Supplementary Information for Survey of Viral Pathogens and Human Fecal Markers in Stormwater Across a Highly Populated Urban Region

Joshua A. Steele[1*], Amy Zimmer-Faust[1&], Thomas J. Clerkin[2], Adriana Gonzalez-Fernandez[1], Sarah Lowry[3], A. Denene Blackwood[2], Kayla Raygoza[1#], Kylie Langlois[1], Alexandria B. Boehm[3], Rachel T. Noble[2], John F. Griffith[1], Kenneth C. Schiff[1]

[1]Southern California Coastal Water Research Project, Costa Mesa, CA, USA
[2]Institute of Marine Sciences, The University of North Carolina at Chapel Hill, Morehead City, NC USA
[3]Department of Civil and Environmental Engineering, Stanford University, Stanford, California, USA
[#]Current address: University of California, Irvine, Irvine, CA, USA
[&]Current address: The Nature Conservancy …

*Corresponding author joshuas@sccwrp.org

Supplementary Material

Table S1. Storm Sample Locations and Dates

Jurisdiction	Site name	Site ID	Receiving Water or MS4 Outfall	Latitude	Longitude	Event 1	Event 2	Event 3
County of San Bernardino	Cuc Creek 3	801CUC3b	Receiving Water	33.9495	-117.6104	12/9/21	2/23/23	
	Cuc Creek 2	801CCCR12	Receiving Water	34.030926	-117.598891	12/9/21	2/23/23	
	SA@Chino	801SACCH17	Receiving Water	34.011076	-117.729943	12/9/21	3/28/22	
	SA@State	801SACST18	Receiving Water	34.059358	-117.717331	12/14/21	3/28/22	
	Santa Ana R.	801SARWA19	Receiving Water	34.069342	-117.280457	12/9/21	3/28/22	
County of Ventura	Bus Cn Drain	MO-SIM	Outfall	34.2721	-118.78374	10/25/21	12/14/21	
	Call Creek @Cam.	ME-CC	Receiving Water	34.17917	-119.03889	10/25/21	12/14/21	
	Santa Clara R.	ME-SCR	Receiving Water	34.29917	-119.10722	10/25/21	12/14/21	
	N Fill Drain	MO-FIL	Outfall	34.40459	-118.93069	10/25/21	12/14/21	
	11th St.	MO-SPA	Outfall	34.34861	-119.05551	10/25/21	12/14/21	
City of San Diego	MB 1	MS4-MB-1 DW053	Outfall	32.83938	-117.28181	10/25/21	2/27/23	
	MB 3	MS4-MB-4 DW171	Outfall	32.77145	-117.20434	10/25/21	2/27/23	
	MB 5	MS4-MB-5 DW275	Outfall	32.82403	-117.17834	10/25/21	2/27/23	
	Los Pen	MS4-LPC-5 DW0839	Outfall	32.89915	-117.11371	10/25/21	2/27/23	

	TRJ @Leon	DW0224	Outfall	32.56471	-117.101447	12/9/21	2/27/23	
County of San Diego	San Vic Creek	MS4-SDR-228	Outfall	32.86917	-116.9216	12/14/21	2/25/23	
	Sweet Water R.	MS4-SLR-150	Outfall	33.283702	-117.217033	12/9/21	2/25/23	
County of Riverside	Magnolia	801MAG364	Outfall	33.965556	-117.415556	12/14/21	2/23/23	
	Univ & Bowling	801UNV702	Outfall	33.997206	-117.372797	12/14/21	3/28/22	2/23/23
	Murrieta Cr @Diaz	902MS43015	Outfall	33.51649	-117.17233	12/14/21	2/23/23	
County of Orange	Aliso Cr	Aliso Creek	Receiving Water	33.54354483	-117.7324621	10/25/21	12/16/21	3/28/22
	SJ Cr	San Juan Creek	Receiving Water	33.467555	-117.681995	10/25/21	12/16/21	3/28/22
	Bolsa	Bolsa Chica	Receiving Water	33.75942	-118.04289	12/14/21	3/28/22	
	Delhi	Santa Ana Delhi	Receiving Water	33.66055	-117.88028	12/14/21	3/28/22	
	SD Cr	San Diego Creek	Receiving Water	33.65555	-117.84556	12/14/21	3/28/22	
County of Los Angeles	LA River Emiss.	Los Angeles River Mass Emission Station	Receiving Water	33.82368	-118.206264	3/28/22	11/8/22	2/24/23
	Malibu Ck. Emission	Malibu Creek Mass Emission Station	Receiving Water	34.077569	-118.701861	3/28/22	11/8/22	2/24/23
	Co. Island	County Island	Outfall	33.825071	-118.108378	3/28/22	11/8/22	2/24/23

	LLAR2	Dominguez Gap Pump Station	Receiving Water	33.83945	-118.2032	3/28/22	11/8/22	2/24/23
	Ballona Ck. Emiss.	Ballona Creek Mass Emission Station	Receiving Water	33.998293	-118.402035	3/28/22	11/8/22	2/24/23
	CCW_SW_LAC	Ballona Creek Stormwater Outfall	Outfall	33.96777	-118.37057	3/28/22	11/8/22	2/24/23

Table S2. Total precipitation at each sampling location for storm events 1-3.

