Supplementary Material

Supplementary Figure

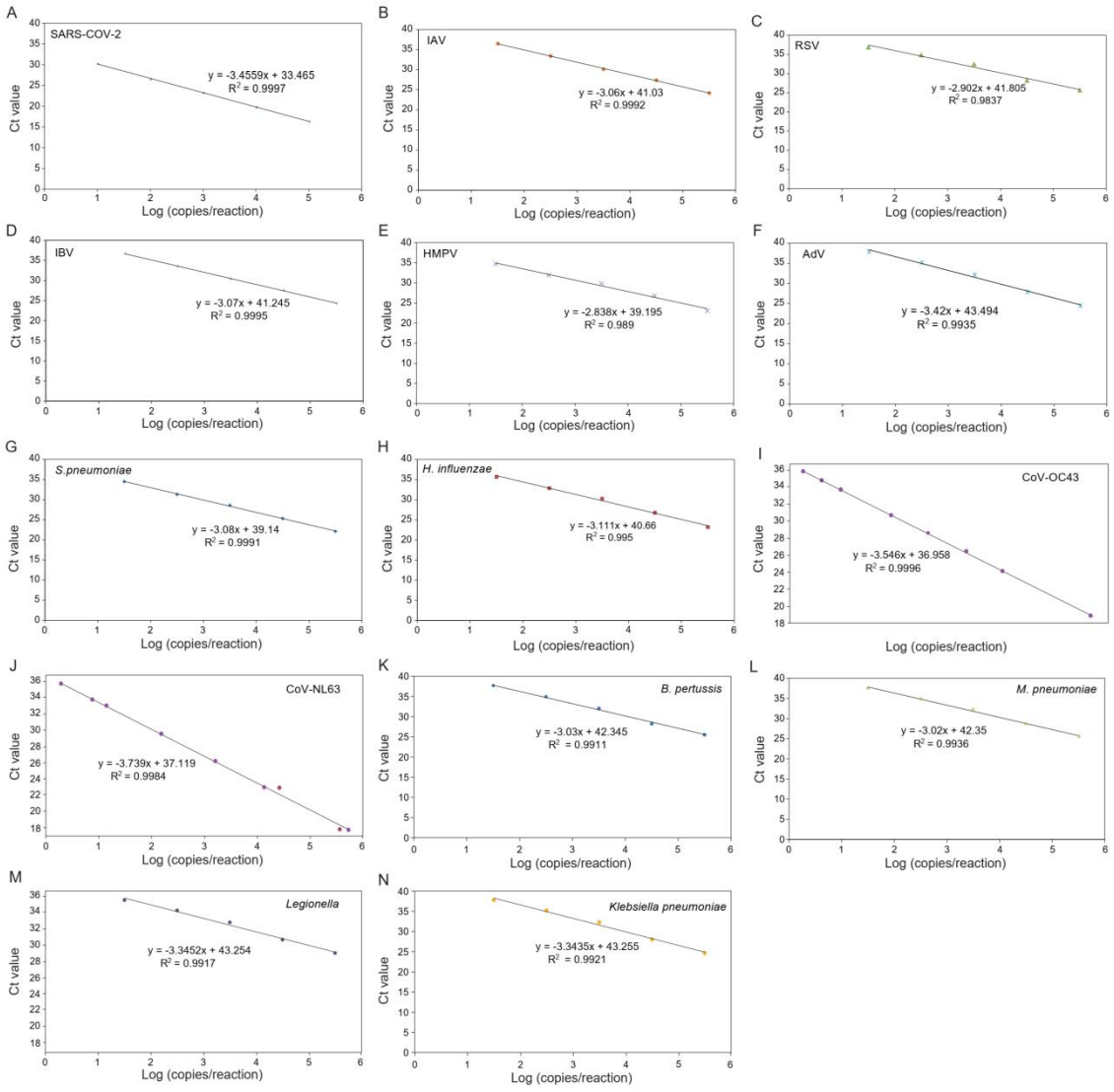

Fig. S1 Standard curves of pathogens used for the determination of the sewage concentrations. (A) Standard curve of SARS-CoV-2; (B) Standard curve of IAV; (C) Standard curve of RSV; (D) Standard curve of IBV; (E) Standard curve of HMPV; (F) Standard curve of AdV; (G) Standard curve of *S. pneumoniae*; (H) Standard curve of *H. influenzae*; (I) Standard curve of CoV-OC43; (J) Standard curve of CoV-NL63; (K) Standard curve of *B. pertussis*; (L) Standard curve of *M. pneumoniae*; (M) Standard curve of *Legionella pneumophila*; (N) Standard curve of *Klebsiella pneumoniae*

