

Supplementary Information

Table S1: Cohort Demographic Data

	Number (N = 48)	Percent
Sex		
Male	21	44
Female	27	56
Age		
1-17	8	17
18-54	32	67
55-70	8	17
Race/Ethnicity		
White	18	38
Asian	13	27
Hispanic/Latino	12	25
Black/African American	2	4
American Indian or Alaska Native	1	2
Pacific Islander/Native Hawaiian	1	2
Declined to Answer	1	2

Table S2: Individual target gene ddPCR operating parameters and primer and probe sequences

Target Gene	Primer Sequence	Cycling Conditions	Amplicon Size	Reference
SARS-CoV-2 N	Fw: CATTACGTTTGGTGGACCCT Rv: CCTTGCCATGTTGAGTGAGA Probe: CGCGATCAAAACAACGTCGG 5'FAM/ZEN/3'IBFQ	50C - 60min 95C - 5min [95C - 30s 56C - 1min] x40 98C - 10min 4C - Hold	143 bp	Wolfe et al. 2021

SARS-CoV 2 ORF1a	Fw: CAGAACTGGAACACCTTGT Rv: TACAGTTGAATTGGCAGGCA Probe: TGCCACAGTACGTCTACAAGC 5'HEX/ZEN/3'IBFQ	50C - 60min 95C - 5min [95C - 30s 56C - 1min] x40 98C - 10min 4C - Hold	179 bp	Wolfe et al. 2021
BCoV	Fw: CTGGAAGTTGGTGGAGTT Rv: ATTATCGGCCTAACATACATC Probe: CCTTCATATCTATACATCAAGTTGTT 5'FAM/ZEN/3'IBFQ	50C - 60min 95C - 5min [95C - 30s 56C - 1min] x40 98C - 10min 4C - Hold	85 bp	Wolfe et al. 2021
PMMoV	Fw: GAGTGGTTTGACCTTAACGTTTGA Rv: TTGTCGGTTGCAATGCAAGT Probe: CCTACCGAAGCAAATG 5'HEX/ZEN/3'IBFQ	50C - 60min 95C - 5min [95C - 30s 56C - 1min] x40 98C - 10min 4C - Hold	68 bp	Wolfe et al. 2021
CrAssphage CPQ064	Fw: TGTATAGATGCTGCTGCAACTGTACTC Rv: CGTTGTTTTCATCTTTATCTTGCCAT Probe: CTGAAATTGTTTATAAGCAA 5'FAM/ZEN/3'IBFQ	95C - 5 min [95C - 30s 56C - 1min] x40 98C - 10min 4C - Hold	126 bp	Stachler et al. 2017

Table S3: Estimates derived from logistic regression of SARS-CoV-2 fecal shedding prevalence for an outcome variable of log10(gc/mg-dw)

Random Effects					
Groups	Name	Variance	Std. Dev		
ID	(Intercept)	13.24	3.638		
Fixed Effects					
Name	Type	Estimate	Std. Error	z-value	p-value
Intercept		2.81747	0.94748	2.974	0.00294
Day after symptom onset	Continuous	-0.22399	0.03611	-6.204	5.52e-10
Sex = Male	Binary	0.14498	1.22669	0.118	0.90592

