GeneRite	N	(Kildare et al. 2007)	ABI environmental	ABI StepOnePlus	S	circ. plas.	3050.7	ND
Horse	HoF597	PCR	1	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	Thermo Hybaid	n.a	circ. plas.	n.a	n.a
			5	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	ABI Veriti	n.a	circ. plas.	n.a	n.a
			4	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a
			3	GeneRite	N,Q	(Dick et al. 2005)	Takara Ex Taq	Biorad CFX	n.a	circ. plas.	n.a	n.a
Sheep	Omvito	PCR	21	Qiagen D-BT	N	(Martellini et al. 2005)	Promega GoTaq Flexi	Eppendorf Mastercycler	n.a	sheep meat DNA	n.a	n.a
General	genbac3	qPCR	3	GeneRite	N,Q	(Seifring 2008)	ABI universal	Biorad CFX	P	gDNA	1300	DNQ
			4	GeneRite	N,Q	(Seifring 2008)	ABI universal	ABI StepOnePlus	P	gDNA	3152	DNQ
			5	GeneRite	N,Q	(Seifring 2008)	ABI universal	ABI StepOnePlus	P	gDNA	1000	DNQ
Human	HF183SYBR	qPCR	8	QIAamp Dm	N	(Seurinck et al. 2005)	Agilent Brilliant SYBR green	Biorad CFX	S	lin. plas.	250	detected
			13	GeneRite	n.a.	(Seurinck et al. 2005)	Eurogentec	Cepheid SmartCycler	S	synthesized oligo	100	DNQ
			1	GeneRite	N,Q	(Seurinck et al. 2005)	AnaSpec/Eurogentec	Biorad CFX	P	lin. plas.	991	DNQ
			3	GeneRite	N,Q	(Seurinck et al. 2005)	ABI Goldstart kit	Biorad CFX	P	lin. plas.	387	DNQ
	HF183Taqman	qPCR	1	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	Biorad CFX	P	lin. plas.	1057	DNQ
			2	GeneRite	N	(Haugland et al. 2010)	ABI universal	ABI StepOnePlus	P	lin. plas.	318	DNQ

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Human (continued)			3	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	Biorad CFX	P	lin. plas.	2222	DNQ
			4	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	ABI StepOnePlus	P	lin. plas.	2948	DNQ
			5	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	ABI StepOnePlus	P	lin. plas.	1000	DNQ
	BacHum	qPCR	11	GeneRite	Q	(Kildare et al. 2007)	Qiagen QuaniTect Probe, ZEN probe	ABI StepOnePlus	S	gDNA	n.d.	n.a
			11	GeneRite	Q	(Kildare et al. 2007)	Qiagen QuaniTect Probe, ZEN probe	ABI StepOnePlus	S	circ. plas.	n.d.	n.a
			6	GeneRite	N	(Kildare et al. 2007)	ABI environmental	ABI StepOnePlus	S	circ. plas.	2404.2	ND
			2	GeneRite	N	(Kildare et al. 2007)	ABI universal	ABI StepOnePlus	P	circ. plas.	288	DNQ
			3	GeneRite	N,Q	(Kildare et al. 2007)	ABI universal	Biorad CFX	P	circ. plas.	644	DNQ
			4	GeneRite	N,Q	(Kildare et al. 2007)	ABI universal	ABI StepOnePlus	P	circ. plas.	3850	DNQ
			5	GeneRite	N,Q	(Kildare et al. 2007)	ABI universal	ABI StepOnePlus	P	circ. plas.	100	DNQ
	HumM2	qPCR	11	GeneRite	Q	(Shanks et al. 2009)	Qiagen QuaniTect Probe, ZEN probe	ABI StepOnePlus	S	lin. plas.	n.d.	n.a
			1	GeneRite	N,Q	(Shanks et al. 2009)	ABI universal	Biorad CFX	P	lin. plas.	1113	DNQ
			2	GeneRite	N	(Shanks et al. 2009)	ABI universal	ABI StepOnePlus	P	lin. plas.	380	DNQ
			3	GeneRite	N,Q	(Shanks et al. 2009)	ABI universal	Biorad CFX	P	lin. plas.	22486	DNQ
			4	GeneRite	N,Q	(Shanks et al. 2009)	ABI universal	ABI StepOnePlus	P	lin. plas.	3407	DNQ
			5	GeneRite	N,Q	(Shanks et al. 2009)	ABI universal	ABI StepOnePlus	P	lin. plas.	1000	DNQ
	BsteriF1	qPCR	1	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	Biorad CFX	P	lin. plas.	957	DNQ

