

## Supporting Information

### Amplitude Multiplexed Wastewater Surveillance for Campus Health:

### Tracking SARS-CoV-2, Influenza A, and Norovirus

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23 **Environmental Microbiology Minimum Information (EMMI)(1) checklist:**

24 • **Environmental Sampling**

- 25 ○ Sampling procedure: section 2.2
- 26 ○ Number of samples: section 3.2
- 27 ○ Sample amount, mean, range: SI Table 8
- 28 ○ Sampling locations, dates: SI Table 9

29 • **Sample Treatment**

- 30 ○ Performed: section 2.3
- 31 ○ Treatment procedure: section 2.3
- 32 ○ Reagents: section 2.3

33 • **Sample Reduction**

- 34 ○ Performed: section 2.3
- 35 ○ Reduction procedure: section 2.3
- 36 ○ Reagents: section 2.3
- 37 ○ Concentration factor: NA

38 • **Nucleic Acid Extraction**

- 39 ○ Extraction procedure: section 2.3
- 40 ○ Amount extracted; amount obtained: section 2.3
- 41 ○ Extract storage conditions: -20°C

42 • **Reverse Transcription**

- 43 ○ One or two-step: two-step
- 44 ○ cDNA storage conditions: -20°C
- 45 ○ Reaction temperatures and times: SI Table 3
- 46 ○ Reaction reagents and concentrations: SI Table 3
- 47 ○ Priming method: SI Table 3 and section 2.3
- 48 ○ Reaction volume; added template amount: SI Table 3

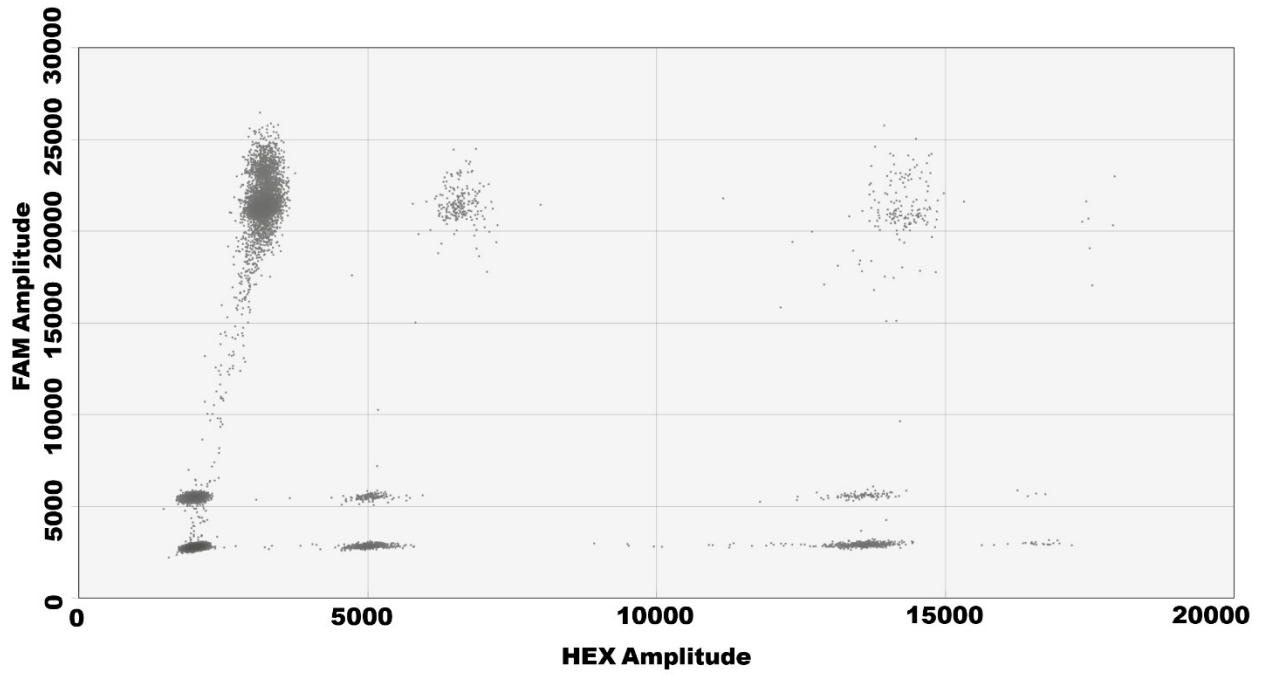
49 • **dPCR**

- 50 ○ Target gene name, amplicon length: SI Table 2
- 51 ○ Thermocycling temperature and times: SI Table 4
- 52 ○ Master mix: composition, vendors, concentrations: SI Table 4 and section 2.3
- 53 ○ Additives: vendors, concentrations: NA
- 54 ○ Template amount added, pre-treatment (if any): SI Table 4 and section 2.3
- 55 ○ Primers and probes—sequences, concentrations, vendors, references: SI Table
- 56 4
- 57 ○ Instrumentation: section 2.3
- 58 ○ Equivalent volume of sample analyzed by PCR: ~2 mL