Jurisdiction	Site name	Site ID	Total Precipitation (cm) for Storm Event 1	Total Precipitation (cm) for Storm Event 2	Total Precipitation (cm) for Storm Event 3
County of San Bernardino	Cuc Creek 3	801CUC3b	0.3048	8.5598	
	Cuc Creek 2	801CCCR12	0.4572	7.239	
	SA@Chino	801SACCH17	0.2794	1.2446	
	SA@State	801SACST18	6.0452	3.175	
	Santa Ana R.	801SARWA19	0.7366	1.143	
County of Ventura	Bus Cn Drain	MO-SIM	2.6162	3.429	
	Call Creek @Cam.	ME-CC	1.6764	3.9878	
	Santa Clara R.	ME-SCR	2.2606	5.334	
	N Fill Drain	MO-FIL	1.8542	6.6802	
	11th St.	MO-SPA	1.9304	5.588	
City of San Diego	MB 1	MS4-MB-1DW053	0.5334	3.2258	
	MB 3	MS4-MB-4 DW171	1.016	1.6002	
	MB 5	MS4-MB-5 DW275	0.3048	2.8956	
	Los Pen	MS4-LPC-5 DW0839	0.7366	4.699	
	TRJ @Leon	DW0224	0.508	3.8608	
County of San Diego	San Vic Creek	MS4-SDR-228	2.794	6.8834	
	Sweet Water R.	MS4-SLR-150	3.2766	7.7216	
County of Riverside	Magnolia	801MAG364	2.9464	4.3942	
	Univ & Bowling	801UNV702	2.54	0.5842	2.9718
	Murrieta Cr @Diaz	902MS43015	4.2926	8.4328	
County of Orange	Aliso Cr	Aliso Cr	0.7112	2.9464	3.0734
	SJ Cr	SJ Cr	0.1016	1.3208	1.0668
	Bolsa	Bolsa	2.54	1.4986	
	Delhi	Delhi	4.0132	1.4478	
	SD Cr	SD Cr	4.0132	1.4478	
County of Los Angeles	Los Angeles River Mass Emission Station	LA R.	1.7018	2.159	5.9944
	Malibu Creek Mass Emission Station	Malibu Ck.	3.7338	4.5466	17.1958
	County Island	Co. Island	2.1844	2.794	8.43534
	Dominguez Gap Pump Station	LLAR2	1.7018	2.159	5.9944
	Ballona Creek Mass Emission Station	Ballona Ck. Emiss.	3.2766	4.3688	16.1798
	Ballona Creek Stormwater Outfall	CCW_SW_LAC	2.6416	3.9624	15.0876

Table S3 Primers and Probes

Gene target	Assay Name	Forward primer	Reverse Primer	Probe	Reference
Bacteroides 16S	HF183	ATCATGAGTTCACATGTCCG	CGTAGGAGTTTGGACCGTGT	[HEX]CTGAGAGGA/ZEN/AGGTCCCCCACATTGGA[3IABkFQ]	Cao et al. 2015, Bernhard & Field 200
Lachnospiraceae 16S	Lachno3	CAACGCGAAGAACCTTACCAAA	CCCAGAGTGCCCACCTTAAAT	[FAM]CTC+TGACCG+GTCTT+TAATCGGA[3IABkFQ]	Feng et al. 2018
Natronomonas pharaonis gyrA	NP	ACGATTACCTGCTCTGCTTTAC	CGTTGAGGTCGAGAACATTGA	[FAM]CAAGGGCAGGTCTATCGGCTGAAG[3IABkFQ]	Steele et al. 2017
Bovine Coronavirus	BoCoV	C+TGGAAGTTGGTGGAGTT	ATTATCGG+CCTAACATAC+ATC	[HEX]CCTT+CATAT+CTATACA+CAT+CAAGTT[3IABKFQ]	Decaro et al. 2008
Mouse lung beta actin	ACTB	proprietary	proprietary	proprietary	Thermo Fisher
Adenovirus	JTVX	GGACGCCTCGGAGTACCTGAG	ACIGTGGGGTTTCTGAACTTGTT	[FAM]CTGGTGCAG/ZEN/TTCGCCCGTGCCA[3IABKFQ]	Jothikumar et al. 2005
Norovirus GII	QNIFS	ATGTTCAGRTGGATGAGRTTCTCWGA	TCGACGCCATCTTCATTCACA	[FAM]AGCACGTGGGAGGGGATCG[TAMRA]	daSilva et al. 2007
Norovirus GI	NV1LC	CGCTGGATGCGNTTCCAT	CCTTAGACGCCATCATCATTTAC	[FAM]TGGACAGGA/ZEN/GAYCGCRATCT[3IABkFQ]	daSilva et al. 2007
Salmonella invA	INVA	CAACGTTTCCTGCGGTACTGT	CCCGAACGTGGCGATAATT	[FAM]CTCTTTCGTCTGGCATTATCGATCAGTACCA[BHQ]	González-Escalona et al. 2009
Campylobacter coli glyA	Cc-glyA	CATATTGTAAAACCAAAGCTTATCGTG	AGTCCAGCAATGTGTGCAATG	[FAM]TAAGCTCCAACTTCATCCGCAATCTCTCTAAATTT[BHQ-1]	LaGier et al. 2006, Vondrakova et al. 2014
Campylobacter jejeuni hipO	Cj-hipO	TGCACCAGTGACTATGAATAACGA	TCCAAAATCCTCACTTGCCATT	[FAM]TTGCAACCTCACTAGCAAAATCCACAGCT[BHQ-1]	He et al. 2010, Vondrakova et al. 2014

Table S4 Correlation of Rainfall with human markers and pathogens

	Pearson's r	**p-value**	**Kendall's Tau**	**p-value**
HF183	-0.03	1	-0.01	1
Lachno3	-0.2	1	-0.17	1
Campylobacter	-0.29	0.56	-0.27	<0.001
Salmonella	-0.37	0.07	-0.35	<0.001
Human Adenovirus	0.07	1	0.06	1
Human Norovirus	0.16	1	0.08	1

Table S5 Correlation matrix of log transformed human marker and pathogen concentrations; the lower matrix are Pearson's r values and the upper matrix (shaded grey) are adjusted p-values.

	Salmonella	Campylobacter	HF183	Lachno3	Human Adenovirus	Human Norovirus
Salmonella		0.669	0.669	0.007	1	1
Campylobacter	0.24		0.326	0.222	0.585	1
HF183	0.25	0.29		<0.001	0.006	1
Lachno3	0.43	0.31	0.72		0.132	1
Human Adenovirus	0.00005	0.26	0.43	0.33		0.6688
Human Norovirus	0.068	-0.00023	0.17	0.19	0.24	

Table S6 Correlation matrix of log transformed human marker and pathogen concentrations; the lower matrix are Kendall's τ values and the upper matrix (shaded grey) are adjusted p-values.