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Human (continued)			2	GeneRite	N	(Haugland et al. 2010)	ABI universal	ABI StepOnePlus	P	lin. plas.	349	DNQ
			3	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	Biorad CFX	P	lin. plas.	1857	DNQ
			5	GeneRite	N,Q	(Haugland et al. 2010)	ABI universal	ABI StepOnePlus	P	lin. plas.	1000	DNQ
	nifH	qPCR	14	MoBio PW	Q	(Johnston et al. 2010)	ABI environmental	ABI StepOnePlus	S	gDNA	n.d.	n.a
			11	GeneRite	Q	(Johnston et al. 2010)	Qiagen QuaniTect Probe, ZEN probe	ABI StepOnePlus	S	gDNA	n.d.	n.a
			15	MoBio PW	n.a.	(Johnston et al. 2010)	Qiagen QuaniTect Probe	Cepheid SmartCycler	S	gDNA	200	ND
			1	GeneRite	N,Q	(Johnston et al. 2010)	Qiagen QuaniTect Probe	Biorad CFX	P	gDNA	3282	DNQ
			3	GeneRite	N,Q	(Johnston et al. 2010)	Qiagen QuaniTect Probe	Biorad CFX	P	gDNA	2450	DNQ
	BacH	qPCR	7	PC	N	(Reischer et al. 2007)	iQ Supermix	Eppendorf Mastercycler	S	circ. plas.	230	detectable
	gyrB	qPCR	12	GeneRite	N	(Lee and Lee 2010)	ABI universal	ABI StepOnePlus	P	gDNA	20000	ND
	Btheta	qPCR	16	GeneRite	N	(Yampara-Iquise et al. 2008)	Roche Taqman480 Probe	Roche LightCycler	S	gDNA	700	DNQ
	GB124 (*Bacteroides* phage)	plaque assay	17	n.a.	n.a.	(Ebdon et al. 2007)	n.a.	n.a.	n.a	n.a.	n.a	n.a.
		plaque assay	26	n.a.	n.a.	(Ebdon et al. 2007)	n.a.	n.a.	n.a	n.a.	n.a	n.a.
	HB73 (*Bacteroides* phage)	plaque assay	18	n.a.	n.a.	(Vijayavel et al. 2010)	n.a.	n.a.	n.a	n.a.	n.a	n.a.

