

Supplementary Material

Droplet Digital PCR Quantification of Norovirus and Adenovirus in Decentralized Wastewater and Graywater Collections: Implications for Onsite Reuse

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Abbreviations

AdV adenovirus

BDL below limit of detection

BLQ below limit of quantification

C_q quantification cycle

ddPCR droplet digital polymerase chain reaction

NoVGI norovirus genogroup I

NoVGII norovirus genogroup II

NTC no-template control

qPCR quantitative polymerase chain reaction

Figure S1. qPCR Standard Curves

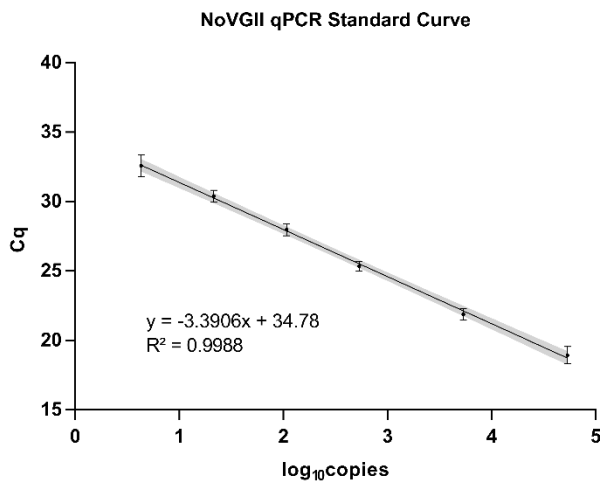
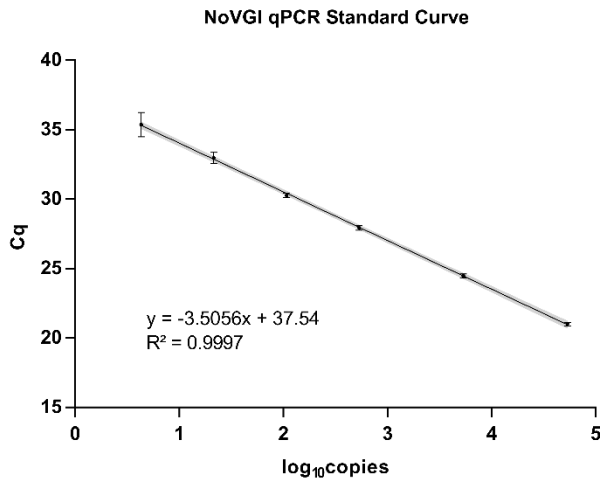
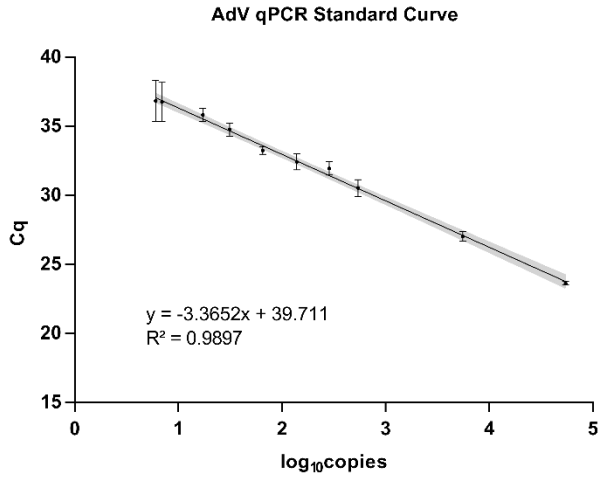


Figure S2. Threshold Plots for Selected ddPCR Settings

Threshold settings were 0.9995 for AdV (top); 0.99995 for NoVGI (center); and 0.9999995 with block size 100 for NoVGII (bottom). Left and right panels represent negative and positive controls, respectively.