	Salmonella	Campylobacter	HF183	Lachno3	Human Adenovirus	Human Norovirus
Salmonella		0.612	1.000	0.002	1.000	1.00
Campylobacter	0.2		0.125	0.210	0.310	1.00
HF183	0.14	0.25		<0.001	<0.001	1.00
Lachno3	0.36	0.23	0.45		0.016	0.39
Human Adenovirus	0.034	0.24	0.39	0.31		0.74
Human Norovirus	0.094	0.018	0.14	0.19	0.18	

Table S7 Test of differences in distribution (Kolmogorov-Smirnov test) from early season and late season samples

	D	p-value
HF183	0.19	1
Lachno3	0.16	1
Campylobacter	0.26	1
Salmonella	0.17	1
Human adenovirus	0.20	1
Human norovirus	0.27	1

Table S8 Whole stormwater human marker and pathogen concentrations in gene copies per 100ml

Site	Storm Event	HF183	Lachno3	Salmon-ella	Campylobacter coli	Campylobacter jejeuni	Adeno-virus	Norovirus GI	Norovirus GII
Los Pen	1	258	296	BD	BD	BD	BD	BD	BD
Los Pen	2	202	BD	BD	BD	BD	BD	BLOQ	BD
MB1	1	1158	37698	BLOQ	BD	BD	BLOQ	BLOQ	BD
MB1	2	158	147817	BD	BD	BD	BD	BD	BD
MB3	1	527	28983	86	BD	BD	BD	BD	BD
MB3	2	BLOQ	163	BLOQ	BD	BD	BD	BD	BD
MB5	1	108	1004	10233	BD	BLOQ	BD	BLOQ	BLOQ
MB5	2	BD	1598	BD	BD	BD	BD	BD	BD
DW0224	1	BLOQ	15851	5499	BD	BD	BD	BD	BD
DW0224	2	760	4168	BLOQ	BD	BD	BD	BD	BLOQ
Ballona	1	35218	51180	BD	BD	304	42	BLOQ	203
Ballona	2	53912	26388	129	BD	BD	BD	BLOQ	BD
Ballona	3	15531	28786	BD	BD	BD	329	BLOQ	BLOQ
CCW_SW_LAC	1	15127	16981	BD	BD	BD	90	BD	BD
CCW_SW_LAC	2	9969	16682	BLOQ	BD	BD	42	BLOQ	BD
CCW_SW_LAC	3	BD	BD	BD	BD	BD	BD	114	BD
Co Island	1	1101	1417	BD	BD	BD	BD	58	101
Co Island	2	794	300	BLOQ	BD	BD	BD	BLOQ	BD
Co Island	3	580	494	BLOQ	BD	BD	BD	BLOQ	BD
LLAR2	1	5158	85625	101	BLOQ	BLOQ	56	BLOQ	321
LLAR2	2	157	1492	BD	BD	BD	BD	BD	BD
LLAR2	3	535	3318	BD	BD	BD	BD	BD	BLOQ
LA R	1	6399	171238	BLOQ	BD	177	BLOQ	BD	BLOQ
LA R	2	7971	13990	99	BLOQ	419	BLOQ	BD	BLOQ
LA R	3	1831	1956	BLOQ	1492	BD	148	BLOQ	276
Malibu Crk	2	243	79	BD	BD	BD	BD	BLOQ	BD
Malibu Crk	3	531	1472	BD	BD	BD	BD	BD	BLOQ
Malibu Crk	1	BD	BD	BD	BD	BD	BLOQ	BD	BD
Bolsa	1	919	3964	182	BD	127	BLOQ	BD	BD
Bolsa	2	1358	4729	BLOQ	BD	261	BD	BLOQ	BLOQ
Delhi	1	2780	9000	BD	BD	100	43	BD	BLOQ
Delhi	2	2271	35900	BLOQ	833	BLOQ	BLOQ	BD	BD
SD creek	1	BD	BD	BD	BD	BD	BD	BD	BD
SD creek	2	BD	528	BD	BD	BD	BD	BLOQ	BD
Aliso Creek	1	4269	3074	87	BLOQ	176	BD	BD	BD
Aliso Creek	2	2648	1219	BD	BLOQ	BD	61	BD	BD
Aliso Creek	3	19646	5033	BD	BLOQ	BD	45	BD	BD

San Juan River	1	3980	4145	BLOQ	BD	BLOQ	BD	49	BLOQ
San Juan River	2	1237	455	BD	BLOQ	BD	BLOQ	BD	BD
San Juan River	3	5923	1368	BLOQ	BLOQ	BLOQ	BLOQ	BD	BD
801MAG364	1	14171	59403	BLOQ	BD	BLOQ	BLOQ	BD	263
801MAG364	2	4798	28958	BD	BD	BD	BLOQ	210	90
902MS43015	1	491	596	BD	BD	BD	BD	BLOQ	BLOQ
902MS43015	2	BLOQ	84	BD	BD	BD	BD	BD	89
801UNV702	1	BD	BLOQ	BD	BD	BLOQ	BD	81	BD
801UNV702	2	1362	143399	526	BD	394	53	BLOQ	3399
801UNV702	3	BD	BD	BD	BD	BD	BD	BD	160
801CCCR12	1	3380	21009	222	BD	189	BD	BD	176
801CCCR12	2	1521	15378	180	BD	BD	BD	BD	147
801CUCb	1	3520	3923	BD	BD	408	BLOQ	BD	117
801CUCb	2	44959	9626	BLOQ	BD	BD	BLOQ	BD	120
801SACCH17	1	298	12944	2595	BD	1020	BD	BD	BD
801SACCH17	2	BLOQ	15280	BLOQ	BD	BD	BD	43	BLOQ
801SACST18	1	211	533	BLOQ	BD	BD	BLOQ	BLOQ	BD
801SACST18	2	1266	17669	294.	BD	BD	BD	BD	BD
801SARWA19	1	748	53260	BLOQ	BD	BD	BD	BLOQ	BD
801SARWA19	2	147	39667	199	BD	BD	BLOQ	74	142
MS4-SDR-228	1	3461	204	BD	BD	BD	BD	BD	BD
MS4-SDR-228	2	BLOQ	BLOQ	BD	BLOQ	BD	BD	BD	BD
MS4-SLR-150	1	BD	BD	BLOQ	BD	BD	BLOQ	BD	65
MS4-SLR-150	2	BD	BD	BD	BD	BD	BD	BD	BD
MO-SPA	1	398	4970	BLOQ	BD	122	BD	BD	92
MO-SPA	2	290	12768	BD	BD	BLOQ	BD	BLOQ	BD
MO-SIM	1	BD	BD	BD	BD	BD	BD	BD	BD
MO-SIM	2	4360	9349	BLOQ	BD	113	BD	BLOQ	BD
ME-CC	1	BLOQ	BD	BD	BD	BD	BLOQ	BD	93
ME-CC	2	BD	BLOQ	BD	BD	BD	BD	225	116
MO-FIL	1	BD	953	113	BD	BLOQ	BD	BD	BD
MO-FIL	2	BLOQ	8714	BD	BD	132	BLOQ	BD	BD
ME-SCR	1	BD	BLOQ	BLOQ	BD	BD	BD	BD	BD