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Human (continued)	F-specific coliphage	plaque assay / PCR	8	n.a.	n.a.	(International Organization for Standardization 1995, Gourmelon et al. 2007, Ogorzaly et al. 2009)	Qiagen Quanti Tech Probe RT-PCR	Stratagene MX3000	n.a	lin. plasmid	n.a	n.a.
	HF183Endpt	PCR	20	Fast DNA	UV	(Santoro and Boehm 2007)	GoTaq Flexi (Promega)	Thermo Hybaid	n.a	raw sewage DNA	n.a	n.a.
			21	Qiagen D-BT	N	(Bernhard and Field 2000a, Bernhard and Field 2000b)	GoTaq Flexi (Promega)	Eppendorf Mastercycler	n.a	lin. plas.	n.a	n.a.
			22	Qiagen D-BT	n.a.	(Bernhard and Field 2000a)	Taq (Promega)	Thermo Hybaid	n.a	lin. plas.	n.a	n.a.
			16	GeneRite	N	(Bernhard and Field 2000a)	Qiagen HotStarTaq	Biorad iCycler	n.a	raw sewage DNA	n.a	n.a.
			1	GeneRite	N,Q	(Bernhard and Field 2000a)	Takara Ex Taq	Thermo Hybaid	n.a	circ. plas.	n.a	n.a.
			5	GeneRite	N,Q	(Bernhard and Field 2000a)	Takara Ex Taq	ABI Veriti	n.a	circ. plas.	n.a	n.a.
			4	GeneRite	N,Q	(Bernhard and Field 2000a)	Takara Ex Taq	GeneAmp 9700	n.a	circ. plas.	n.a	n.a.
	Enterovirus	RT-qPCR	23	Qiagen R-MT	n.a.	(Fuhrman et al. 2005)	ABI one-step RT-PCR	Stratagene Mx3000P	S	virion	100	negative
		RT-PCR,	15	Qiagen R-MT	n.a.	(Donaldson et al. 2002)	Qiagen QuantiTect RT-PCR	Cepheid SmartCycler	S	gRNA echovirus	n.a	n.a.
			5	QIAamp MEV	N	(DeLeon et al. 1990, Gregory et al. 2006, Walters et al. 2009)	ABI RNA to Ct One step	ABI StepOnePlus	P	in vitro trans. RNA	42-904	ND
			3	QIAamp MEV	N	(DeLeon et al. 1990, Gregory et al. 2006, Walters et al. 2009)	ABI Taqman RTPCR	Biorad CFX	P	in vitro trans. RNA	921	ND
			3	QIAamp MEV	N	(DeLeon et al. 1990, Gregory et al. 2006, Walters et al. 2009)	ABI Taqman RTPCR	Biorad CFX	P	in vitro trans. RNA	921	ND

Table SI-2 Continued

Host	Assay	Type	Lab	DNA/RNA extract. method	DNA/RNA quant. method	Reference	Mastermix, probe	Instrument	SC	Standard	LLOQ	BLOQ reported
Human (continued)	AdV	qPCR	9	QIAamp MEV	N	(Jothikumar et al. 2005)	QuantiFast Pathogen PCR + IC Kit	Qiagen RotorGene-Q	S	circ. plas.	n.d.	n.a
			5	QIAamp MEV	N	(Jothikumar et al. 2005)	ABI universal	ABI StepOnePlus	P	circ. plas.	87-996	ND
		PCR	13	GeneRite	n.a.	(Xu et al. 2000)	Platinum high fidelity Taq	Cepheid SmartCycler	n.a	lin. plas.	n.a	n.a.
	Norovirus I	RT-qPCR	15	Qiagen R-PM	n.a.	(Jothikumar et al. 2005)	Qiagen QuantiTect RT-PCR	Cepheid SmartCycler	S	in vitro trans. RNA	6000	ND
	Norovirus II	RT-qPCR	15	Qiagen R-PM	n.a.	(Jothikumar et al. 2005)	Qiagen QuantiTect RT-PCR	Cepheid SmartCycler	S	in vitro trans. RNA	300	ND
			5	QIAamp MEV	N	(Da Silva et al. 2007)	ABI RNA to Ct One step	ABI StepOnePlus	P	in vitro trans. RNA	n.d.	ND
	PyV	RT-qPCR	9	QIAamp MEV	N	(Aksamit 1993, McQuaig et al. 2009)	QuantiFast Pathogen PCR + IC Kit	Qiagen RotorGene-Q	S	circ. plas.	n.d.	n.a
	MB55 (*Ent. faecium* phage)	plaque assay	18	n.a.	n.a.	(Vijayavel et al. 2010)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Canine Scent Tracking	n.a.	24	n.a.	n.a.	(Murray 2011)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Comm-unity methods	PhyloChip	Micro-array	25			(Dubinsky et al. 2012)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Bac TRFLP		1			(Cao et al. 2012)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			3			(Cao et al. 2012)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Univ TRFLP		1			(Cao et al. 2012)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			3			(Cao et al. 2012)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table SI-3. For each quantitative assay, the number of target and non-target challenge samples, the number of each that were classified as ND (not detected), DNQ (detected, but not quantified), or ROQ (within the range of quantification), and the median of each group (see main text for more on ND, DNQ, ROQ definitions). The median is reported as the log-base 10 of the median in units of copies per CFU ENT.