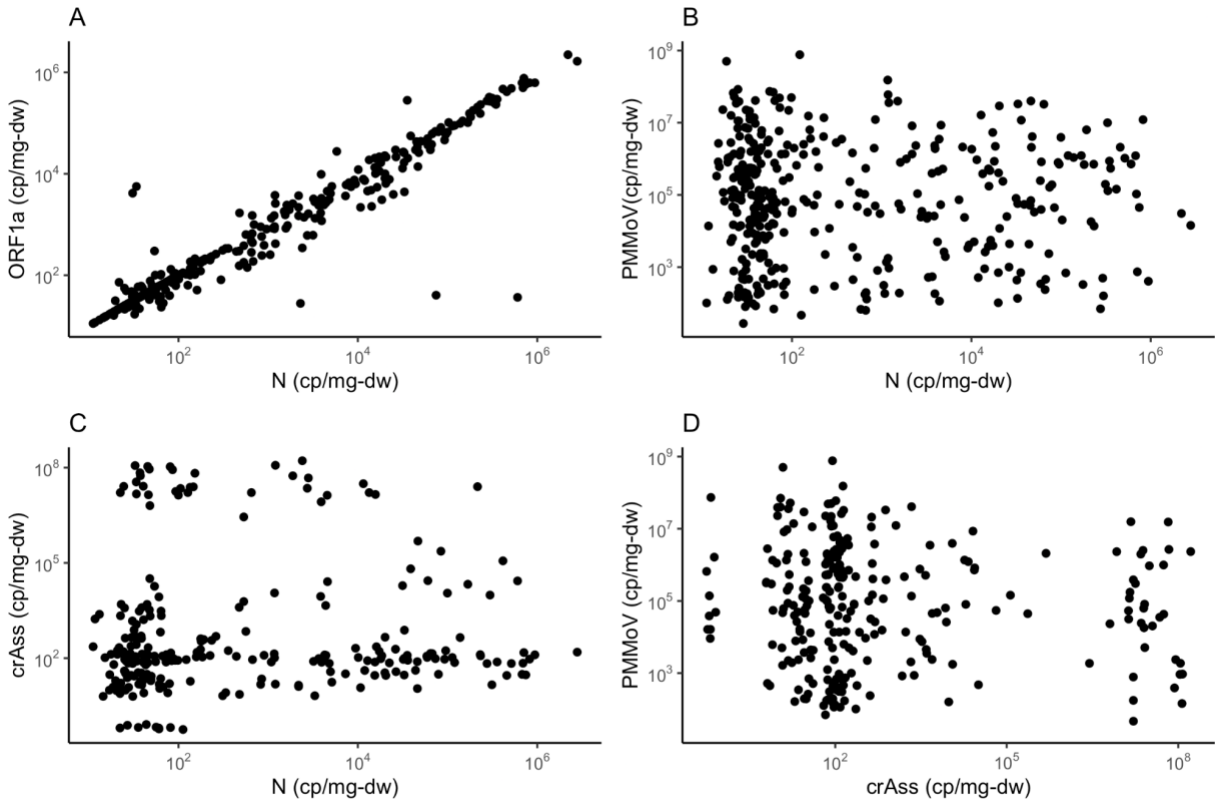
Table S4: Estimates derived from linear regression of concentrations of PMMoV and crAssphage fecal shedding for an outcome variable of log10(gc/mg-dw).

crAssphage						
Random Effects						
Groups	Name	Variance	Std. Dev			
ID	(Intercept)	4.6816	2.1637			
Residual		0.4219	0.6495			
Fixed Effects						
Name	Type	Estimate	Std. Error	df	t-value	p-value
Intercept		2.70163	0.44172	55.18068	6.116	1.03e-07
Sex = Male	Binary	-0.51143	0.63581	45.44786	-0.804	0.425
Positive SARS-CoV-2 Fecal Shedding	Binary	0.09748	0.10196	318.64308	0.956	0.340
log10(PMMoV_conc	Continuous	0.02513	0.02587	312.35886	0.971	0.332
PMMoV						
Random Effects						
Groups	Name	Variance	Std. Dev			
ID	(Intercept)	0.6787	0.8238			
Residual		2.0021	1.4149			
Fixed Effects						
Name	Type	Estimate	Std. Error	df	t-value	p-value

Intercept		4.78069	0.27345	64.19427	17.483	<2e-16
Sex = Male	Binary	-0.02717	0.29561	45.30766	-0.092	0.927
Positive SARS-CoV-2 Fecal Shedding	Binary	0.07691	0.19406	313.24624	0.396	0.692
log10(crAss_conc)	Continuous	0.00974	0.06179	88.00628	0.158	0.875

Equation S1: Calculation converting the ddPCR generated concentration of gc/uL extract to gc/mg-dw.

$$\begin{aligned}
 & \frac{\text{Gene Copies}}{\text{mg dry weight stool}} \\
 &= \frac{\text{Gene Copies}}{\mu\text{L Reaction}} \times \frac{22 \mu\text{L Reaction}}{5.5 \mu\text{L RNA Extract}} \times \frac{100 \mu\text{L RNA Extract}}{300 \mu\text{L Homogenate}} \\
 & \times \frac{1200 \mu\text{L Homogenate}}{\text{mg Stool}} \times \frac{\text{mg stool}}{\text{mg dry weight stool}}
 \end{aligned}$$



S1. Plots showing correlations between the different gene targets investigated in this study. A) SARS-CoV-2 N vs ORF1a, B) N vs PMMoV, C) N vs. crAss, D) crAss vs PMMoV