Table S9 Separated sediment virus concentrations

Site	Date	Adenovirus pellet copies per 100ml	Adenovirus supernatant copies per 100ml	Norovirus GI pellet copies per 100ml	Norovirus GI supernatant copies per 100ml	Norovirus GII pellet copies per 100ml	Norovirus GI supernatant copies per 100ml
Los Pen	10/25/21	BD	BD	BD	BD	BD	BD
MB1	10/25/21	BLOQ	BD	BD	BD	BD	BD
MB3	10/25/21	BD	BD	BD	BD	BD	BD
MB5	10/25/21	BD	BD	BD	BD	BD	BLOQ
ME-CC	10/25/21	BD	BD	BD	BD	BD	BLOQ
ME-SCR	10/25/21	BD	BD	BD	BD	BD	BD
MO-FIL	10/25/21	BD	BD	BD	BD	BLOQ	BD
MO-SIM	10/25/21	BD	BD	BD	BD	BD	BD
MO-SPA	10/25/21	BD	BD	BD	BD	BD	BLOQ
Aliso Cr	10/26/21	BD	BD	BD	BD	BD	BD
SJ R	10/26/21	BD	BD	BD	BLOQ	BLOQ	BD
801CCCR12	12/9/21	BD	BD	BD	BD	62	BLOQ
801CUCb	12/9/21	BD	BD	BD	BD	BD	BLOQ
801SACCH17	12/9/21	BD	BD	BD	BD	BD	BD
801SARWA19	12/9/21	BD	BD	BLOQ	BD	BD	BD
DW0224	12/9/21	BD	BD	BD	BD	BD	BD
MS4-SLR-150	12/9/21	BLOQ	BD	BD	BD	BD	BLOQ
801MAG364	12/14/21	BD	BD	BLOQ	BD	BD	262
801SACST18	12/14/21	BLOQ	BD	BLOQ	BLOQ	BD	BD
801UNV702	12/14/21	BD	BD	BD	BD	BD	BD
902MS43015	12/14/21	BD	BD	BD	BD	BLOQ	BD
Bolsa	12/14/21	BD	BD	BD	BD	BLOQ	BD
Delhi	12/14/21	BLOQ	BD	BD	BD	BLOQ	BD
ME-CC	12/14/21	BD	BD	BD	BLOQ	BD	BLOQ
MO-FIL	12/14/21	BD	BD	BD	BD	BD	BD
MO-SIM	12/14/21	BD	BD	BLOQ	BD	BD	BD
MO-SPA	12/14/21	BD	BD	BLOQ	BD	BD	BD
SD Cr	12/14/21	BD	BD	BD	BD	BD	BD
801SACCH17	3/28/22	BD	BD	43	BD	BLOQ	BD
801SARWA19	3/28/22	BD	BD	74	BD	BLOQ	BLOQ
801UNV702	3/28/22	53	BD	BLOQ	BD	916	1136
Ballona	3/28/22	BLOQ	BD	BLOQ	BD	BLOQ	BLOQ
Co Island	3/28/22	BD	BD	58	BD	BD	BD
LA R	3/28/22	BLOQ	BD	BD	BD	BLOQ	BD
LLAR2	3/28/22	BD	BD	BLOQ	BD	146	BD
Malibu Crk	3/28/22	BLOQ	BD	BD	BD	BD	BD

Ballona	11/8/22	BD		BD		BD		BD		BD		BD		
CCW_SW_LAC	11/8/22		42	BD		BD		BD		BD		BD		
Co Island	11/8/22	BD		BD		BD		BD		BD		BD		
LA R	11/8/22	BD		BD		BLOQ		BD		BD		BD		
LLAR2	11/8/22	BD		BD		BD		BD		BLOQ		BD		
Malibu Crk	11/8/22	BD		BD		BD		BD		BD		BD		
801CCCR12	2/24/23	BD		BD		BD		BD		BD		BD		
801MAG364	2/24/23	BD		BLOQ		BD			1543	BD				246
801UNV702	2/24/23	BD		BD		BD		BD		BD		BLOQ		
902MS43015	2/24/23	BD		BD		BD		BD		BD		BLOQ		
Ballona	2/24/23	BD			328	BLOQ		BD		BD		BD		
CCW_SW_LAC	2/24/23	BD		BD		BD		BLOQ		BD		BD		
Co Island	2/24/23	BD		BD		BLOQ		BD		BD		BD		
LA R	2/24/23		148	NA		BD					117			
Malibu Crk	2/24/23	BD		BD		BD		BD		BD		BD		
MS4-SDR-228	2/25/23	BD		BD		BD		BD		BD		BD		
MS4-SLR-150	2/25/23	BD		BD		BD		BD		BD		BD		
801CUCb	2/27/23	BLOQ		BD		BD		BD		BLOQ		BD		
Los Pen	2/27/23	BD		BD		BD		BD		BD		BD		

Table S10 RNA viruses extraction kit comparison

		Biomerieux		MagMax	
Site	Date	GI copies per 100ml	GII copies per 100ml	GI copies per 100ml	GII copies per 100ml
Ballona	11/8/22	BLOQ	BD	100	BLOQ
Malibu Crk	11/8/22	BLOQ	BD	BD	BD
LA R	11/8/22	BLOQ	BD	160	31
Co Island	11/8/22	BLOQ	BD	BD	BD
CCW_SW_LAC	11/8/22	BLOQ	BD	BD	BD
LLAR2	11/8/22	BD	BD	40	31
San Vic Creek	2/25/23	BLOQ	BD	BD	BD
Ballona	2/24/23	BLOQ	BLOQ	BLOQ	224
Malibu Crk	2/24/23	BLOQ	BD	BD	32
LA R	2/24/23	BLOQ	276	40	744
Co Island	2/24/23	BLOQ	BD	BLOQ	BLOQ
LLAR2	2/24/23	BD	BLOQ	BD	BLOQ
Magnolia	2/24/23	210	90	2425	12800
Cuc Ck 2	2/24/23	BLOQ	BD	BLOQ	147
Cuc Ck 3	2/24/23	BD	BD	BLOQ	120
Los Pen	2/27/23	BD	BD	BLOQ	BD
MB 1	2/27/23	BD	BD	BD	BLOQ
DW2204	2/27/23	BD	BLOQ	BLOQ	499