Assay	Host	Target # samples	Target # (%) ND	Target # (%) DNQ	Target # (%) ROQ	Target median	Non-target # samples	Non-target # (%) ND	Non-target # (%) DNQ	Non-target # (%) ROQ	Non-target median
BacH	Human	12	0 (0)	3 (25)	9 (75)	1.9	26	20 (77)	2 (8)	4 (15)	ND
BacHum	Human	84	3 (4)	0 (0)	81 (96)	2.5	181	75 (41)	39 (22)	67 (37)	DNQ
BsteriF1	Human	48	0 (0)	2 (4)	46 (96)	2.1	103	45 (44)	17 (17)	41 (40)	DNQ
Btheta	Human	12	0 (0)	1 (8)	11 (92)	1.1	26	14 (54)	11 (42)	1 (4)	ND
gyrB	Human	12	6 (50)	0 (0)	6 (50)	-2.6	26	25 (96)	0 (0)	1 (4)	ND
HF183SYBR	Human	48	0 (0)	4 (8)	44 (92)	1.7	103	80 (78)	12 (12)	11 (11)	ND
HF183Taqman	Human	60	0 (0)	3 (5)	57 (95)	2.1	129	59 (46)	59 (46)	11 (9)	DNQ
HumM2	Human	72	5 (7)	17 (24)	50 (69)	0.8	155	116 (75)	25 (16)	14 (9)	ND
nifH	Human	60	14 (23)	8 (13)	38 (63)	1.5	129	88 (68)	8 (6)	33 (26)	ND
BacCow	Cow	20	0 (0)	0 (0)	20 (100)	4.1	169	84 (50)	28 (17)	57 (34)	DNQ
CowM2	Cow	20	0 (0)	1 (5)	19 (95)	1.2	169	169 (100)	0 (0)	0 (0)	ND
CowM3	Cow	4	0 (0)	1 (25)	3 (75)	0.1	34	34 (100)	0 (0)	0 (0)	ND
BacR	Ruminant	12	0 (0)	0 (0)	12 (100)	3.0	63	50 (79)	12 (19)	1 (2)	ND
Rum2Bac	Ruminant	6	0 (0)	0 (0)	6 (100)	2.9	32	32 (100)	0 (0)	0 (0)	ND
Gull2SYBR	Gull	16	1 (6)	1 (6)	14 (88)	-0.4	136	118 (87)	0 (0)	18 (13)	ND
Gull2Taqman	Gull	24	3 (13)	0 (0)	21 (88)	0.9	203	125 (62)	30 (15)	48 (24)	ND
LeeSeaGull	Gull	4	0 (0)	0 (0)	4 (100)	1.7	34	32 (94)	0 (0)	2 (6)	ND
DogBact	Dog	20	0 (0)	0 (0)	20 (100)	2.2	170	93 (55)	11 (6)	66 (39)	ND
BacCan	Dog	4	0 (0)	0 (0)	4 (100)	3.7	34	28 (82)	0 (0)	6 (18)	ND
Pig2Bac	Pig	20	0 (0)	0 (0)	20 (100)	5.0	169	124 (73)	44 (26)	1 (1)	ND

Table SI-4. The number and percent of non-target challenge samples for which source-specific quantitative assays measured normalized gene abundances per copies genbac3 at levels higher than those measured in the target samples in the range of quantification (ROQ). Results are broken down by fecal source, with the total and percent of non-target challenge samples that amplified in the ROQ shown in the bottom row. Top panel shows the human assays, bottom panel shows the remaining assays. Shaded cells indicate that these are the target challenge samples for the particular assay.

Sample	BacH	BacHum	BsteriF1	Btheta	gyrB	HF183SYBR	HF183Taqman	HumM2	*nifH*
chicken	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
cow	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (15)
deer	0 (0)	2 (14)	0 (0)	0 (0)	0 (0)	5 (63)	0 (0)	0 (0)	4 (40)
dog	0 (0)	17 (61)	16 (100)	0 (0)	0 (0)	3 (19)	0 (0)	0 (0)	1 (5)
goose	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
gull	0 (0)	2 (7)	0 (0)	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)
horse	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
pig	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (25)
pigeon	0 (0)	3 (21)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
human									
septage									
sewage									
total	**0 (0)**	**28 (15)**	**16 (16)**	**0 (0)**	**0 (0)**	**11 (11)**	**0 (0)**	**0 (0)**	**13 (10)**