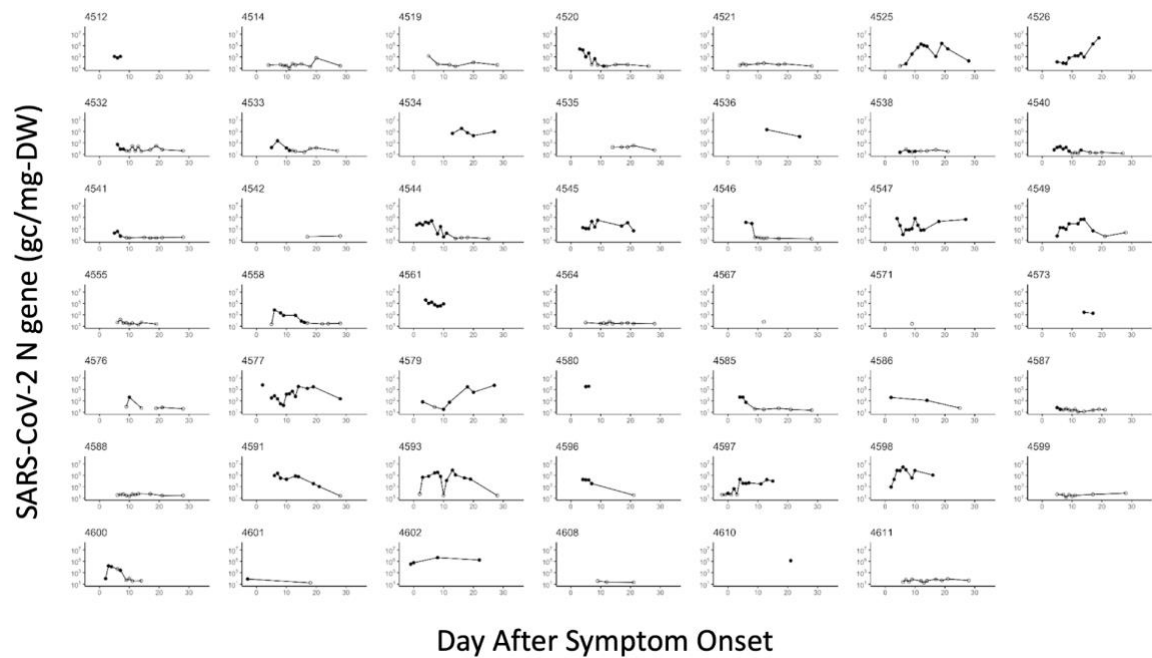


Figure S2. Individual SARS-CoV-2 Trajectories