ITEM TO CHECK	PROVIDED	COMMENT
	Y/N	
1. SPECIMEN		
Detailed description of specimen type and numbers	y	
Sampling procedure (including time to storage)	y	
Sample aliquotation, storage conditions and duration	y	
2. NUCLEIC ACID EXTRACTION		
Description of extraction method including amount of sample processed	y	
Volume of solvent used to elute/resuspend extract	y	
Number of extraction replicates	y	
Extraction blanks included?	y	
3. NUCLEIC ACID ASSESSMENT AND STORAGE		
Method to evaluate quality of nucleic acids	y	Qubit Flex, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018186_Qubit_Flex_Fluorometer_UG.pdf
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	y	Qubit Flex, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018186_Qubit_Flex_Fluorometer_UG.pdf
Storage conditions: temperature, concentration, duration, buffer, aliquots	y	Filters flash frozen in liquid nitrogen post filtration. -80C storage of filters and extracts.
Clear description of dilution steps used to prepare working DNA solution	y	DNA undiluted unless exceeded the upper limit of quantification, then diluted with DNA/RNA free water.
4. NUCLEIC ACID MODIFICATION		
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	n	No modifcations were made.
Details of repurification following modification if performed	n	No modifcations were made.
5. REVERSE TRANSCRIPTION		
cDNA priming method and concentration	y	One-Step RT ddPCR, https://www.bio-rad.com/en-us/life-science/digital-pcr/digital-pcr-supermixes/one-step-rt-ddpcr-advanced-kit-for-probes/500-x-20-reaction-kit
One or two step protocol (include reaction details for two step)	y	One step
Amount of RNA added per reaction	y	5ul
Detailed reaction components and conditions	y	
Estimated copies measured with and without addition of RT*	n	RNA samples were run using RT, DNA samples were run using non-RT. We did not run any RNA samples with non-RT nor any DNA samples with RT.
Manufacturer of reagents used with catalogue and lot numbers	y	
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	Y	
6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION		
Sequence accession number or official gene symbol	y	
Method (software) used for design and *in silico* verification	y	IDT online tools, Geneious Prime v.2022.2.1
Location of amplicon	y	Varies depending on primer/probe.
Amplicon length	y	15-25 nt
Primer and probe sequences (or amplicon context sequence)**	y	
Location and identity of any modifications	y	No modifications unless otherwise specified (e.g. LNA mofidication).
Manufacturer of oligonucleotides	y	IDT, Eurofins
7. dPCR PROTOCOL		
Manufacturer of dPCR instrument and instrument model	y	Bio-Rad (Hercules, CA), QX200 or QX600
Buffer/kit manufacturer with catalogue and lot numbers	y	One-Step RT ddPCR advanced kit for Probes (1864022), ddPCR Multiplex Supermix 4X (12005911)
Primer and probe concentration		100uM
Pre-reaction volume and composition (incl. amour		
Template treatment (initial heating or chemical c		l treatment to template.
Polymerase identity and concentration, Mg++ an		ietary to Bio-Rad
Complete thermocycling parameters		nin, and a final enzyme deactivation step at 98°C for 10 min. RNA targets: reverse 5°C for 30s, 58°C 1 min, and a final enzyme deactivation step at 98°C for 10 min.
8. ASSAY VALIDATION		
Details of optimisation performed		o et al. (2015)
Analytical specificity (vs. related sequences) and l		late controls (NTC) were run every plate, required to contain less than 3 positive roplets (LOQ)
Analytical sensitivity/LoD and how this was eval		e in QX Manager v.1.2 = 0.0085, droplet size in v.2.0 = 0.00795
Testing for inhibitors (from biological matrix/extr		controls: mouse lung beta actin RNA.
9. DATA ANALYSIS		
Description of dPCR experimental design		
Comprehensive details negative and positive of c		ntamination. 2 positive control reactions run every assay to validate primer/probe.
Partition classification method (thresholding)		ard deviation from baseline amplitude.
Examples of positive and negative experimental r		pos neg example.png
Description of technical replication		ate and merged by QX Manager software.
Repeatability (intra-experiment variation)		10-15 samples, required to contain less than 3 positive droplets (LOQ)
Reproducibility (inter-experiment/user/lab etc. va		personnel, ddPCR personnel, or filtering personnel.
Number of partitions measured (average and sta		ed by QX Manager software) required for each reaction.
Partition volume		40ul
Copies per partition (λ or equivalent) (average a		varied
dPCR analysis program (source, version)		ersion 1.2 or version 2.0
Description of normalisation method		, no other normalization.
Statistical methods used for analysis		
Data transparency		

Environmental Microbiology Minimum Information Checklist

Study Description

Study: SMC
Date: 5-15-23
Completed by:

Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	Analysis
2L collection in PP bottles, sterilized with 10% HCl Vacuum filtration	☐ Performed	☐ Performed	ThermoFisher MagMax performed on KingFisher Flex	■ Performed One-step RT on Bio-Rad ddPCR system	☐ qPCR ■ dPCR Bio-Rad ddPCR system	R

Control Checklist

	Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	
Step performed	■	☐	☐	■	■	■	
Step has control info	■	☐	☐	■	■	■	Negative Controls
# control replicates			0				
Control result reported	■	☐	☐	■	■	■	
Data handling reported	■	☐	☐	■	■	■	
Control introduced	■	☐	☐	■	☐	■	Positive Controls
Internal/External	Internal	N/A	N/A	External	External	External	
Independant/Parallel	Parallel	N/A	N/A	Parallel	Parallel	Parallel	
Step has control info	■	☐	☐	■	■	■	
# control replicates	1	1	0	1	1	1	
Control result reported	■	☐	☐	■	■	■	
Data Handling reported	■	☐	☐	■	■	■	

Process Checklist

Environmental Sampling
- ■ Sampling Procedure
- ■ Number of samples
- ■ Sample amount, mean, range
- ■ Sampling locations, dates, times

Sample Treatment
- ☐ Performed
- ☐ Treatment procedure
- ☐ Reagents

Sample Reduction
- ☐ Performed
- ☐ Reduction procedure
- ☐ Reagents
- ☐ Concentration Factor