Sample	BacCow	CowM2	CowM3	BacR	Rum2Bac	Gull2SYBR	Gull2Taqman	LeeSeaGull	DogBact	BacCan	Pig2Bac
chicken	3 (33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (40)	0 (0)	0 (0)
cow						0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
deer	10 (100)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
dog	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)
goose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
gull	4 (20)	0 (0)	0 (0)	1 (13)	0 (0)				6 (30)	0 (0)	0 (0)
horse	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
pig	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
pigeon	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)	6 (75)	12 (100)	2 (100)	4 (40)	0 (0)	1 (10)
human	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
septage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
sewage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (10)	0 (0)	0 (0)
total	**18 (11)**	**0 (0)**	**0 (0)**	**1 (2)**	**0 (0)**	**6 (4)**	**12 (6)**	**2 (6)**	**16 (9)**	1 (3)	1 (1)

Table SI-5. The number and percent of non-target challenge samples for which source-specific quantitative assays measured normalized gene abundances per mass of DNA at levels higher than those measured in the target samples in the range of quantification (ROQ). Results are broken down by fecal source, with the total and percent of non-target challenge samples that amplified in the ROQ shown in the bottom row. Top panel shows the human assays, bottom panel shows the remaining assays. Shaded cells indicate that these are the target challenge samples for the particular assay.

Sample	BacH	BacHum	BsteriF1	Btheta	gyrB	HF183SYBR	HF183Taqman	HumM2	*nifH*
chicken	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
cow	0 (0)	4 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (50)
deer	0 (0)	12 (86)	0 (0)	0 (0)	0 (0)	5 (63)	0 (0)	2 (17)	4 (40)
dog	2 (50)	28 (100)	16 (100)	0 (0)	0 (0)	3 (19)	0 (0)	0 (0)	2 (10)
goose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
gull	0 (0)	2 (7)	0 (0)	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)
horse	0 (0)	2 (14)	2 (25)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
pig	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (70)
pigeon	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
human									
septage									
sewage									
total	**2 (8)**	**49 (27)**	**18 (17)**	**0 (0)**	**0 (0)**	**11 (11)**	**0 (0)**	**2 (1)**	**30 (23)**

Sample	BacCow	CowM2	CowM3	BacR	Rum2Bac	Gull2SYBR	Gull2Taqman	LeeSeaGull	DogBact	BacCan	Pig2Bac
chicken	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
cow						0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
deer	10 (100)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
dog	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)	0 (0)	0 (0)			0 (0)
goose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
gull	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)				0 (0)	0 (0)	0 (0)
horse	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
pig	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
pigeon	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (63)	12 (100)	0 (0)	0 (0)	0 (0)	0 (0)
human	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
septage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
sewage	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
total	**10 (6)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**8 (6)**	**13 (6)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**

Table SI-6. Number (and percent) of non-target samples for which each assay measured copies per CFU ENT-MF, copies genbac3, and ng DNA at levels greater than or equal to those measured in target samples. Results for assays deemed 'specific' when results were normalized by ENT-MF are highlighted. Note that results provided in the column for ENT-MF are also reported in the bottom row of Table 4 in the main text.

Assay	ENT-MF	genbac3	DNA
BacH	0 (0)	0 (0)	2 (8)
BacHum	20 (11)	28 (15)	49 (27)
BsteriF1	10 (10)	16 (16)	18 (17)
Btheta	0 (0)	0 (0)	0 (0)
gyrB	0 (0)	0 (0)	0 (0)
HF183SYBR	7 (7)	11 (11)	11 (11)
HF183Taqman	0 (0)	0 (0)	0 (0)
HumM2	0 (0)	0 (0)	2 (1)
nifH	24 (19)	13 (10)	30 (23)
BacCow	12 (7)	18 (11)	10 (6)
CowM2	0 (0)	0 (0)	0 (0)
CowM3	0 (0)	0 (0)	0 (0)
BacR	0 (0)	1 (2)	0 (0)
Rum2Bac	0 (0)	0 (0)	0 (0)
Gull2SYBR	14 (10)	6 (4)	8 (6)
Gull2Taqman	21 (10)	12 (6)	13 (6)
LeeSeaGull	0 (0)	2 (6)	0 (0)
DogBact	25 (15)	16 (9)	0 (0)
BacCan	2 (6)	1 (3)	0 (0)
Pig2Bac	0 (0)	1 (1)	0 (0)