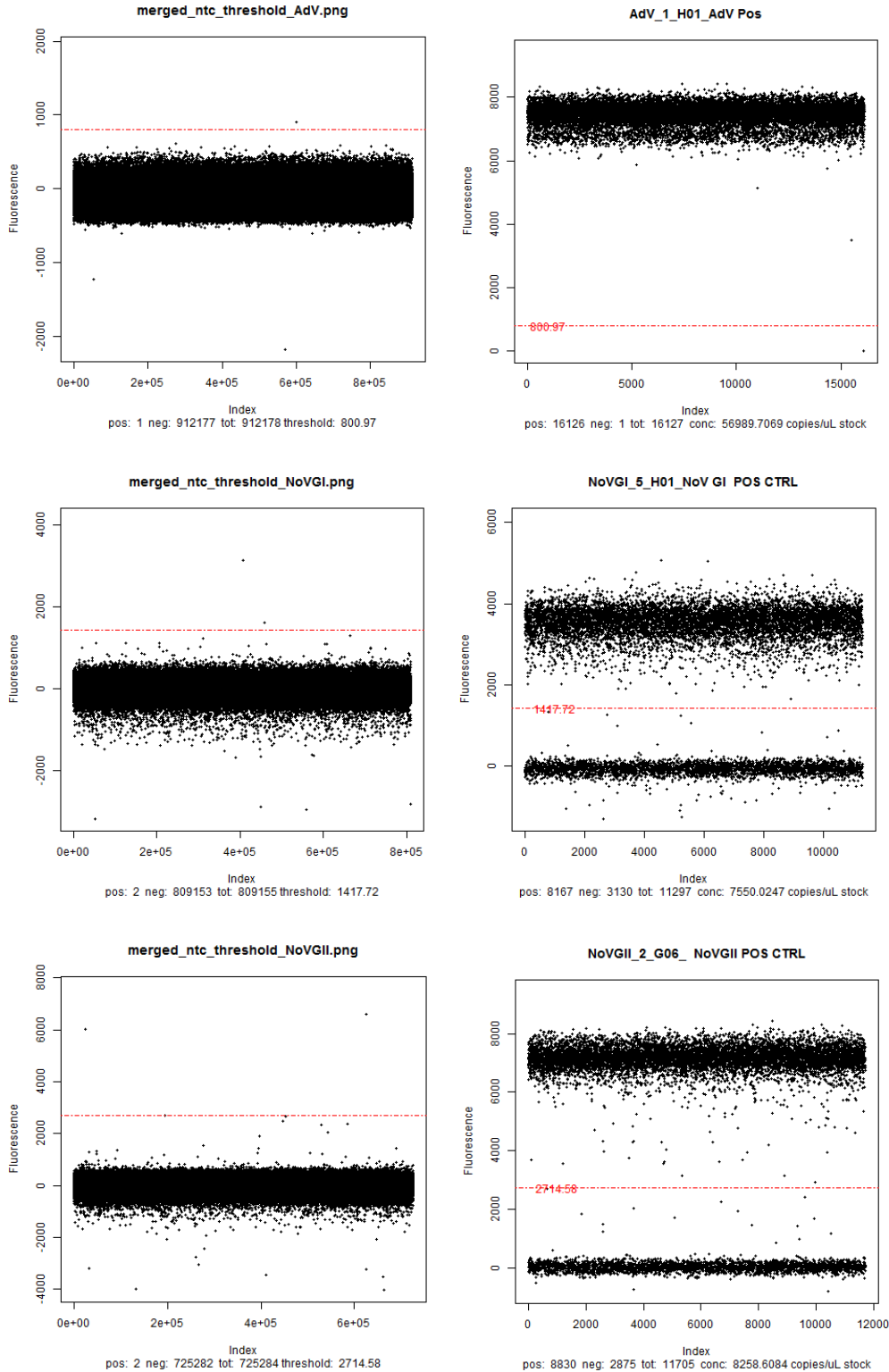


Table S1. Droplet counts for ddPCR

Total positive (+) and negative (-) droplet counts from 3 pooled ddPCR wells for each sample (9 wells for WW1 from 2017). Also shown are merged negative (NTC) and positive (POS) controls (varying number of wells per assay). Refer to main text for sample site descriptions.