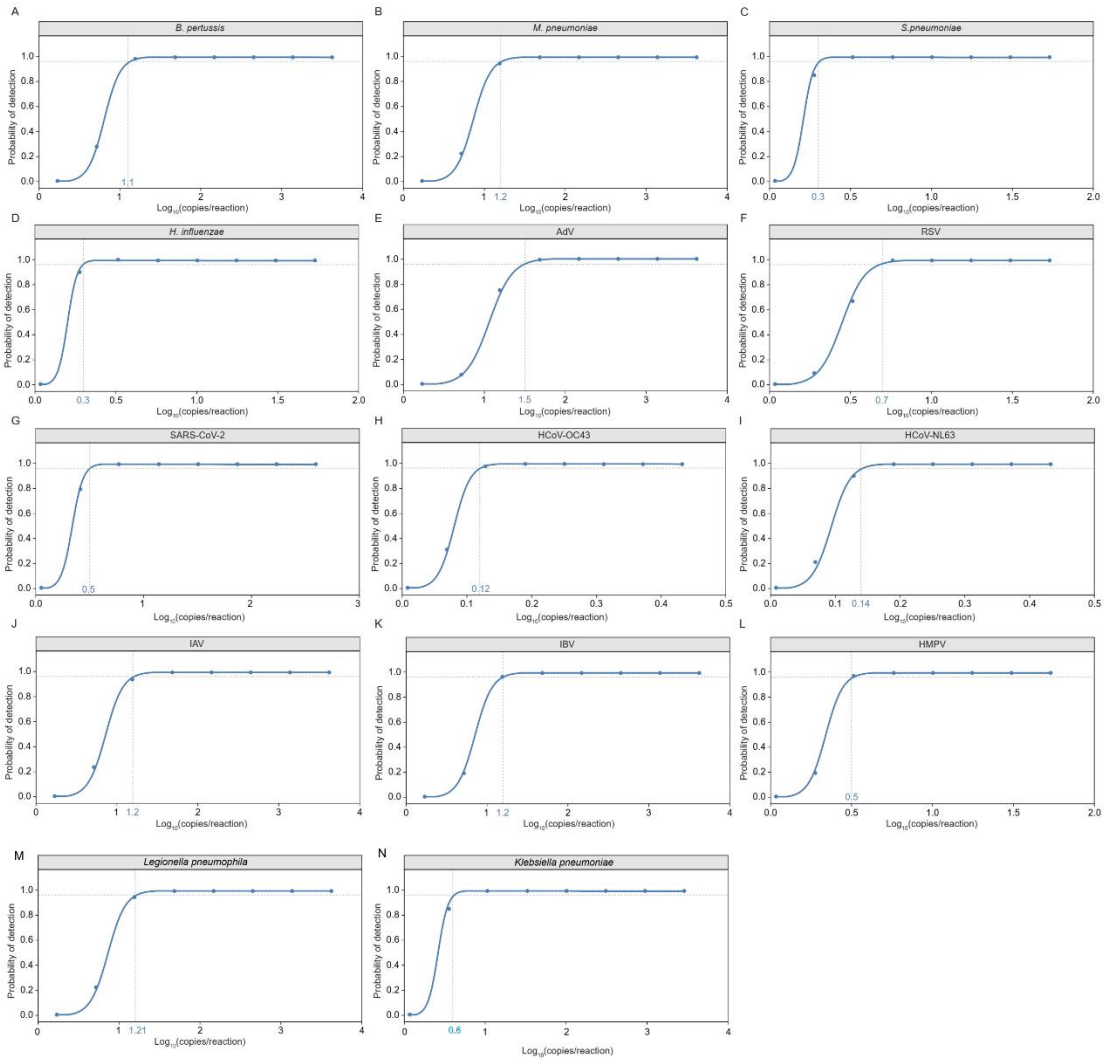

Fig. S2 The relationship between concentration of 14 pathogens measured by singleplex qPCR and probability of detection. Data represent serial dilutions over four orders of magnitude. Dashed line indicates the 95% LOD. (A) Prohit analysis of the LOD of *B. pertussis*; (B) Prohit analysis of the LOD of *M. pneumoniae*; (C) Prohit analysis of the LOD of *S. pneumoniae*; (D) Prohit analysis of the LOD of *H. influenzae*; (E) Prohit analysis of the LOD of AdV; (F) Prohit analysis of the LOD of RSV; (G) Prohit analysis of the LOD of SARS-CoV-2; (H) Prohit analysis of the LOD of HCoV-OC43; (I) Prohit analysis of the LOD of HCoV-NL63; (J) Prohit analysis of the LOD of IAV; (K) Prohit analysis of the LOD of IBV; (L) Prohit analysis of the LOD of HMPV; (M) Prohit analysis of the LOD of *Legionella pneumophila*; (N) Prohit analysis of the LOD of *Klebsiella pneumoniae*