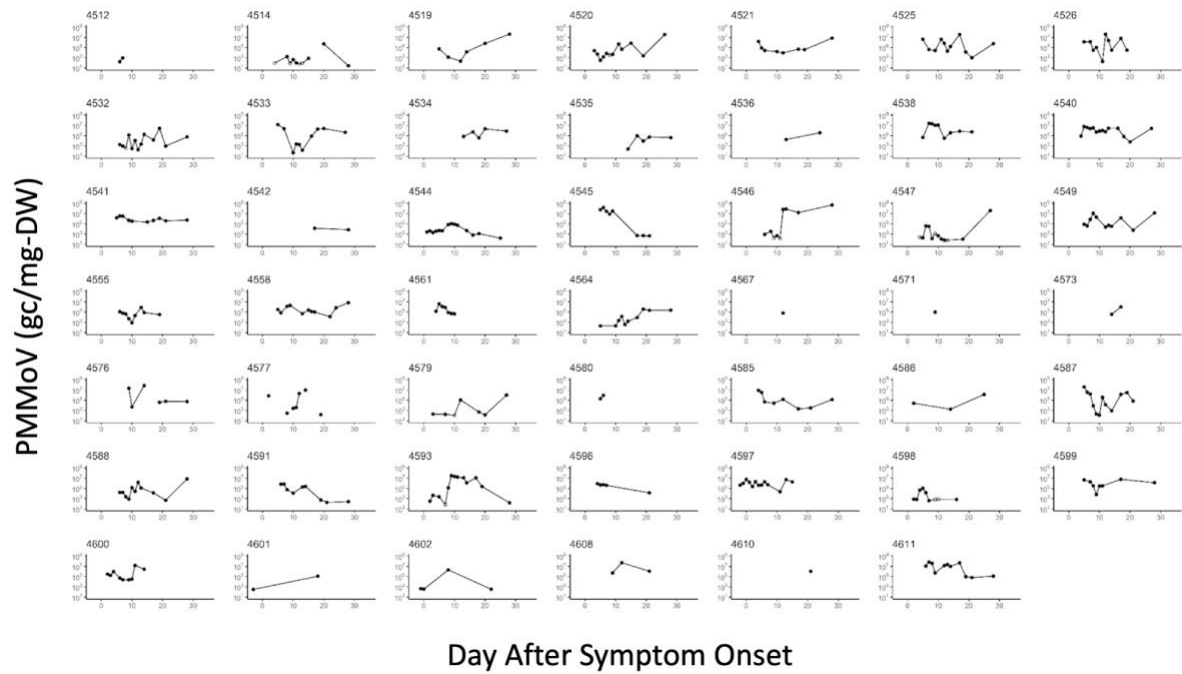


Figure S3: Individual PMMoV shedding trajectories