Nucleic Acid Extraction
- ■ Extraction procedure
- ■ Amount extracted, amount obtained
- ■ Extract storage conditions

qPCR or dPCR
- ■ Target gene name, amplicon length
- ■ Thermocycling temperatures and times
- ■ Master mix: composition, vendors, concentrations
- ☐ Additives: vendors, concentrations
- ■ Template amount added, pre-treatment (if any)
- ■ Primers: sequences, concentrations, vendors, references
- ■ Amplicon confirmation method (probe, melt curve, etc)
- ■ Probe sequence, concentration, vendor, reference
- ■ Instrumentation
- ■ Equivalent volume of sample analyzed by PCR
- ■ Inhibition assessment procedure
- ■ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Reverse Transcription
- ■ Performed
- ■ One or two step
- ☐ cDNA storage conditions (if two step)
- ■ Reaction temperatures and times
- ■ Reaction reagents and concentrations
- ■ Priming method
- ■ Reaction volume, added template amount
- ■ Inhibition assessment procedure
- ■ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Analysis – dPCR
- ■ Threshold settings
- ■ Technical replicates, number, well merging
- ■ Partitions measured, number, mean, variance
- ■ Partition volume
- ■ Target copies per partition, mean, variance
- ■ Program used for dPCR analysis
- ■ Explanation of control results, example plots

Analysis – qPCR
- ☐ Method for handling failed negative controls
- ☐ Technical replicates, number, calculations
- ☐ Calibration standards: description and source
- ☐ Method of quantifying standards
- ☐ Calibration curve slope
- ☐ Calibration curve R2
- ☐ Lowest standard measured or 95% LOD
- ☐ Cq value determination method

Figure S1. dMIQE checklist (A) and Environmental Microbiology Minimum Information Checklist (B).