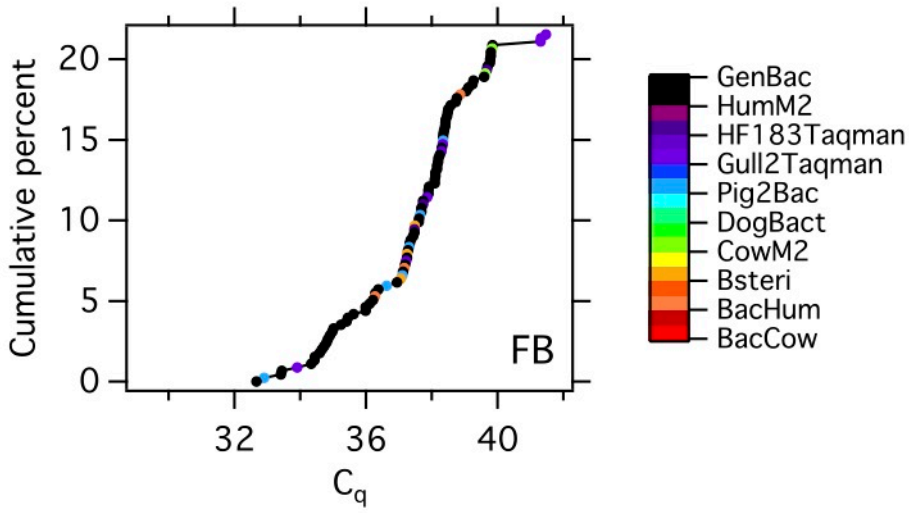

Figure SI-1. Cumulative frequency distribution for filtration blank (FB) samples. Each dot indicates the percentage of the FB assays run that returned Cq lower than the value where the dot appears. A total of 22% of the FB assays were positive. The color of the dot indicates the assay that returned the Cq.

References

Aksamit, A.J. (1993) Diagnostic molecular microbiology principles and applications. Persing, D.H., Smith, T.F., Tenover, F.C. and White, T.J. (eds), p. 364, Mayo Foundation, Rochester.

Bernhard, A.E. and Field, K.G. (2000a) A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. Applied and Environmental Microbiology 66(10), 4571-4574.

Bernhard, A.E. and Field, K.G. (2000b) Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology 66(4), 1587-1594.

Cao, Y., Van De Werfhorst, L.C., Scott, E., Raith, M., Holden, P.A. and Griffith, J.F. (2012) Bacteroidales terminal restriction fragment length polymorphism (TRFLP) for fecal source differentiation in comparison to and in combination with universal bacteria TRFLP. Water Research, this issue.

Da Silva, A.K., Le Saux, J.C., Parnaudeau, S., Pommepuy, M., Elimelech, M. and Le Guyader, F.S. (2007) Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Applied and Environmental Microbiology 73(24), 7891-7897.

DeLeon, R., Shieh, C., Baric, R.S. and Sobsey, M.D. (1990) Advances in water analysis and treatment, Proceedings of the Water Quality Technology Conference, San Diego, CA, pp. 833–853, American Water Works Association, Denver.

Dick, L.K., Bernhard, A.E., Brodeur, T.J., Domingo, J.W.S., J. M. Simpson, Walters, S.P. and Field., K.G. (2005) Host distributions of uncultivated fecal Bacteroides bacteria reveal genetic markers for fecal source identification. Applied and Environmental Microbiology 71, 3184-3191.

Donaldson, K.A., Griffin, D.W. and Paul, J.H. (2002) Detection, quantitation and identification of enteroviruses from surface waters and sponge tissue from the Florida Keys using real-time RT–PCR. Water Research 36(10), 2505–2514.