59 • **Analysis—dPCR**

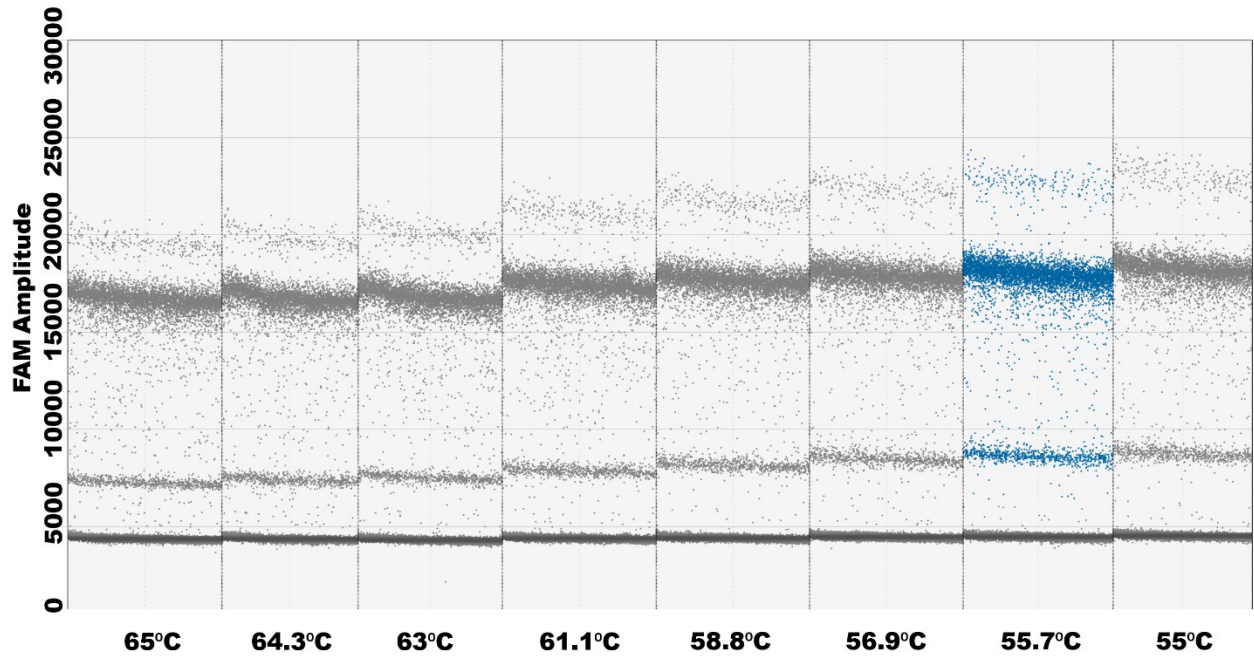
- 60 ○ Threshold settings: SI Table 7
- 61 ○ Technical replicates, number, well merging: section 2.3
- 62 ○ Partitions measured, number, mean, variance: Not analyzed
- 63 ○ Partition volume: Not analyzed
- 64 ○ Target copies per partition, mean, variance: Not analyzed
- 65 ○ Program used for dPCR analysis: Section 2.3
- 66 ○ Explanation of control results, example plots: Figure 2 and SI Figure 5

67 **SI Figure 1: Graphical output of amplitude multiplex with FAM and HEX at 20x and 10x**  
68 **probe concentrations.**

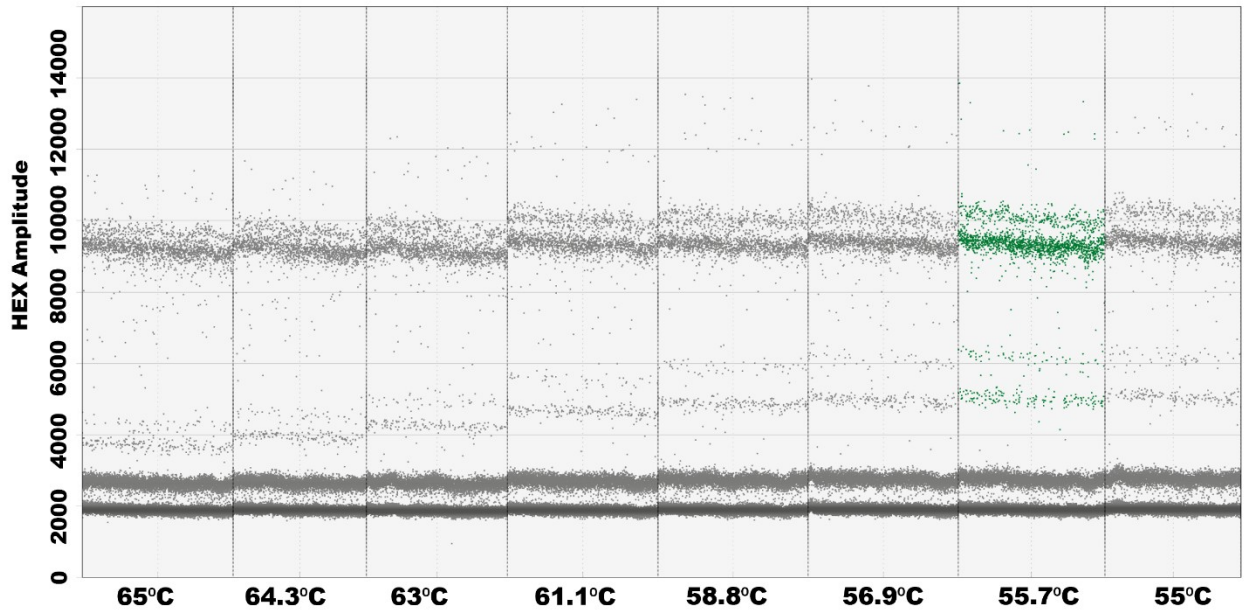


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85 SI Figure 2: Temperature gradient of amplitude multiplex with FAM and HEX at 20x and  
86 12.5x probe concentrations. The selected temperature for use is highlighted.