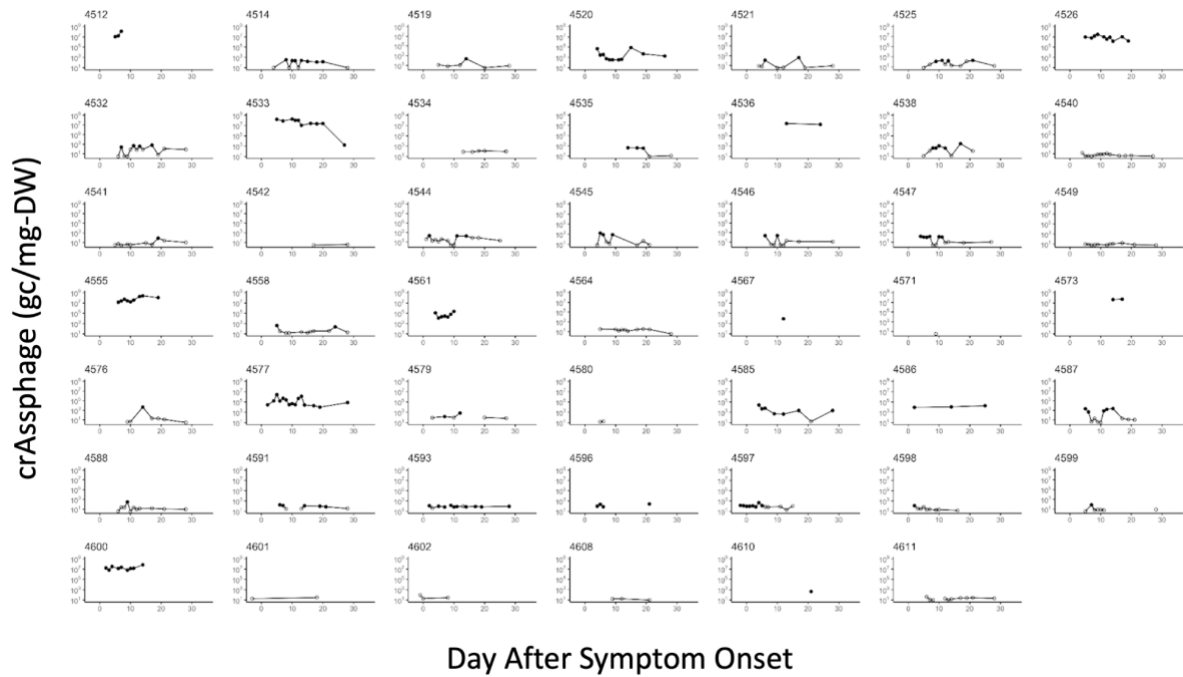
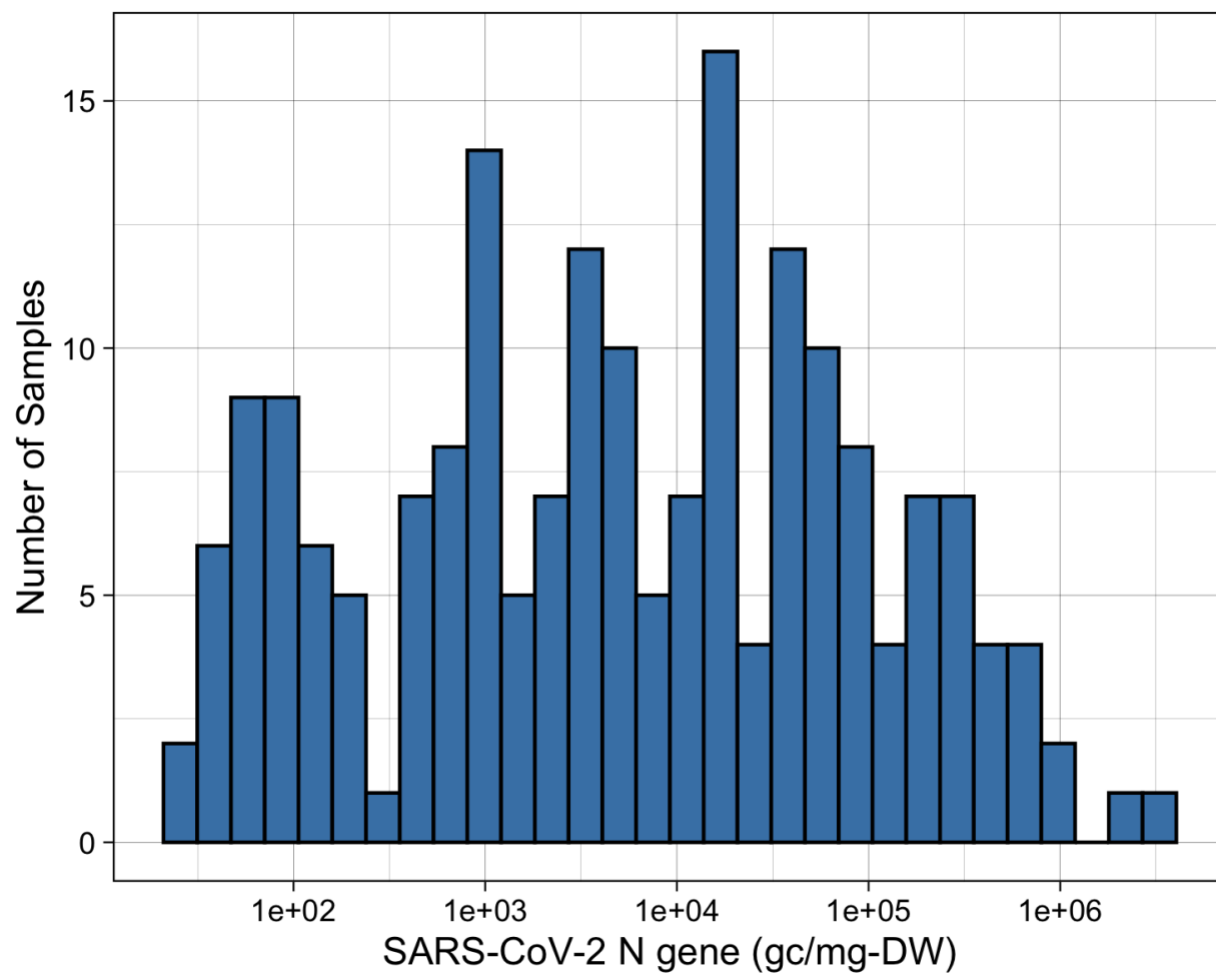