Site	Date	AdV (+)	AdV (-)	NoVGI (+)	NoVGI (-)	NoVGII (+)	NoVGII (-)
NTC	n/a	1	912177	2	809153	2	725282
POS	n/a	240827	61	138274	53916	150870	41907
WW1	1/11/2016	0	36987	0	29854	4301	34886
WW1	1/13/2016	0	43614	0	32633	916	29171
WW1	1/20/2016	1	39423	2	35386	1126	35383
WW1	1/27/2016	1	38534	0	32198	430	31835
WW1	2/1/2016	1	38983	18	27148	3710	30347
WW1	2/3/2016	0	43578	1	37720	2224	41065
WW1	2/8/2016	1	43015	0	34480	288	40887
WW1	2/10/2016	0	42030	0	35690	32438	1628
WW1	2/16/2016	0	44695	0	31062	26761	6996
WW1	2/17/2016	0	44129	0	37348	27085	6354
WW1	2/22/2016	1	43227	3	33099	868	30143
WW1	2/24/2016	2	40584	2	38387	15547	19590
WW1	2/29/2016	0	39615	1	28612	4642	24544
WW1	3/2/2016	0	42897	0	40416	3737	28586
WW1	3/7/2016	0	41323	0	42160	1062	33921
WW1	3/9/2016	0	40783	0	34463	1661	30778
WW1	3/14/2016	0	45482	0	35938	6211	25713
WW1	3/16/2016	0	41634	0	42298	3699	36774
WW1	3/21/2016	0	45196	4	36965	995	39433
WW1	3/23/2016	0	38942	2	33920	547	37003
WW1	3/28/2016	0	42966	0	36587	1	33767
WW1	3/30/2016	2	42494	1	41113	15743	18519
WW1	4/4/2016	0	41287	8	34739	24138	6728
WW1	4/6/2016	1	40287	4	36198	13460	22000
WW1	6/12/2017	3	130320	3	103235	22547	89980
WW1	6/19/2017	2	81312	17	82026	1728	79965
WW1	6/26/2017	2	121953	4	112687	924	119726
WW1	7/10/2017	15	119391	0	120294	861	103960
GW1	12/16/2014	0	36455	0	26080	1	28165
GW1	12/18/2014	0	39481	0	26795	1	31614
GW1	1/6/2015	1	39789	0	24357	0	29292
GW1	1/8/2015	0	45645	0	28566	0	29445
GW1	1/12/2015	0	44574	0	29136	0	32299
GW1	1/13/2015	0	41839	0	29958	0	34194
GW1	1/21/2015	0	44111	0	29777	0	31925