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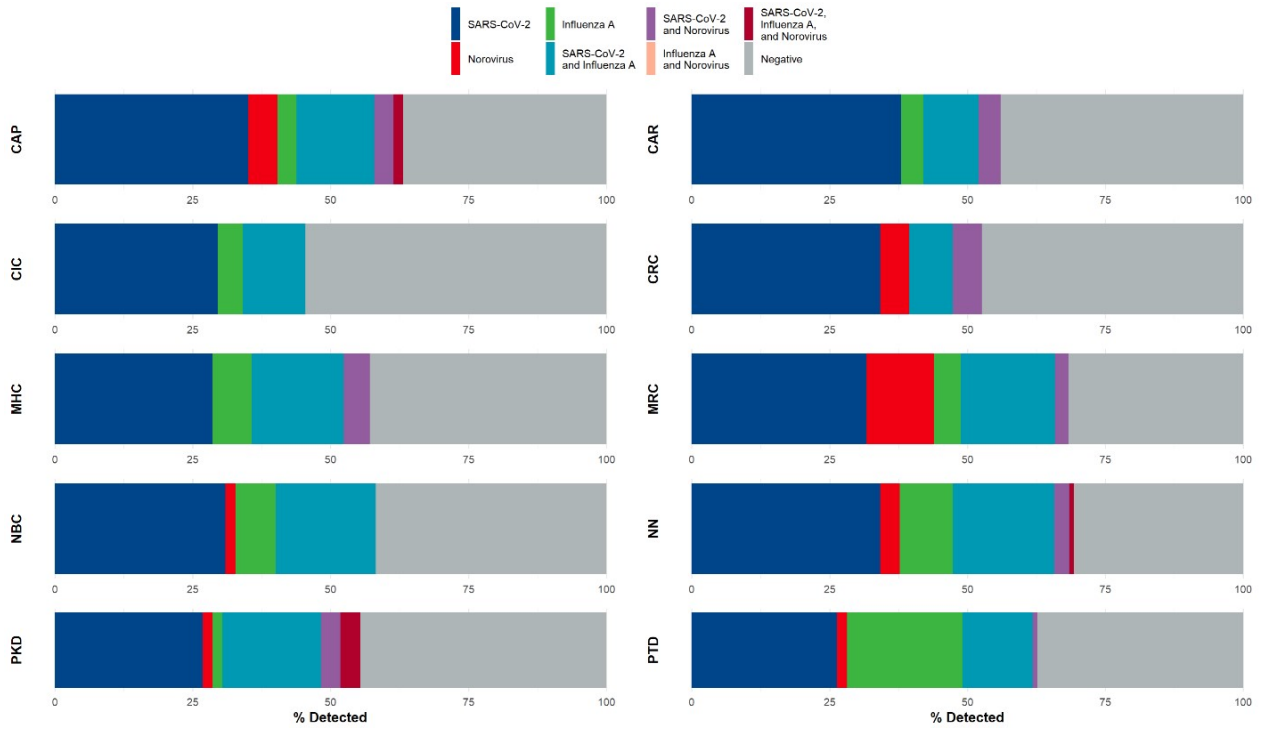
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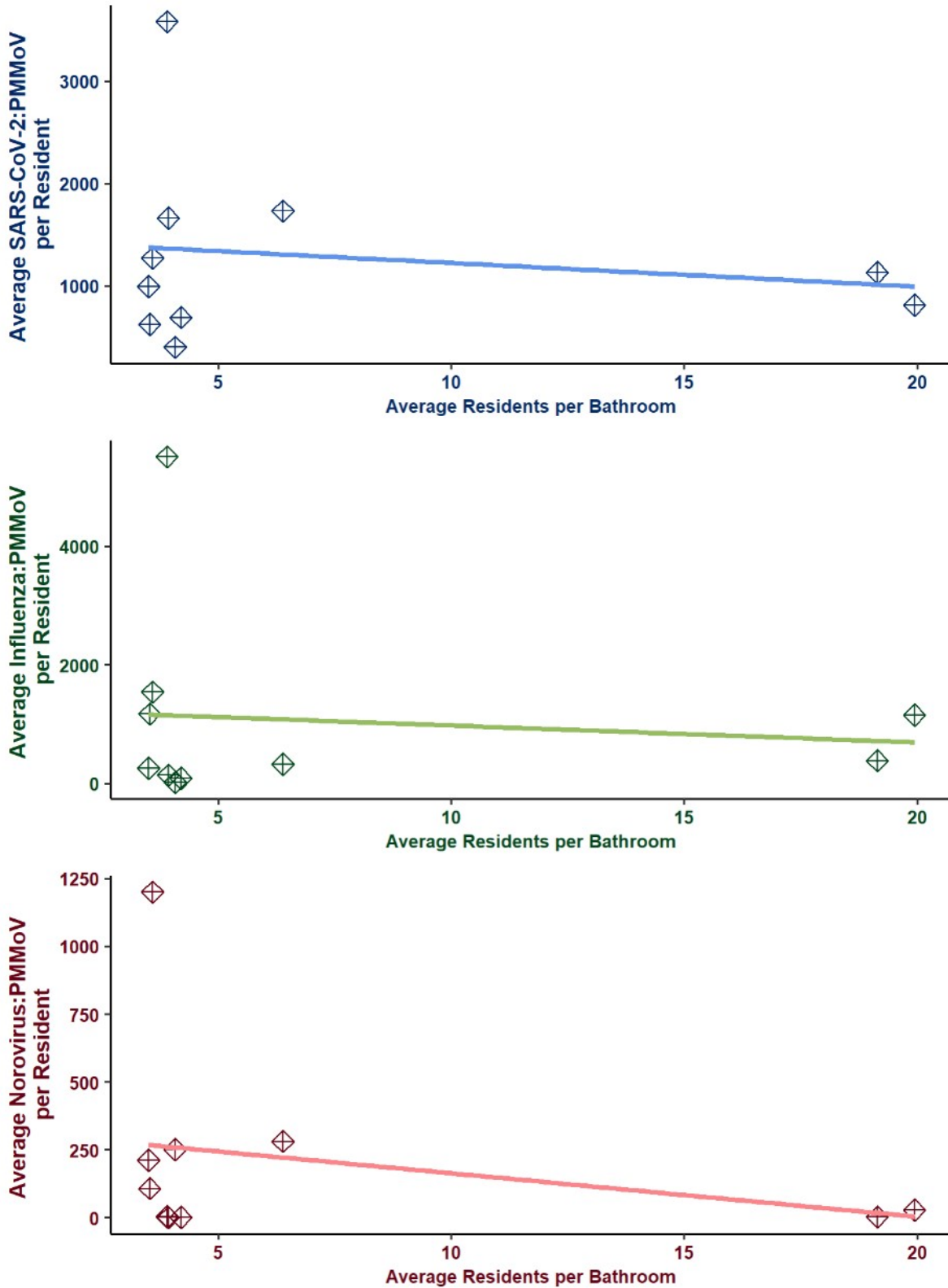
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93 **SI Figure 3: Every monitored building's result described as percentage of the total**  
 94 **samples collected at each site.**