Fig. S3 Concurrent real-time PCR and melting curve analysis in three tubes. Nucleic acid reference material of 17 species (IBV Victoria, HMPV, HCoV-NL63, HCoV-OC43, *Cryptococcus*, HPIV-3, Bocavirus, IAV H1N1, RSV A, Enterovirus, AdV, *C. pneumoniae*, *S.pneumoniae*, *B. pertussis,* SARS-CoV-2 N, *M. pneumoniae*, *H. influenzae*) were quantitated by determination of the quantification cycle (Cq value) that occurred during real-time PCR, and were each identified by the unique combination of the fluorescent color and the Tm value of their melting peak at 5×10^3, 5×10^2, 5×10^1, 5×10^0 copies/reaction.

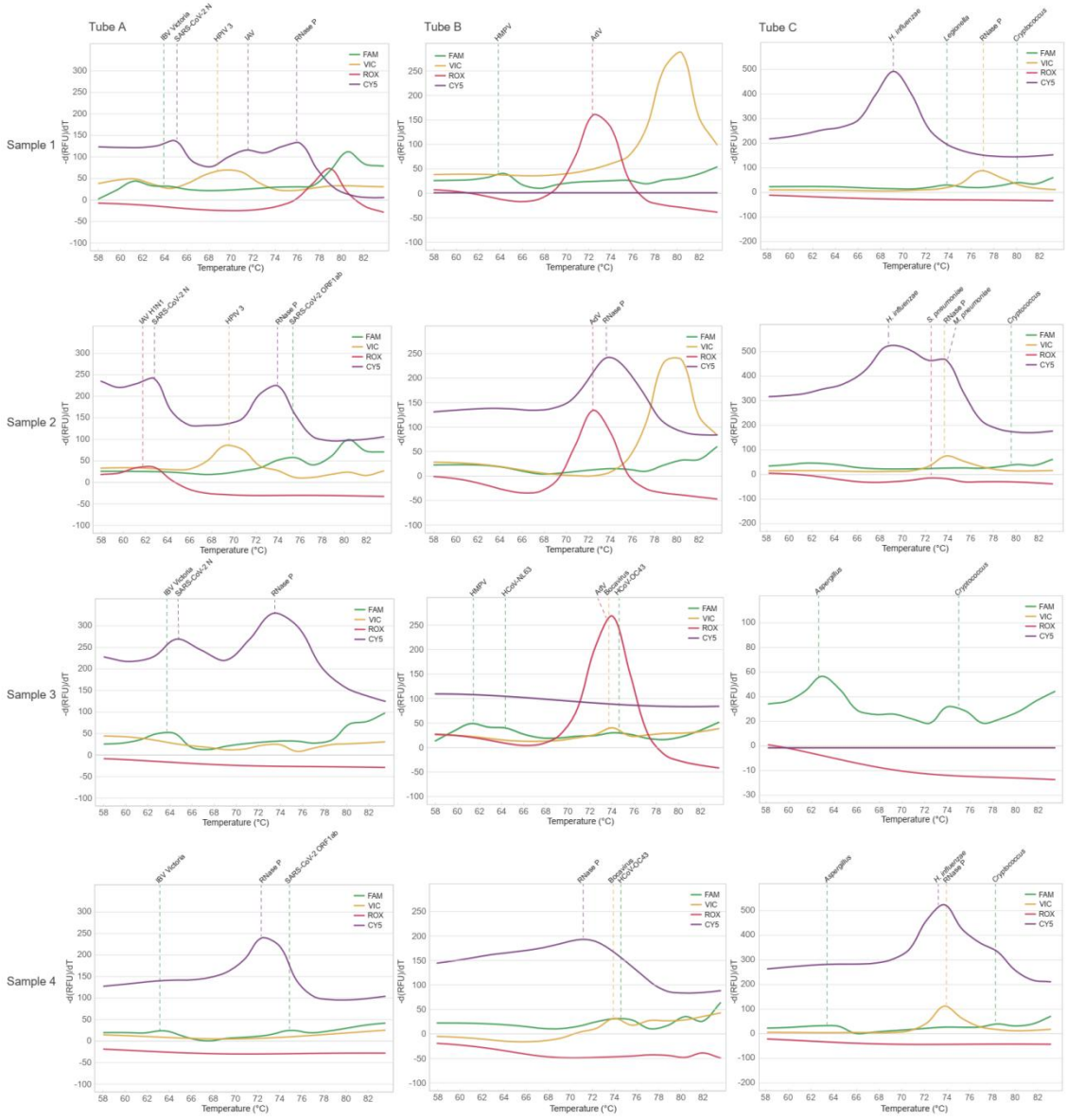

Fig. S4 Concurrent real-time PCR and melting curve analysis in three tubes. SARS-CoV-2, HMPV, Enterovirus, and AdV were quantitated by determination of the quantification cycle (Cq value) that occurred during real-time PCR, and were each identified by the unique combination of the fluorescent color and the Tm value of their melting peak.