GW1	1/22/2015	0	48068	0	30101	0	32624
GW1	1/26/2015	0	44016	0	29430	3	31655
GW1	1/28/2015	1	42232	0	34707	2	35191
GW1	2/4/2015	0	42356	0	32489	0	35547
GW1	2/5/2015	0	50059	0	33336	0	31976
GW1	2/9/2015	0	44009	0	28893	1	30779
GW1	2/11/2015	0	41372	0	31229	0	32302
GW1	2/17/2015	0	46271	0	33703	0	35945
GW1	2/19/2015	0	44801	0	30867	1	31259
GW1	2/23/2015	0	42253	0	29877	0	31181
GW1	2/25/2015	0	41513	0	37967	1	33466
GW1	3/3/2015	6	44013	0	31595	1	31401
GW1	3/4/2015	8	44610	0	33477	1	30972
GW1	3/10/2015	0	39743	0	37669	0	30533
GW1	3/12/2015	2	43323	1	39867	1	35474
GW1	3/16/2015	35	47267	1	35279	1	32123
GW1	3/18/2015	10	46105	0	28691	0	32431
GW1	3/24/2015	0	45485	0	32845	0	30710
GW1	3/25/2015	0	39739	0	31974	0	33866
GW1	3/31/2015	1	45268	0	34968	2	34201
GW1	4/2/2015	0	45755	0	29708	0	29908
GW1	4/6/2015	0	39618	0	34634	0	35957
GW1	4/8/2015	1	39163	0	30166	0	36130
GW1	4/15/2015	0	49919	1	35618	0	27052
GW1	4/20/2015	0	43519	0	33882	0	30696
GW1	4/22/2015	1	37766	0	36850	0	34178
GW2	2/22/2016	1	43841	0	33548	0	31599
GW2	2/24/2016	0	43744	0	41584	0	32027
GW2	2/29/2016	0	44005	0	36680	0	40309
GW2	3/7/2016	5	43599	0	35054	0	38306
GW2	3/14/2016	2	46291	1	41425	0	32583
GW2	3/16/2016	0	45666	0	39520	1	39079
GW2	3/30/2016	0	45582	0	34030	1	37540
GW2	4/4/2016	0	43839	0	33677	0	38469
GW2	4/6/2016	0	48410	0	34066	0	32211
GW2	4/11/2016	1	41681	0	37929	0	39026
GW2	4/13/2016	0	42414	0	38885	0	37770
GW2	4/18/2016	0	42794	0	34380	0	37044
GW2	4/20/2016	0	44656	0	34480	0	34464
GW2	4/25/2016	0	42870	0	33294	0	29911
GW2	4/27/2016	0	46806	0	40252	0	36321
GW2	5/2/2016	0	44845	0	35614	1	28300
GW2	5/4/2016	0	46159	0	36311	0	30408

Table S2. Complete PCR Results

Units are log₁₀ genome copies/L. BLD indicates non-detection and BLQ indicates putative detection beyond the qPCR standard curve range of quantification or within the 95% credible interval of ddPCR NTCs. Refer to main text for sample site descriptions.

Site	Date	AdV-qPCR	AdV-ddPCR	NoVGI-qPCR	NoVGI-ddPCR	NoVGII-qPCR	NoVGII-ddPCR
WW1	1/11/2016	BLD	BLD	BLD	BLD	5.93	6.12
WW1	1/13/2016	BLD	BLD	BLD	BLD	5.43	5.53
WW1	1/20/2016	BLD	BLQ	BLD	2.13	5.46	5.54
WW1	1/27/2016	BLQ	BLQ	BLD	BLD	5.54	5.46
WW1	2/1/2016	BLQ	BLQ	BLD	3.98	6.28	6.42
WW1	2/3/2016	BLQ	BLD	BLD	BLQ	6.05	6.08
WW1	2/8/2016	BLD	BLQ	BLD	BLD	4.89	5.17
WW1	2/10/2016	BLQ	BLD	BLD	BLD	7.64	7.85
WW1	2/16/2016	BLQ	BLD	BLQ	BLD	7.32	7.56
WW1	2/17/2016	BLD	BLD	BLD	BLD	7.53	7.59
WW1	2/22/2016	4.26	BLQ	BLQ	2.78	5.59	5.80
WW1	2/24/2016	4.02	2.38	BLQ	2.41	6.87	7.13
WW1	2/29/2016	BLQ	BLD	BLD	BLQ	6.36	6.60
WW1	3/2/2016	BLD	BLD	BLD	BLD	6.56	6.45
WW1	3/7/2016	BLD	BLD	BLD	BLD	5.80	5.83
WW1	3/9/2016	BLD	BLD	BLD	BLD	5.55	6.07
WW1	3/14/2016	BLD	BLD	BLD	BLD	6.53	6.70
WW1	3/16/2016	BLQ	BLD	BLD	BLD	6.33	6.34
WW1	3/21/2016	BLD	BLD	BLQ	2.93	5.78	5.74
WW1	3/23/2016	BLD	BLD	BLD	2.46	5.66	5.50
WW1	3/28/2016	BLD	BLD	BLD	BLD	BLD	BLQ
WW1	3/30/2016	BLD	2.36	BLD	BLQ	7.32	7.15
WW1	4/4/2016	BLD	BLD	BLD	3.41	7.61	7.55
WW1	4/6/2016	BLQ	BLQ	BLD	2.94	7.14	7.04
WW1	6/12/2017	BLQ	2.18	BLQ	2.28	6.55	6.72
WW1	6/19/2017	BLQ	BLQ	BLD	3.47	5.45	5.68
WW1	6/26/2017	BLQ	BLQ	BLQ	2.45	5.30	5.23
WW1	7/10/2017	BLQ	3.24	BLQ	BLD	5.33	5.26
GW1	12/16/2014	BLD	BLD	BLQ	BLD	BLD	BLQ
GW1	12/18/2014	BLD	BLD	BLD	BLD	BLD	BLQ
GW1	1/6/2015	BLD	BLQ	BLD	BLD	BLQ	BLD
GW1	1/8/2015	BLD	BLD	BLD	BLD	BLQ	BLD
GW1	1/12/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	1/13/2015	BLD	BLD	BLQ	BLD	BLD	BLD
GW1	1/21/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	1/22/2015	BLD	BLD	BLD	BLD	BLQ	BLD