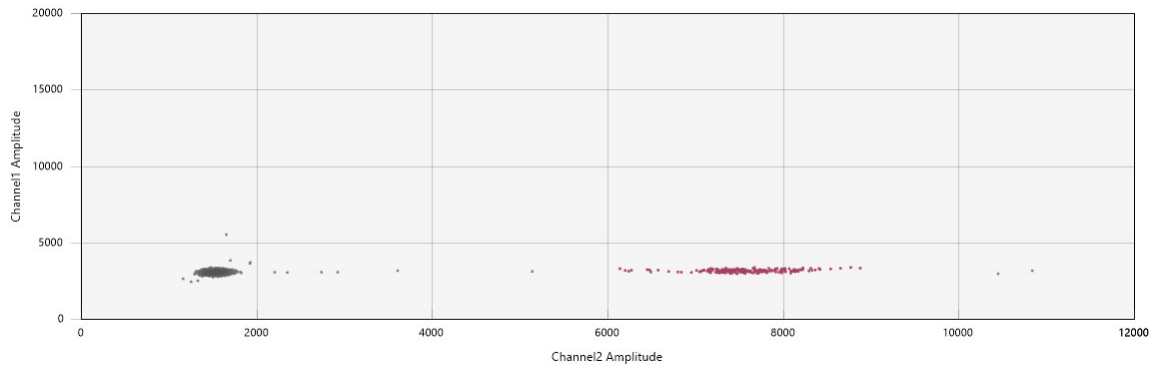
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111 **SI Figure 4: No correlation is found when comparing the average number of residents per**  
112 **bathroom against the average viral load per resident in each building.**



114 **SI Figure 5: Possible 2D graphical outcomes from amplitude multiplex. Text above each**  
115 **image describes the positives and fluorophore designation.**

116 PMMoV (HEX-Lo)



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118 SARS-CoV-2 (FAM-Hi)

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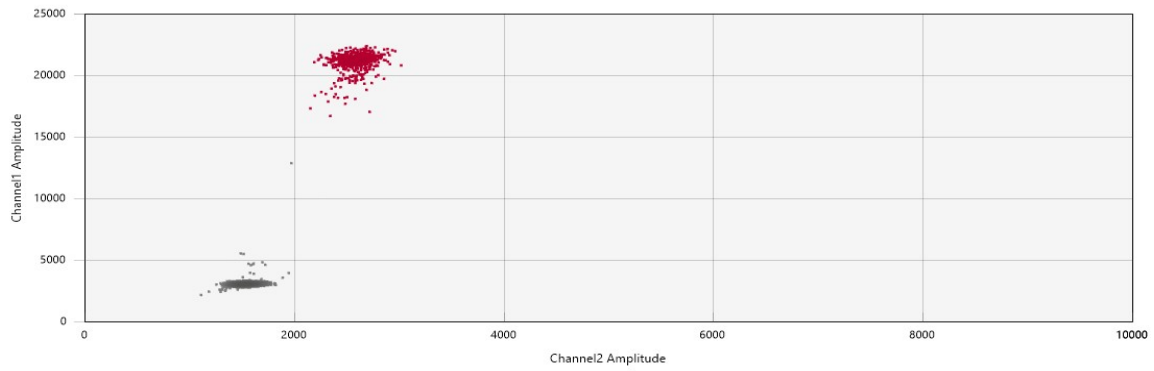
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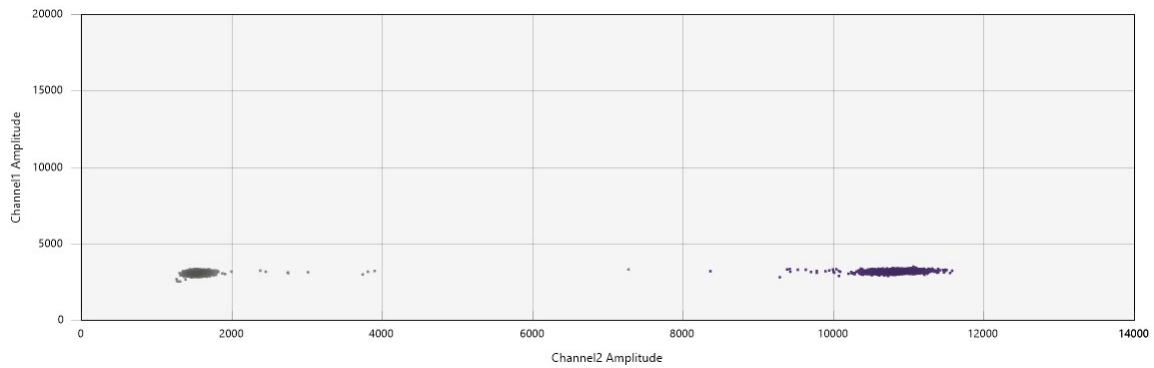
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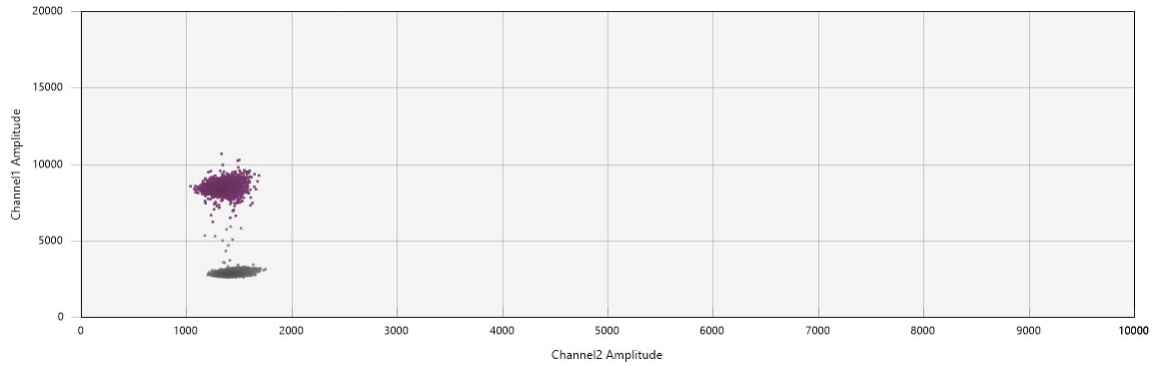
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126 Influenza A (HEX-Hi)