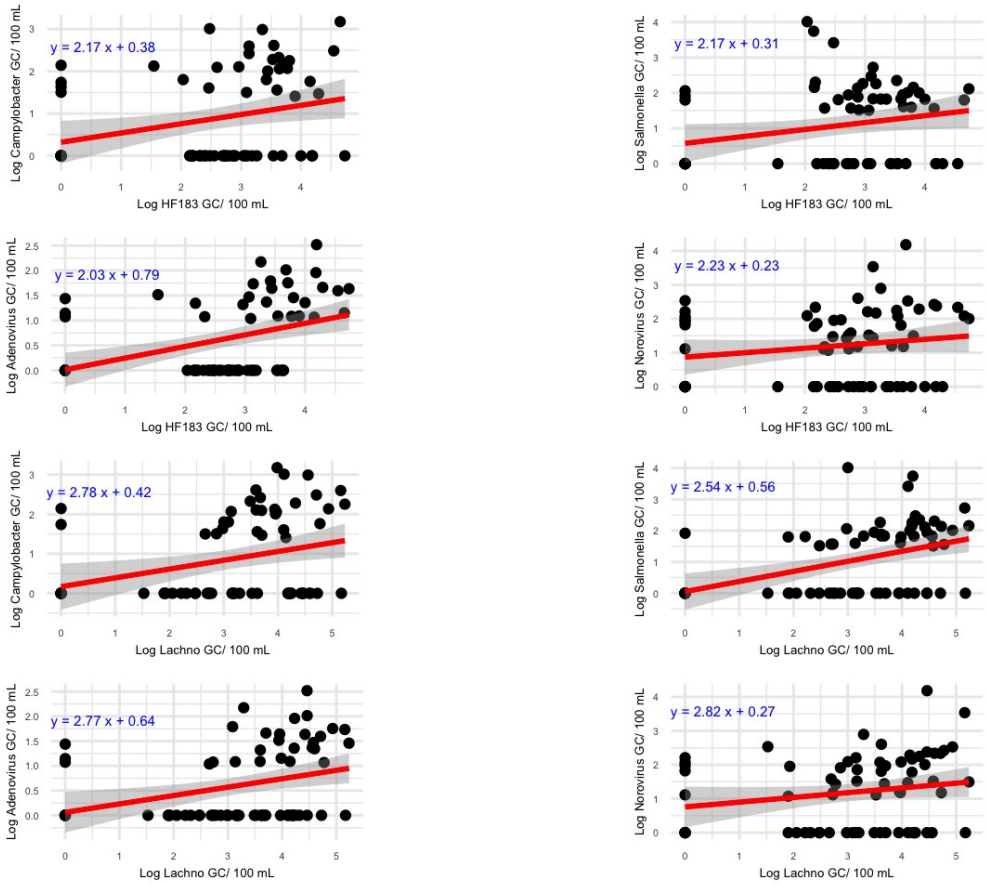
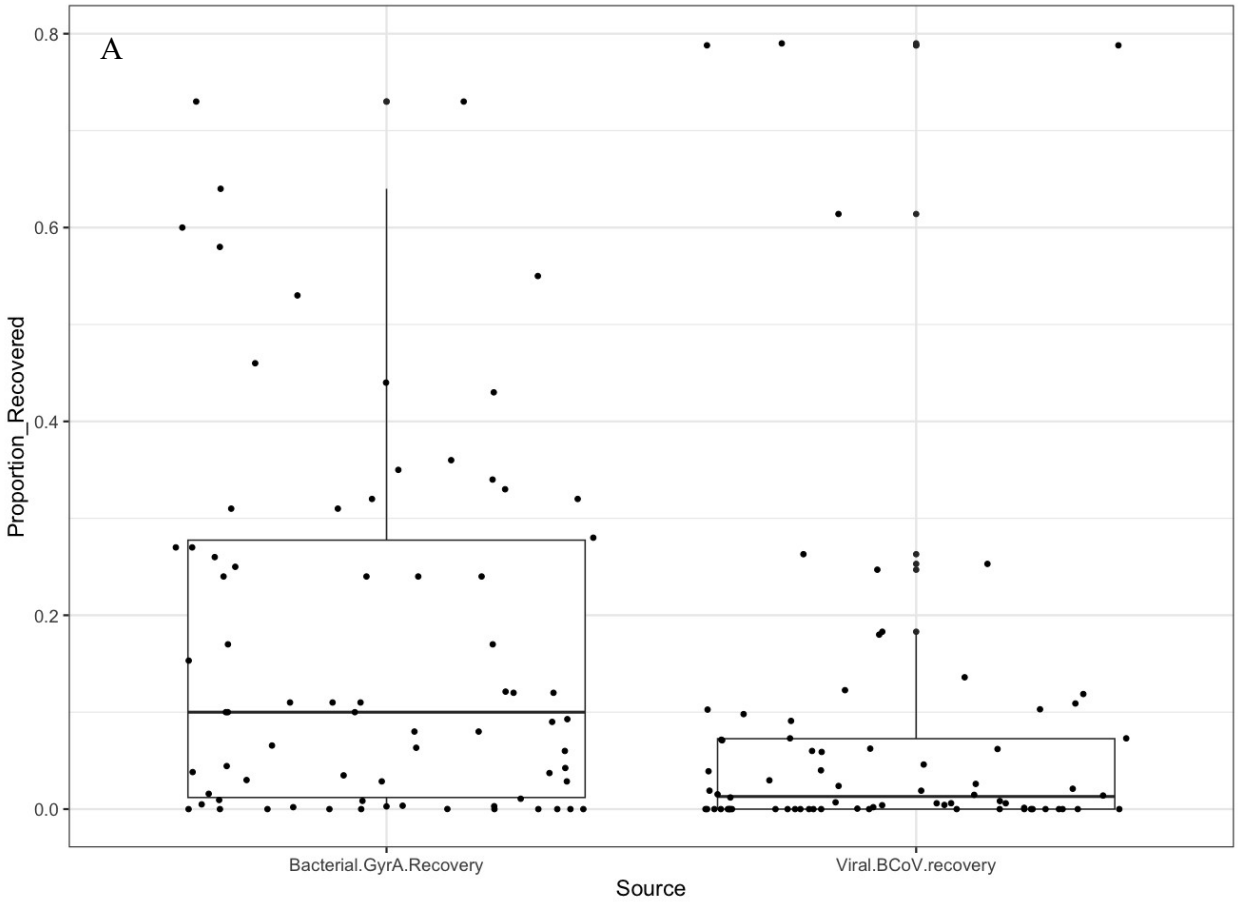
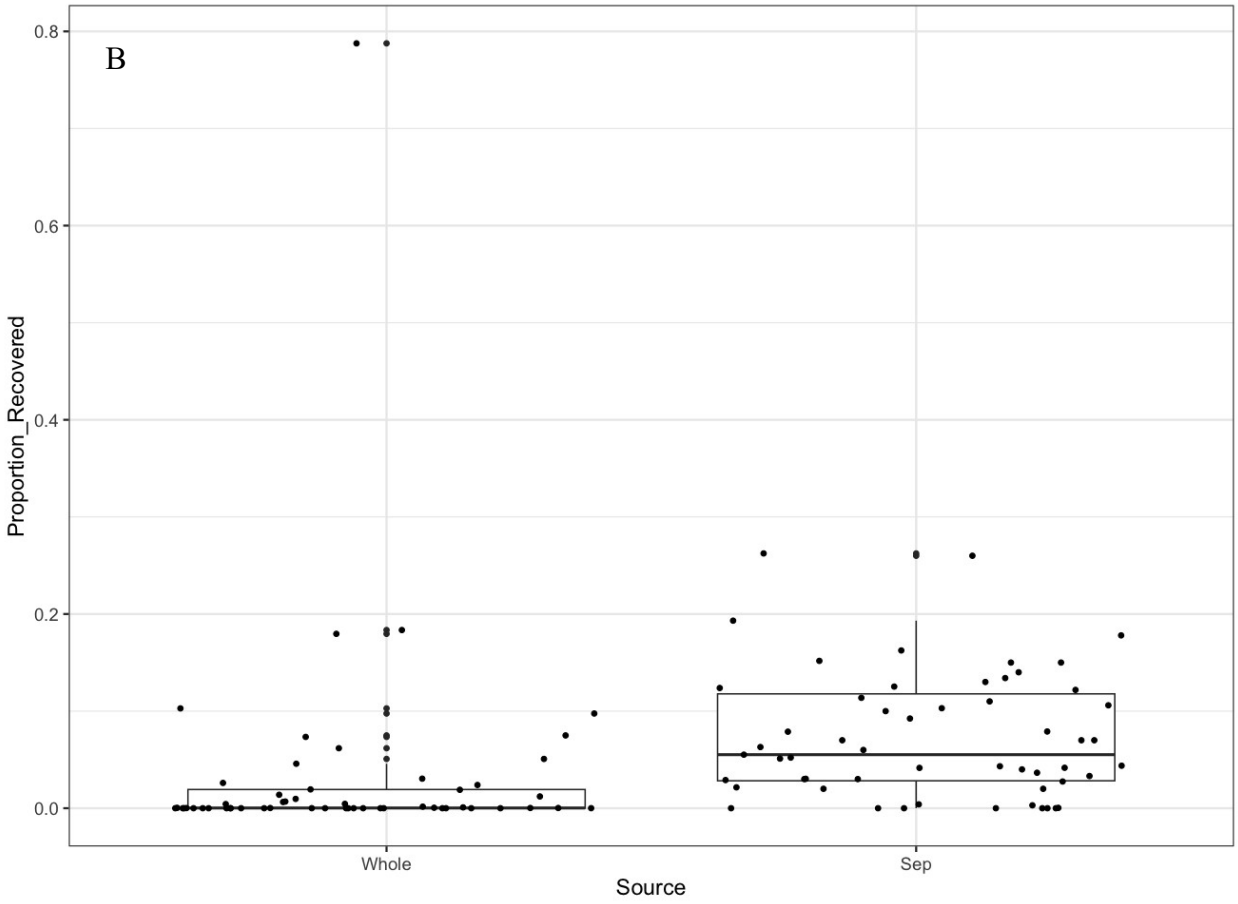


Figure S2. Scatter plots of human markers and pathogens with least squares regression line and 95% CI.