Figure S4. Individual crAssphage shedding trajectories



S5: Histogram showing the distribution of all above-LOB SARS-CoV-2 nucleocapsid gene measurements.

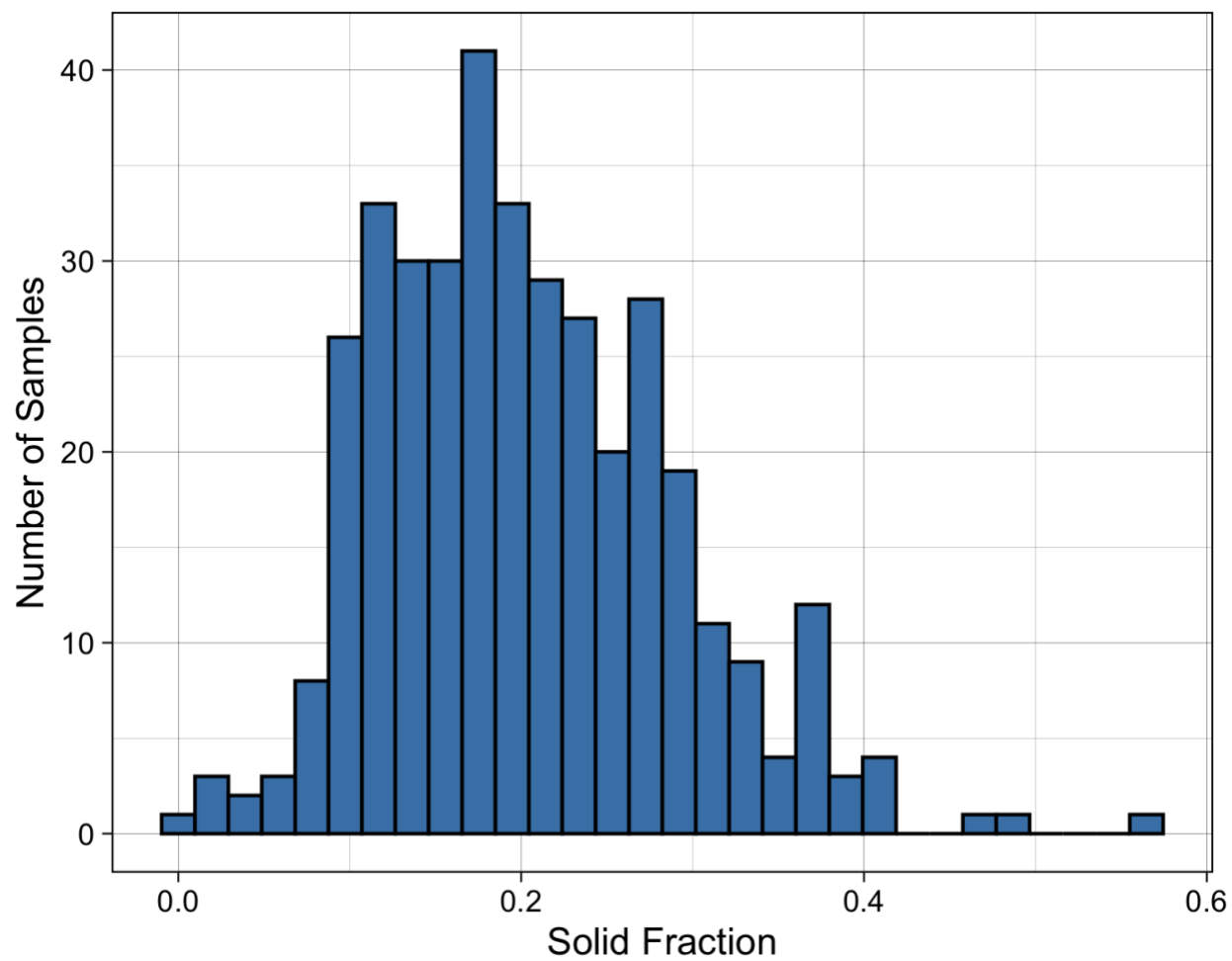


Figure S6: Histogram of the distribution of the solid fraction of all stool samples measured.

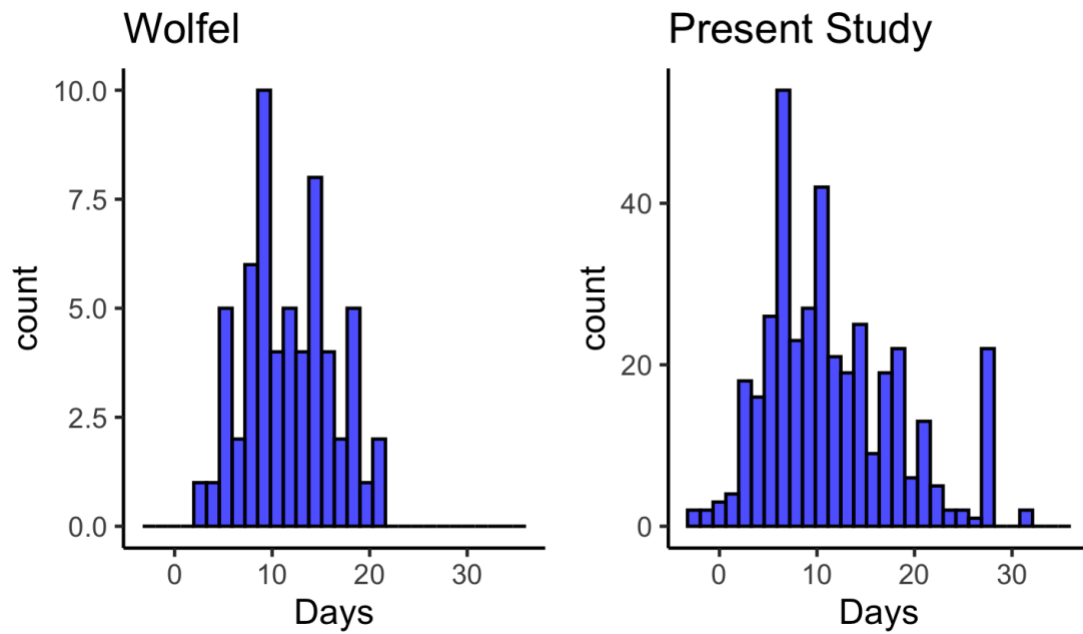


Figure S7: Histogram comparing the sampling coverage (post symptom onset) between Wölfel et al 2020, and the present study.