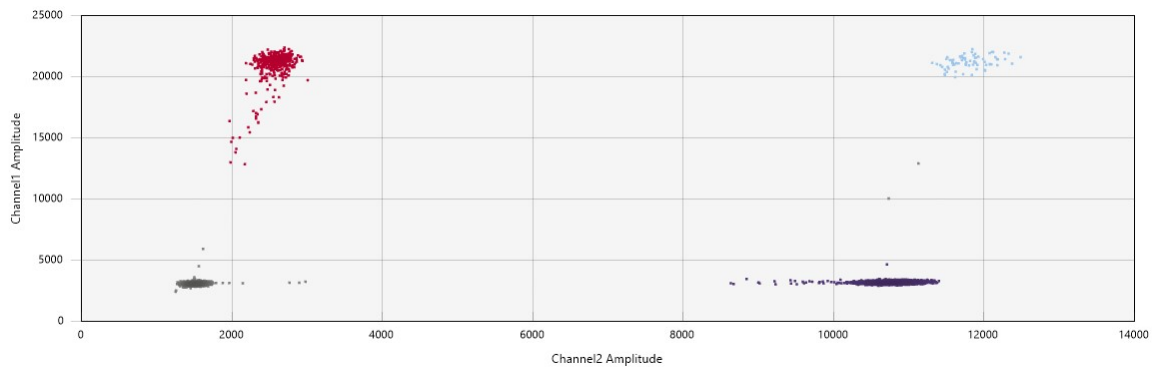
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128 Norovirus (FAM-Lo)



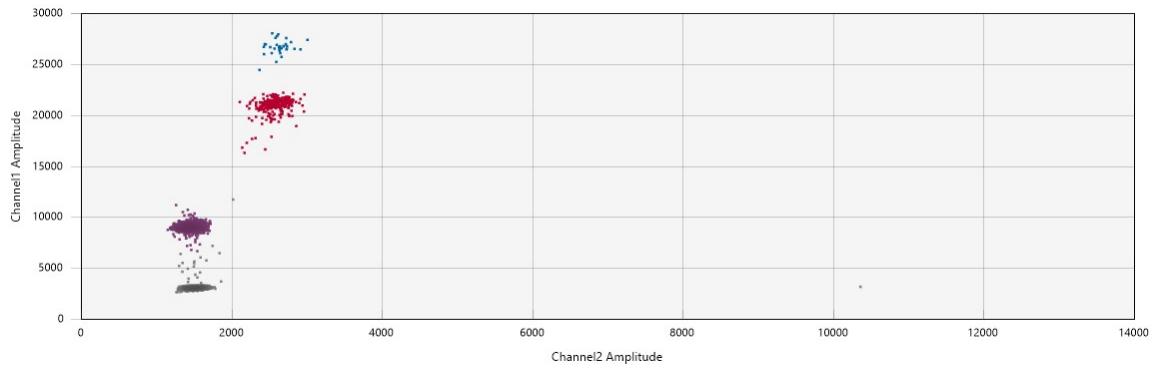
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130 SARS-CoV-2 (FAM-Hi), influenza A (HEX-Hi)



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132 SARS-CoV-2 (FAM-Hi), norovirus (FAM-Lo)



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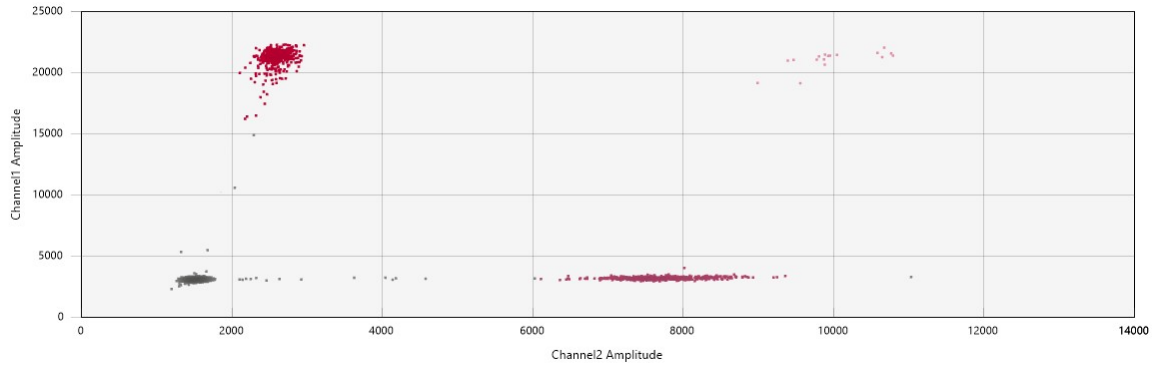
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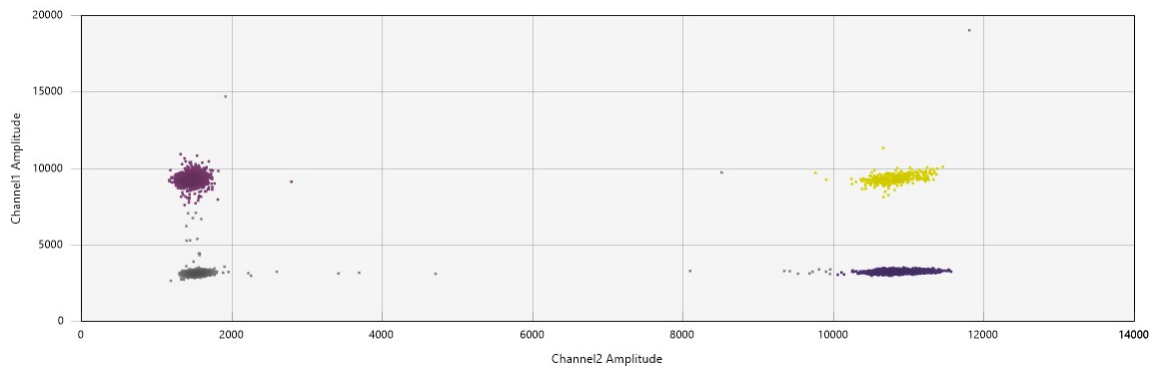