GW1	1/26/2015	BLD	BLD	BLD	BLD	BLQ	2.50
GW1	1/28/2015	BLQ	BLQ	BLD	BLD	BLQ	2.14
GW1	2/4/2015	BLD	BLD	BLD	BLD	BLQ	BLD
GW1	2/5/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	2/9/2015	BLD	BLD	BLD	BLD	BLD	BLQ
GW1	2/11/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	2/17/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	2/19/2015	BLD	BLD	BLD	BLD	BLD	BLQ
GW1	2/23/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	2/25/2015	BLD	BLD	BLD	BLD	BLD	BLQ
GW1	3/3/2015	BLD	2.83	BLD	BLD	BLQ	BLQ
GW1	3/4/2015	BLQ	3.00	BLD	BLD	BLD	BLQ
GW1	3/10/2015	BLQ	BLD	BLD	BLD	BLQ	BLD
GW1	3/12/2015	BLQ	2.05	BLD	BLQ	BLQ	BLQ
GW1	3/16/2015	4.09	3.79	BLD	BLQ	BLD	BLQ
GW1	3/18/2015	BLQ	3.12	BLD	BLD	BLD	BLD
GW1	3/24/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	3/25/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	3/31/2015	BLQ	BLQ	BLD	BLD	BLD	2.15
GW1	4/2/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	4/6/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	4/8/2015	BLD	BLQ	BLD	BLD	BLD	BLD
GW1	4/15/2015	BLD	BLD	BLD	BLQ	BLD	BLD
GW1	4/20/2015	BLD	BLD	BLD	BLD	BLD	BLD
GW1	4/22/2015	BLQ	BLQ	BLD	BLD	BLD	BLD
GW2	2/22/2016	BLD	BLQ	BLD	BLD	BLD	BLD
GW2	2/24/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	2/29/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	3/7/2016	BLQ	2.71	BLD	BLD	BLQ	BLD
GW2	3/14/2016	BLD	2.03	BLD	BLQ	BLQ	BLD
GW2	3/16/2016	BLD	BLD	BLD	BLD	BLQ	BLQ
GW2	3/30/2016	BLD	BLD	BLD	BLD	BLQ	BLQ
GW2	4/4/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	4/6/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	4/11/2016	BLD	BLQ	BLD	BLD	BLD	BLD
GW2	4/13/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	4/18/2016	BLD	BLD	BLD	BLD	BLD	BLD
GW2	4/20/2016	BLD	BLD	BLD	BLD	BLQ	BLD
GW2	4/25/2016	BLD	BLD	BLD	BLD	BLQ	BLD
GW2	4/27/2016	BLD	BLD	BLD	BLD	BLQ	BLD
GW2	5/2/2016	BLD	BLD	BLD	BLD	BLD	BLQ
GW2	5/4/2016	BLD	BLD	BLD	BLD	BLD	BLD