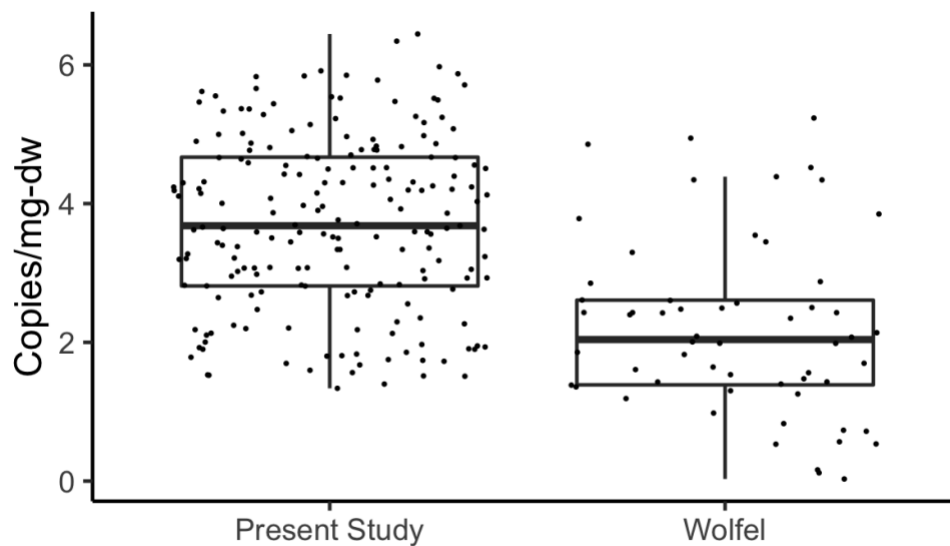


Figure S8: Comparison of the magnitudes of all positive measurements collected from this study with those collected in Wölfel et al 2020, assuming 20% dry mass samples.

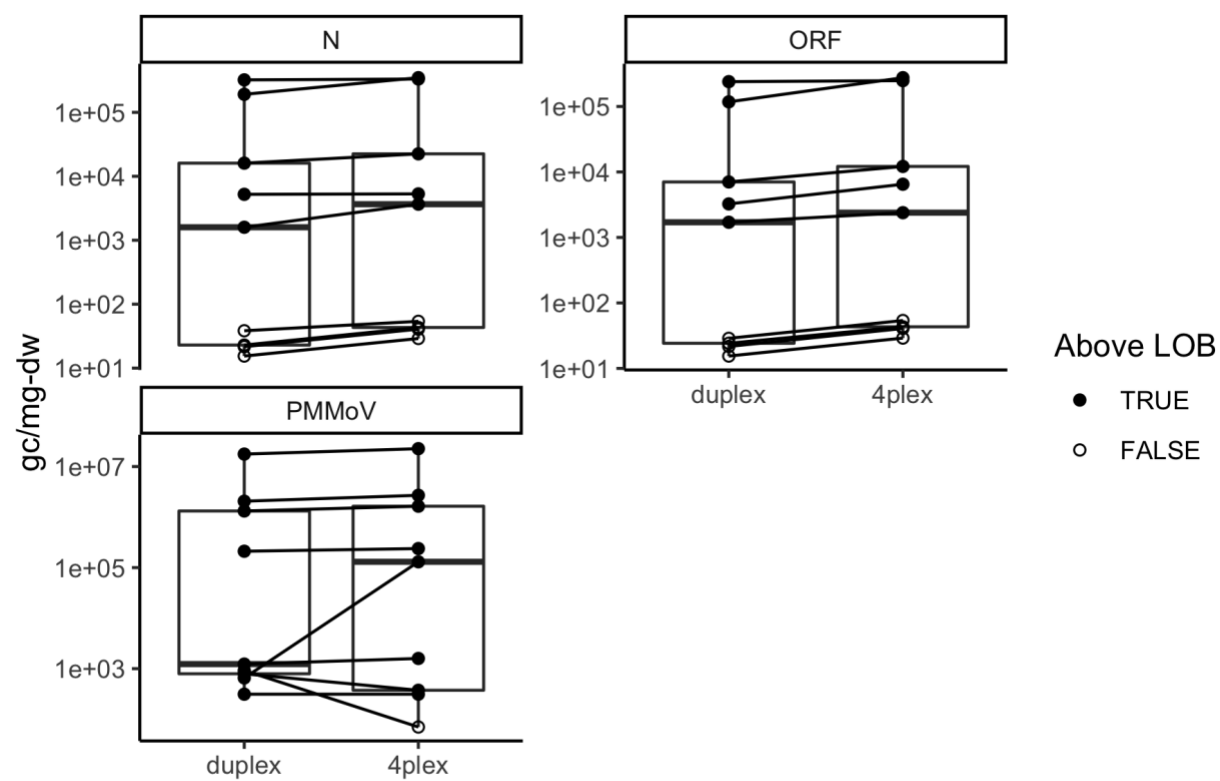


Figure S9: Side-by-side comparison of the RT-ddPCR 4plex assay with two duplex assays for the targets of interest.