140 SARS-CoV-2 (FAM-Hi), PMMoV (HEX-Lo)



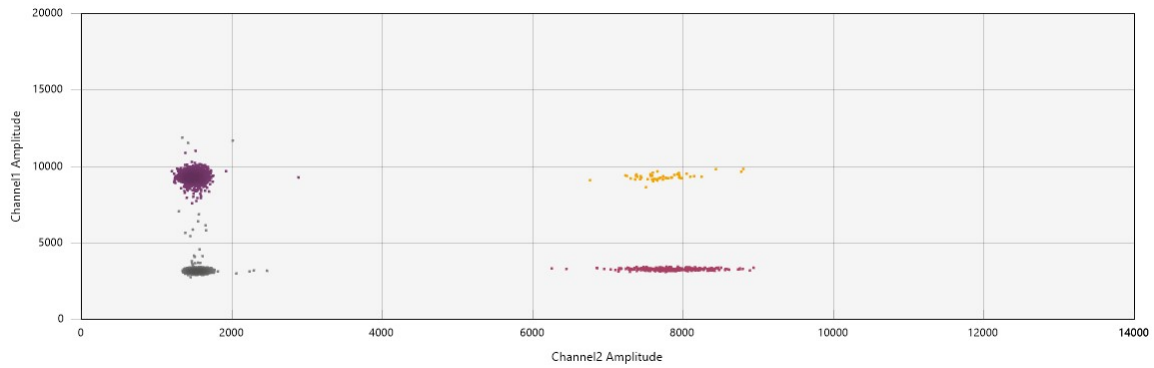
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142 Norovirus (FAM-Lo), influenza A (HEX-Hi)



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144 Norovirus (FAM-Lo), PMMoV (HEX-Lo)



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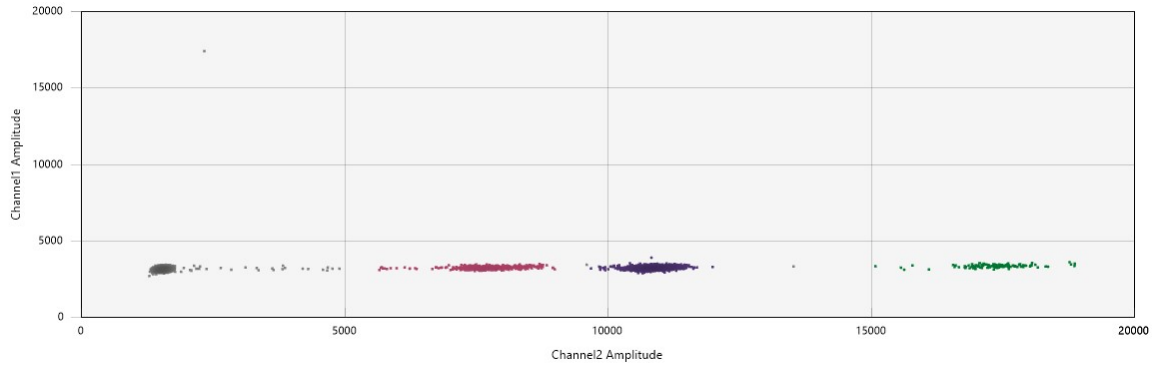
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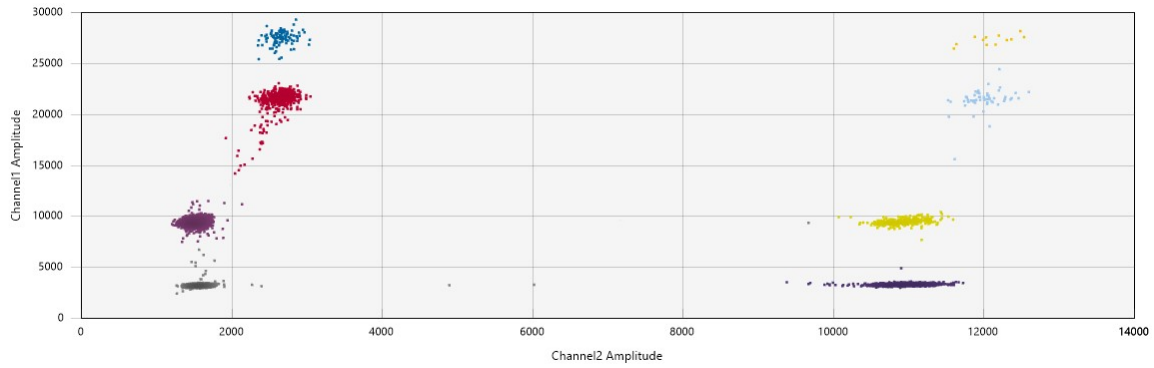
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152 Influenza A (HEX-Hi), PMMoV (HEX-Lo)



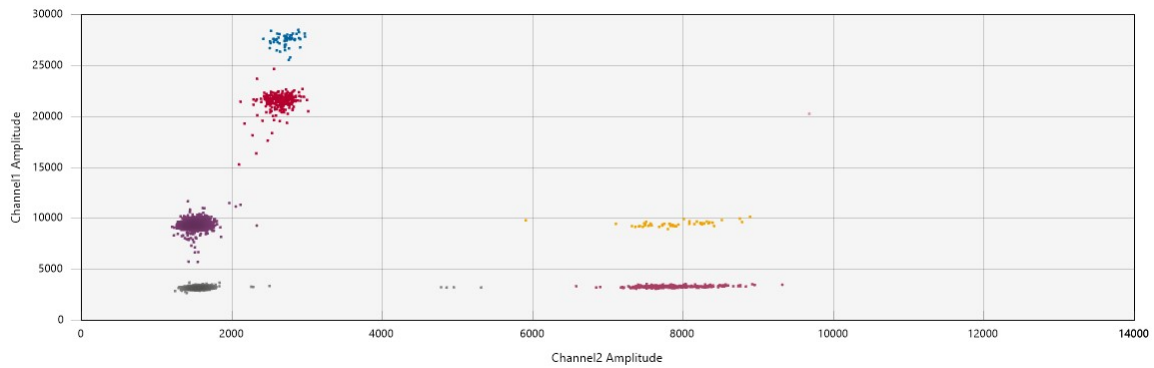
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154 SARS-CoV-2 (FAM-Hi), norovirus (FAM-Lo), influenza A (HEX-Hi)