A
0.8
0.6
0.4
0.2
0.0
Proportion_Recovered
Bacterial.GyrA.Recovery
Viral.BCoV.recovery
Source


B
0.8
0.6
0.4
0.2
0.0
Proportion_Recovered
Whole
Sep
Source

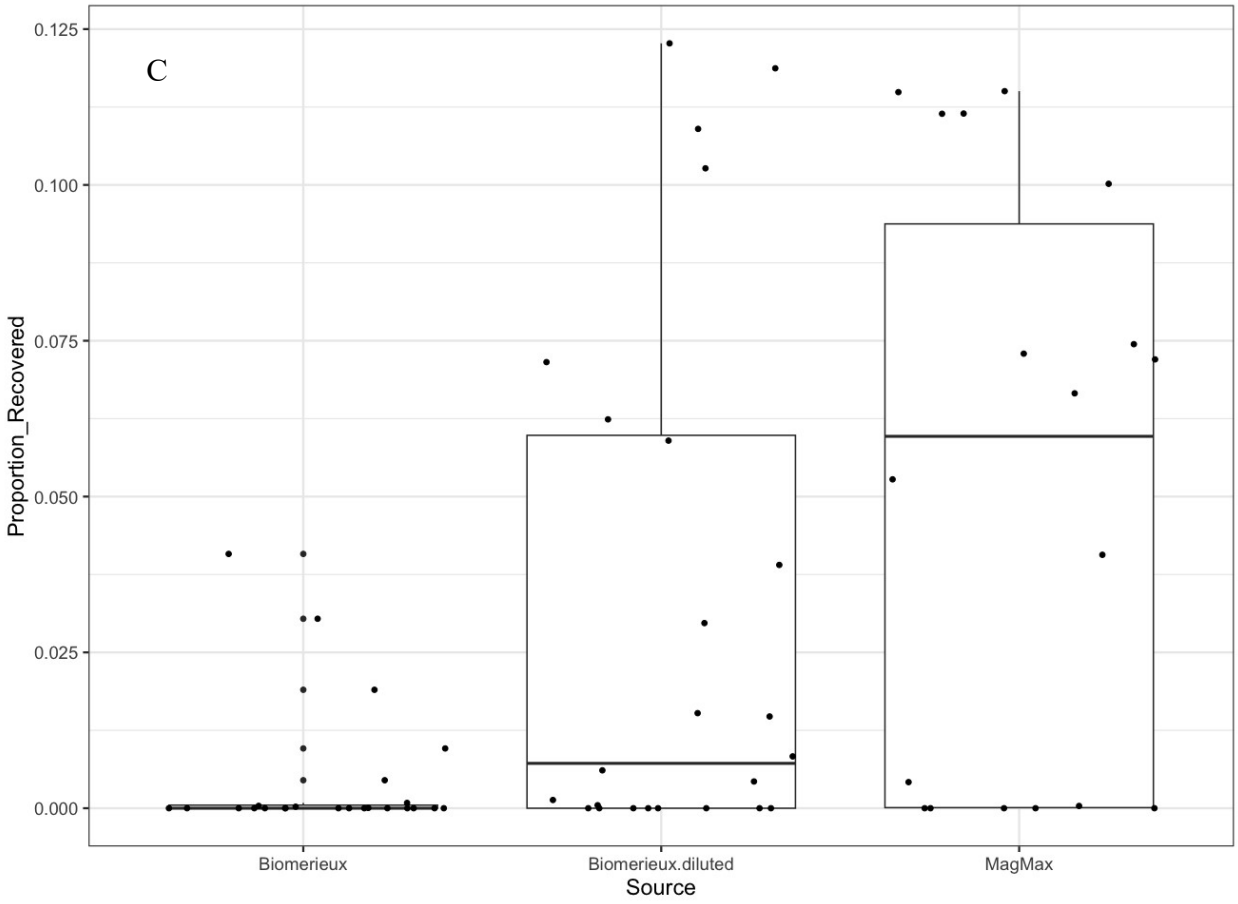


Figure S3. Boxplots of sample process recovery for stormwater filters from whole stormwater for bacteria and viruses (A), whole stormwater vs separated stormwater (B), and comparing extractions (C).

References

Bernhard, A. E., and Field, K. G. (2000). 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, 4571–4574.

Cao, Y., M.R. Raith, J.F. Griffith. 2015. Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research* 70:337–349

Bernhard, A. E., and Field, K. G. (2000). 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, 4571–4574.

da Silva AK, Le Saux JC, Parnaudeau S, Pommepuy M, Elimelech M, Le Guyader FS. 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* 73:7891–7897

Decaro, N., Elia, G., Campolo, M., Desario, C., Mari, V., Radogna, A., et al. (2008). Detection of bovine coronavirus using a TaqMan-based real-time RT-PCR assay. *J. Virol. Methods* 151, 167–171. doi: 10.1016/j.jviromet.2008.05.016

Feng, S., Bootsma, M., and McLellan, S. L. (2018). Human-Associated LachnospiraceaeGenetic Markers Improve Detection of Fecal Pollution Sources in Urban Waters. *Applied and Environmental Microbiology* 84, 1765–14. doi: 10.1128/aem.00309-18

González-Escalona N, Hammack TS, Russell M, Jacobson AP, De Jesús AJ, Brown EW, Lampel KA (2009) Detection of Live Salmonella sp. Cells in Produce by a TaqMan-Based Quantitative Reverse Transcriptase Real-Time PCR Targeting invA mRNA. Applied and Environmental Microbiology 75:3714–3720

He, Y., Yao, X., Gunther, N.W., Xie, Y., Tu, S.-I., and Shu, S.X. 2010. Simultaneous Detection and Differentiation of *Campylobacter jejuni*, *C. coli*, and *C. lari* in Chickens Using a Multiplex Real-Time PCR Assay. *Food Anal Methods* 3**:**321–329.

Jothikumar N, Cromeans TL, Hill VR, Lu X, Sobsey MD, Erdman DD (2005) Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41. Applied and Environmental Microbiology 71:3131–3136

LaGier MJ, Joseph LA, Passaretti TV, Musser KA, Cirino NM (2004) A real-time multiplexed PCR assay for rapid detection and differentiation of Campylobacter jejuni and Campylobacter coli. Molecular and Cellular Probes 18:275–282

Lund M, Nordentoft S, Pedersen K, Madsen M (2004) Detection of Campylobacter spp. in Chicken Fecal Samples by Real-Time PCR. *Journal of Clinical Microbiology* 42:5125–5132

Malorny B, Lofstrom C, Wagner M, Kramer N, Hoorfar J (2008) Enumeration of Salmonella Bacteria in Food and Feed Samples by Real-Time PCR for Quantitative Microbial Risk Assessment. *Applied and Environmental Microbiology* 74:1299–1304

Vondrakova L., Pazelrova, J., Demnerova, K. 2014. Detection, identification and quantification of Campylobacter jejuni, coli and lari in food matrices all at once using multiplex qPCR. *Gut Pathogens*

6:12, doi: 10.1186/1757-4749-6-12**)**.