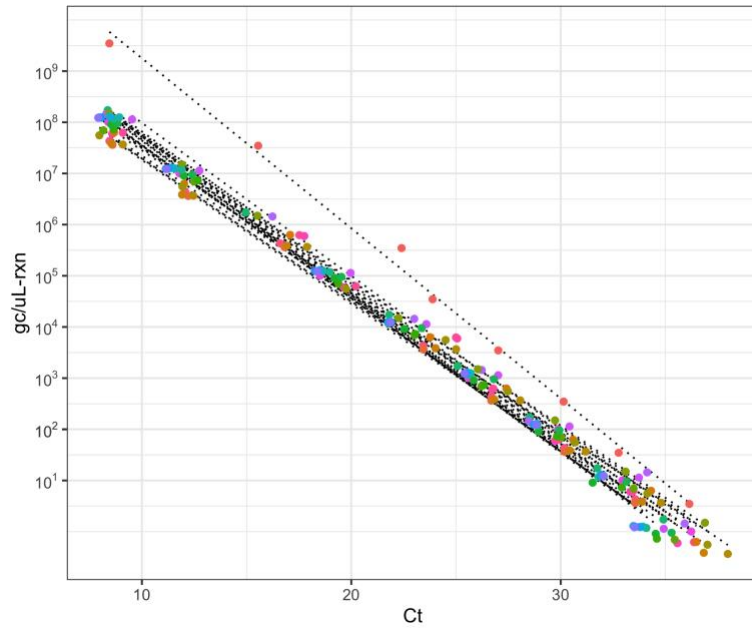


Figure S10: Standard curves correlating the Ct of crAssphage gene fragment dilutions as measured by qPCR to gene copies per microliter of reaction, measured by ddPCR.

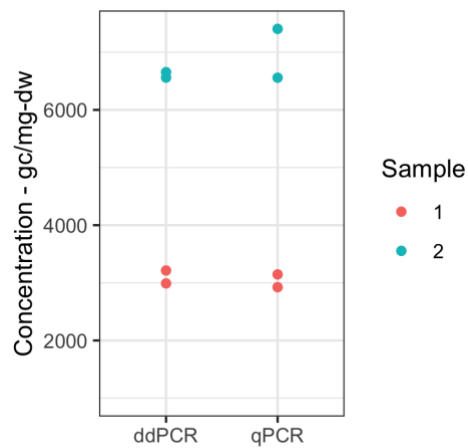


Figure S11: Comparison of quantified concentration of crAssphage positive stool samples from ddPCR vs qPCR with a ddPCR quantified standard curve. For each sample, extraction duplicates were measured using each method.

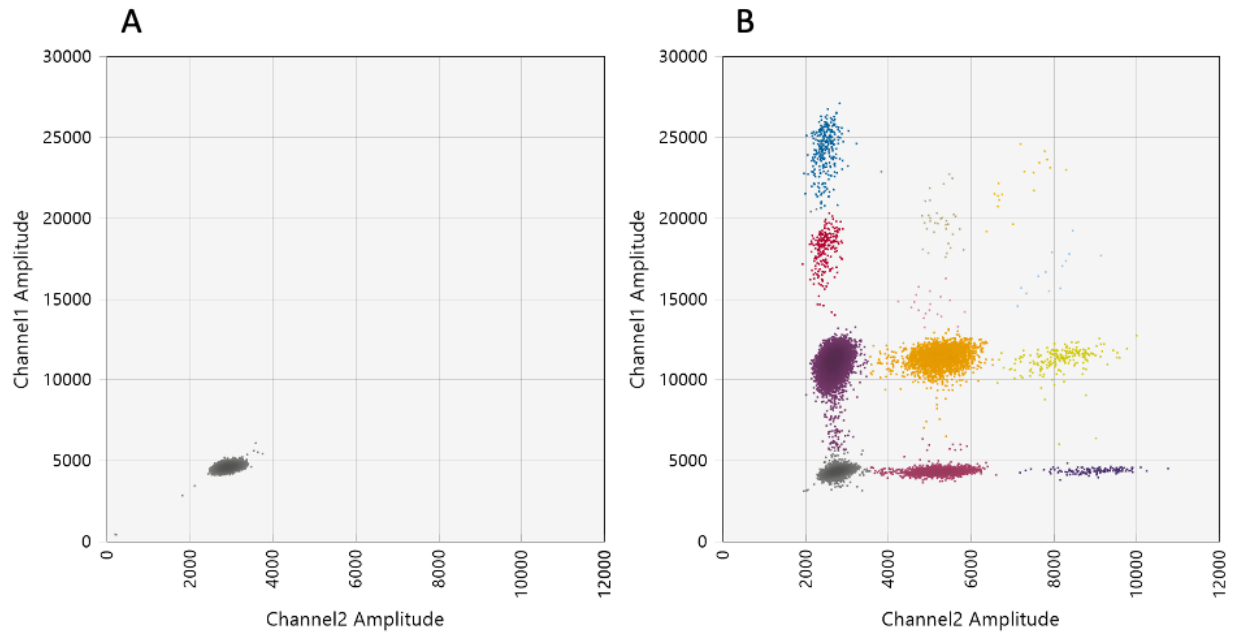


Figure S12: Examples of negative (A), and positive (B) samples measured by the 4 multiplexed ddPCR assay. The positive example exhibits a sample that was positive for all 4 targets.