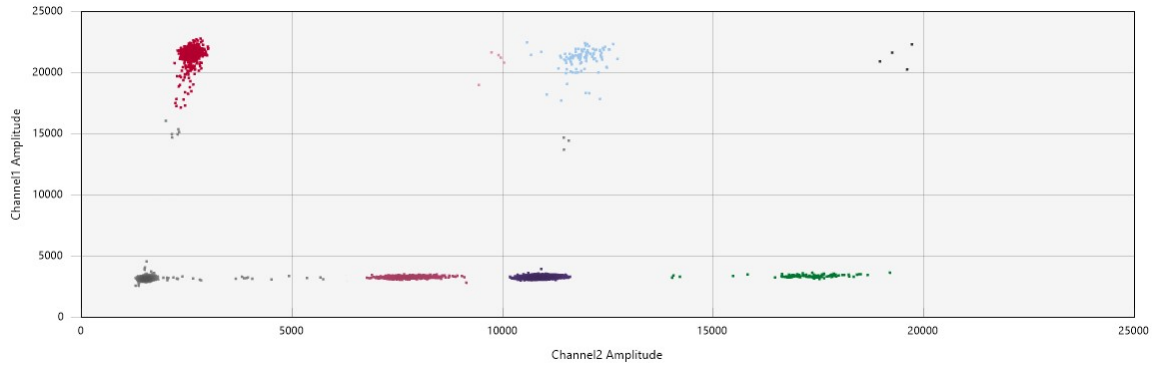
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156 SARS-CoV-2 (FAM-Hi), norovirus (FAM-Lo), PMMoV (HEX-Lo)



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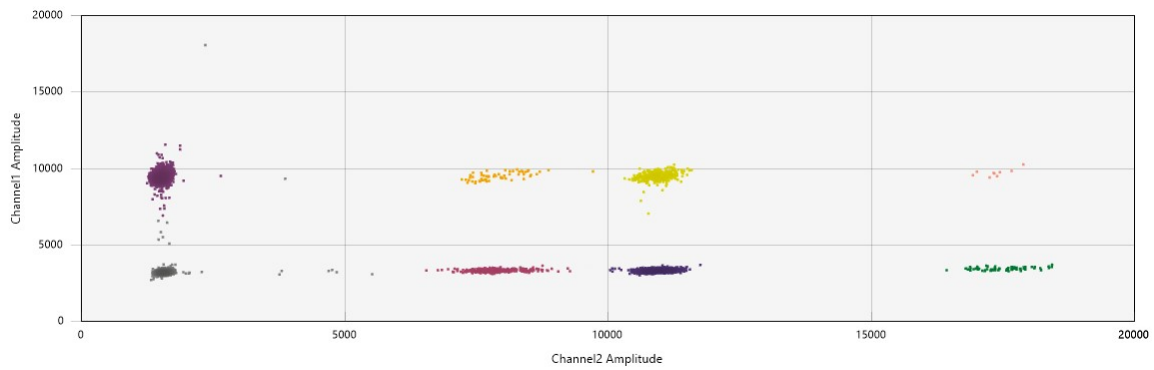
158 SARS-CoV-2 (FAM-Hi), norovirus (FAM-Lo), influenza A (HEX-Hi)



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Norovir

160 us (FAM-Lo), influenza A (HEX-Hi), PMMoV (HEX-Lo)



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176 **SI Table 1: Naming and abbreviation of each building along with the associated sampling**  
 177 **frequency.**

<i>Building Name</i>	<i>Building Abbreviation</i>	<i>Building Type</i>	<i>Days Sampled</i>
<i>New North Residential College</i>	NN	Dormitory	M, T, W, Th, F
<i>Pardee/Mark Tower</i>	PTD	Dormitory	M, T, W, Th, F
<i>McCarthy Honors Residential College</i>	MHC	Dormitory	M, W
<i>Cowlings and Ilium Residential College</i>	CRC	Dormitory	M, W
<i>Cale and Irani Residential College</i>	CIC	Dormitory	M, W
<i>Nemirovsky and Bohnett Residential College</i>	NBC	Dormitory	M, W
<i>McMorrow Residential College</i>	MRC	Dormitory	M, W
<i>Cardinal Gardens</i>	CAR	Apartments	T, Th
<i>Century Apartments</i>	CAP	Apartments	T, Th
<i>Parkside Complex</i>	PKD	Dormitory	T, Th

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179 **SI Table 2: Primer and probe composition for ddPCR**

<i>AMPLICON</i>	<i>FORWARD PRIMER</i>	<i>REVERSE PRIMER</i>	<i>SIZE</i>	<i>PROBE SEQUENCE</i>	<i>PRIMER:PROBE CONCENTRATION (nm)</i>	<i>REFERENCE</i>
<i>SARS-CoV-2_N1</i>	5'-/GAC CCC AAA ATC AGC GAA AT/-3'	5'-/TCT GGT TAC TGC CAG TTG AAT CTG/-3'	73	5'-/FAM/ACC CCG CAT/ZEN/TAC GTT TGG TGG ACC/IABkFQ/-3'	900:250	(2)
<i>Influenza-A_M</i>	5'-/CAA GAC CAA TCY TGT CAC CTC TGA C/-3'	5'-/GCA TTY TGG ACA AAV CGT CTA CG/-3'	106	5'-/HEX/TG CAG TCC T/ZEN/CG CTC ACT GGG CAC G/IABkFQ/-3'	900:250	(3)
<i>Norovirus-GI/GII_ORF2</i>	5'-/CGC TGG ATG CGN TTC CAT/-3'	5'-/CCT TAG ACG CCA TCA TCA TTT AC/-3'	86	5'-/FAM/TGG ACA GGA/ZEN/GAY CGC RAT CT/IABkFQ/-3'	562.5:156.25	(4)

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5'-/GAG TGG  
 TTT GAC CTT  
 AAC GTT TGA/-  
 3'