Dubinsky, E.A., Esmaili, L., Hulls, J.R., Cao, Y., Griffith, J.F. and Andersen, G.L. (2012) Application of phylogenetic microarray analysis to discriminate sources of fecal pollution. Environmental Science & Technology 46(8), 4340-4347.

Ebdon, J.E., Muniesa, M. and Taylor, H.D. (2007) The application of a recently isolated strain of *Bacteroides* (GB124) to identify human sources of faecal pollution in a temperate river catchment. Water Research 41, 3683-3690.

Fuhrman, J.A., Liang, X. and Noble, R.T. (2005) Rapid detection of enteroviruses in small volumes of natural waters by real-time quantitative reverse transcriptase PCR. Applied and Environmental Microbiology 71(8), 4523-4530.

Gourmelon, M., Caprais, M.P., Segura, R., Le Mennec, C., Lozach, S., Piriou, J.Y. and Rince, A. (2007) Evaluation of two library-independent microbial source tracking methods to identify sources of fecal contamination in French estuaries. Applied and Environmental Microbiology 73, 4857-4866.

Gregory, J.B., Litaker, R.W. and Noble, R.T. (2006) Rapid one-step quantitative reverse transcriptase PCR Assay with competitive internal positive control for detection of enteroviruses in environmental samples. Applied and Environmental Microbiology 72(6), 3960-3967.

Haugland, R.A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L. and Shanks, O.C. (2010) Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected Bacteroidales species and human fecal waste by qPCR. Systematic and Applied Microbiology 33(6), 348–357.

International Organization for Standardization (1995) Detection and enumeration of bacteriophages. Part 1: enumeration of F-specific RNA bacteriophages. ISO 10705-1, International Organization for Standardization, Geneva, Switzerland.

Johnston, C., Ufnar, J.A., Griffith, J.F., Gooch, J.A. and Stewart, J.R. (2010) A real-time qPCR assay for the detection of the nifH gene of *Methanobrevibacter smithii*, a potential indicator of sewage pollution. Journal of Applied Microbiology 109(6), 1946-1956.

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. (2005) Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Applied and Environmental Microbiology 71(6), 3131-3136.

Kildare, B.J., Leutenegger, C.M., McSwain, B.S., Bambic, D.G., Rajal, V.B. and Wuertz, S. (2007) 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal *Bacteroidales*: A Bayesian approach. Water Research 41, 3701-3715.

Lee, C., Marion, J.W. and Lee, J. (2013) Development and application of a quantitative PCR assay targeting *Catellicoccus marimammalium* for assessing gull-associated fecal contamination at Lake Erie beaches. Science of the Total Environment 454-455, 1-8.

Lee, C.S. and Lee, J. (2010) Evaluation of new gyrB-based real-time PCR system for the detection of *B. fragilis* as an indicator of human-specific fecal contamination. Journal of Microbiology Methods 82(3), 311–318.

Lu, J., Santo Domingo, J., Lamendella, R., Edge, T. and Hill, S. (2008) Phylogenetic diversity and molecular detection of bacteria in gull feces. Applied and Environmental Microbiology 74(13), 3969-3976.

Martellini, A., Payment, P. and Villemur, R. (2005) Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. Water Research 39(4), 541-548.

McQuaig, S.M., Scott, T.M., Lukasik, J.O., Paul, J.H. and Harwood, V.J. (2009) Quantification of human polyomaviruses JC Virus and BK Virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. Applied and Environmental Microbiology 75(11), 3379-3388.

Mieszkin, S., Furet, J.P., Corthier, G. and Gourmelon, M. (2009) Estimation of pig fecal contamination in a river catchment by real-time PCR using two pig-specific *Bacteroidales* 16S rRNA genetic markers. Applied and Environmental Microbiology 75(10), 3045-3054.

Mieszkin, S., Yala, J.F., Joubrel, R. and Gourmelon, M. (2010) Phylogenetic analysis of Bacteroidales 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. Journal of Applied Microbiology 108(3), 974–984.

Murray, J. (2011) Canine Scent and Microbial Source Tracking in Santa Barbara, CA, U2R09.

Ogorzaly, L., Tissier, A., Bertrand, I., Maul, A. and C., G. (2009) Relationship between F-specific RNA phage genogroups, faecal pollution indicators and human adenoviruses in river water. Water Research 43, 1257-1264.

Reischer, G., Kasper, D., Steinborn, R., Mach, R. and Farnleitner, A. (2006) Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of this method in alpine karstic regions. Applied and Environmental Microbiology 72(8), 5610-5614.

Reischer, G., Kasper, D., Steinborn, R., Farnleitner, A. and Mach, R. (2007) A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. Letters in Applied Microbiology 44(4), 351-356.

Santoro, A.E. and Boehm, A.B. (2007) Frequent occurrence of the human-specific *Bacteroides* fecal marker at an open coast marine beach: Relationship to waves, tides, and traditional indicators. Environmental Microbiology 9(8), 2038-2049.

Seifring, S.C., M. Varma, E. Atikovic, L. J. Wymer, and R. A. Haugland (2008) Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. Journal of Water and Health 6, 225-237.

Seurinck, S., Defoirdt, T., Verstraete, W. and Siciliano, S.D. (2005) Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environmental Microbiology 7(2), 249–259.

Shanks, O.C., Atikovic, E., Blackwood, A.D., Lu, J., Noble, R.T., Santo Domingo, J., Siefring, S., Sivaganesan, M. and Haugland, R.P. (2008) Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Applied and Environmental Microbiology 74(3), 745-752.

Shanks, O.C., Kelty, C.A., Sivaganesan, M., Varma, M. and Haugland, R.A. (2009) Quantitative PCR for genetic markers of human fecal pollution. Applied and Environmental Microbiology 75(17), 5507-5513.

Shibata, T., Solo-Gabriele, H.M. and C. D. Sinigalliano, M.L.G., L. R.W. Plano, J. M. Fleisher, J. D. Wang, S. M. Elmir, G. He, M. E. Wright, A. M. Abdelzaher, C. Ortega, D. Wanless, A. C. Garza, J. Kish, T. Scott, J. Hollenbeck, L. C. Backer, and L. E. Fleming (2010) Evaluation of Conventional and Alternative Monitoring Methods for a Recreational Marine Beach with Nonpoint Source of Fecal Contamination. Environmental Science & Technology 44(21), 8175-8181.

Sinigalliano, C., Ervin, J., Van De Werfhorst, L., Lee, J., Goodwin, K., Wanless, D., Bartkowiak, J., Wuertz, S., Schriewer, A., Holden, T., Jay, J., Boehm, A.B., Layton, B., Raith, M., Meijer, W., Sadowsky, M., Gourmelon, M., Noble, R., Griffith, J., Byappanahalli, M., Wang, D. and SantoDomingo, J. (2013) A multi-laboratory comparative assessment on the performance of

seabird-host-specific microbial source tracking PCR assays targeting *Catellicoccus marimammalium*. Water Research, this issue.

Sivaganesan, M., Haugland, R.A., Chern, E.C. and Shanks, O.C. (2010) Improved strategies and optimization of calibration models for real-time PCR absolute quantification. Water Research 44, 4726-4735.

Vijayavel, K., Fujioka, R., Ebdon, J. and Taylor, H. (2010) Isolation and characterization of *Bacteroides* host strain HB-73 used to detect sewage specific phages in Hawaii. Water Research 44(12), 3714-3724.

Walters, S.P., Yamahara, K.M. and Boehm, A.B. (2009) Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: Implications for their use in assessing risk in recreational waters. Water Research 43(19), 4929-4939.

Xu, W., McDonough, M.C. and Erdman, D.D. (2000) Species-specific identification of human adenoviruses by a multiplex PCR assay. Journal of Clinical Microbiology 38, 4114-4129.

Yampara-Iquise, H., Zheng, G., Jones, J.E. and Carson, C.A. (2008) Use of a *Bacteroides thetaiotaomicron*-specific α-1-6, mannanase quantitative PCR to detect human faecal pollution in water. Journal of Applied Microbiology 105(5), 1686-1693.