5'-/TTG TCG GTT  
 GCA ATG CAA  
 GT/-3'

5'-/HEX/CCT ACC  
 203 GAA/ZEN/GCA AAT  
 G /IABKFQ/-3'

PMMoV 562.5:156.25 (5)

180 **SI Table 3: cDNA reaction mix volumes and thermocycling conditions**

181	<i>Reagent</i>	<i>Volume w/ 10% overage (µL)</i>
182	<i>iScript Reaction Mix</i>	4.4
183	<i>Reverse transcriptase</i>	1.1
184	<i>Nuclease free water</i>	14.3

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<i>Sample volume required per well</i>	<i>Reaction mix volume per well</i>
2 µL	18 µL

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191	<i>Thermocycler step</i>	<i>Time</i>
192	<i>iScript Reaction Mix</i>	4.4
193	<i>Reverse transcriptase</i>	1.1
194	<i>Nuclease free water</i>	14.3

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198 **SI Table 4: ddPCR reaction mix volumes**

<i>Reagent</i>	<i>Volume w/ 10% overage (µL)</i>
SARS-CoV-2_N1 primer/probe	2.2
Influenza-A_M primer/probe	2.2
Norovirus-GI_ORF2 primer/probe	2.2

199	<i>PMMoV primer/probe</i>	2.2
200	<i>ddPCR Multiplex Supermix</i>	6.1
201	<i>Nuclease free water</i>	8.3

	<i>Sample volume required per well</i>	<i>Reaction mix volume per well</i>
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204	1 µL	21 µL
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**SI Table 5: Positive control amplicon sequences**

<i>Target</i>	<i>Positive control amplicon</i>
<i>SARS-CoV-2</i>	gaccccaaaatcagcgaatgcaccccgattacgtttggtggaccctcagattcaactggcagtaaccagaa
<i>Influenza A</i>	caagaccaatctgtcacctctgactaagggaaatgagggttggttcacgctcaccgtgccagtgagcga ggactgcagcgtagacgtttgcctcaaaatgc
<i>Norovirus</i>	cgctggatgcgcttccatgacctcggattgtggacaggagatcgcgatcttctgcccgaattcgtaaattgatga tggcgtctaagg
<i>PMMoV</i>	gagtggttgacctaacgttgagaggcctaccgaagcaaatgtcgcactgcattgcaaccgacaattacat caaaggaggaagggtcgtgaagattgtgcgacgtaggtgagtcctcaatcaaggaagtgggtcgaa aatcagagatttctatgcttggctaacaggcaacacagtgccgatgagttccaaa

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**SI Table 6: Limit of detection for viral targets.**

<i>Target</i>	<i>LOD (gc/uL)</i>
<i>SARS-CoV-2</i>	0.121
<i>Influenza A</i>	0.191
<i>Norovirus</i>	0.186

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215 Calculated using:

216 LoB = mean blank + 1.645(SD blank)

217 LoD = LoB + 1.645(SD low concentration of target)

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221 **SI Table 7: Threshold settings for ddPCR partition identification**

<i>Target</i>	<i>x-axis RFU</i>	<i>y-axis RFU</i>
<i>Negative</i>	0-5000	0-6000
<i>Norovirus</i>	0-5000	6000-15000
<i>SARS-CoV-2</i>	0-5000	15000-25000
<i>SARS-CoV-2, norovirus</i>	0-5000	25000-30000
<i>PMMoV</i>	5000-9000	0-6000
<i>Norovirus, PMMoV</i>	5000-9000	6000-15000
<i>SARS-CoV-2, PMMoV</i>	5000-9000	15000-25000
<i>SARS-CoV-2, norovirus, PMMoV</i>	5000-9000	25000-30000
<i>Influenza A</i>	9000-15000	0-6000
<i>Influenza A, norovirus</i>	9000-15000	6000-15000
<i>SARS-CoV-2, influenza A</i>	9000-15000	15000-25000
<i>SARS-CoV-2, influenza A, norovirus</i>	9000-15000	25000-30000
<i>Influenza A, PMMoV</i>	15000-20000	0-6000
<i>Norovirus, influenza A, PMMoV</i>	15000-20000	6000-15000
<i>SARS-CoV-2, influenza A, PMMoV</i>	15000-20000	15000-25000
<i>SARS-CoV-2, influenza A, norovirus, PMMoV</i>	15000-20000	25000-30000

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225 **SI Table 8: Log10 of SARS-CoV-2, influenza A, norovirus, and PMMoV per resident of the**  
 226 **sampled building. All pathogens were normalized by PMMoV. ND represents non-detect.**

Site ID	SARS-CoV-2		Influenza A		Norovirus		PMMoV	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
<i>CAP</i> (n=57)	3.10	ND-4.35	3.19	ND-4.76	3.08	ND-4.75	5.71	3.91-6.73
<i>CAR</i> (n=50)	2.80	ND-3.84	3.07	ND-4.71	2.03	ND-3.73	5.61	3.42-7.00
<i>CIC</i> (n=44)	3.22	ND-4.62	2.14	ND-3.67	ND	ND	6.03	3.36-7.46
<i>CRC</i> (n=38)	2.60	ND-3.87	1.15	ND-2.68	2.40	ND-3.97	6.38	4.09-7.32
<i>MHC</i> (n=42)	2.84	ND-4.05	1.94	ND-3.28	-0.25	ND-1.14	5.95	3.40-6.69
<i>MRC</i> (n=41)	3.00	ND-4.28	2.40	ND-3.84	2.33	ND-3.85	5.85	3.39-6.68
<i>NBC</i> (n=55)	3.55	ND-4.67	3.74	ND-5.09	0.51	ND-2.24	5.81	3.44-7.01
<i>NN</i> (n=114)	3.05	ND-4.64	2.58	ND-4.22	0.45	ND-2.12	6.00	3.52-7.29
<i>PKD</i> (n=56)	3.24	ND-4.86	2.51	ND-3.97	2.45	ND-4.18	5.56	3.33-7.29
<i>PTD</i> (n=110)	2.91	ND-4.25	3.06	ND-4.97	1.44	ND-3.41	5.56	3.49-6.56

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240 **References**

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