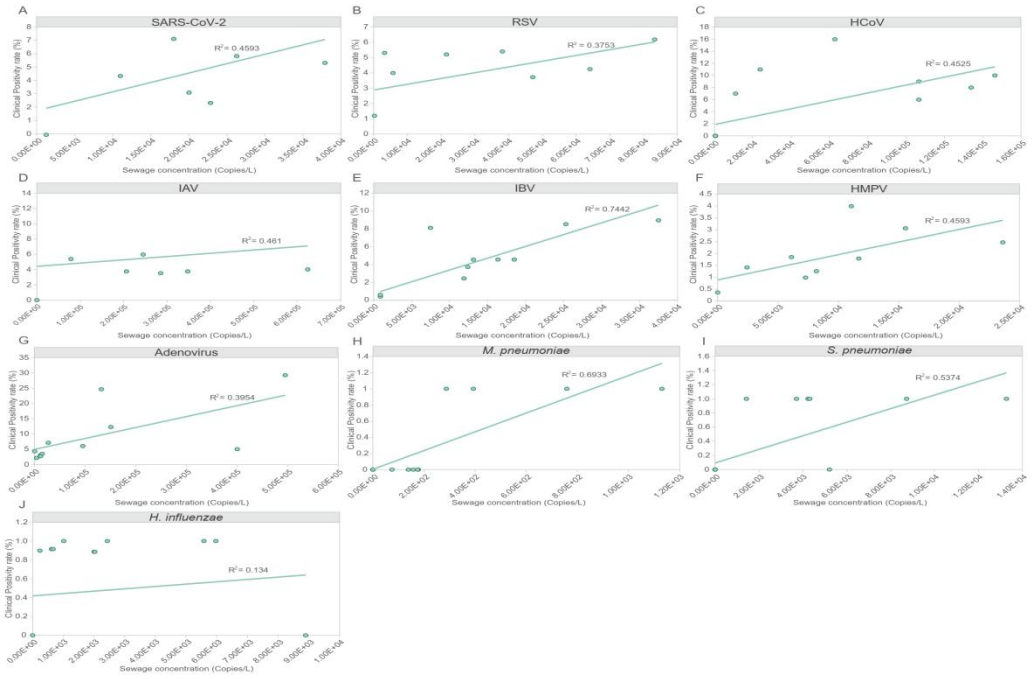

Fig. S5 Correlations between pathogens concentration and clinical positivity rate. (A) Correlations between sewage SARS-CoV-2 concentration and its clinical positivity rate; (B) Correlations between sewage RSV concentration and its clinical positivity rate; (C) Correlations between sewage HCoV concentration and its clinical positivity rate; (D) Correlations between sewage IAV concentration and its clinical positivity rate; (E) Correlations between sewage IBV concentration and its clinical positivity rate; (F) Correlations between sewage HMPV concentration and its clinical positivity rate; (G) Correlations between sewage adenovirus concentration and its clinical positivity rate; (H) Correlations between sewage *M. pneumoniae* concentration and its clinical positivity rate; (I) Correlations between sewage *S.pneumoniae* concentration and its clinical positivity rate; (J) Correlations between sewage *H. influenzae* concentration and its clinical positivity rate.

Supplementary Table

Table S1 Primers for amplification of genes for the construction of plasmid.

Gene	Primer	Sequences (5′–3′)	Size of amplicon (bp)	Reference
IAV/IBV	HA1-F	GAGGTCAATGTGACCGGTGT	809	(Dastyar et al., 2024)
	HA1-R	TACCTTGCTCCTGCCACTTG		
	HA2-F	TCAYCTGCCAACGGAGGAGT		
	HA2-R	TACAGCCAAAGTGGAGGCAG		
RSV	RSV-F	TTYGTTCCCTGTAGTATATGTG	670	(Allen et al., 2024)
	RSV-R	GTTATGACACTGGTATACCAACC		
HCoV-OC43/ NL63	pan-CoVinF	GGTTGGGAYTAYCCHAARTGTGA	600	(Fu et al., 2024)
	pan-CoVoutF	CCAARTTYTAYGGHGGITGG		
Adenovirus	HexonF	GCCCCARTGGGCRTACATGCACATC	300	(Han et al., 2013)

	HexonR	AGCACSCCSCGRATGTCAAAG		
S.pneumoniae	LytA-F	ATGCAGTTGGCTCAGTATGTA	400	(Zhang et al., 1995)
	LytA-R	CACCCAGTCCTCCCTTATCA		
M. pneumoniae	P1-F	AGCACCTTGCACGATGAAGA	551	(Xu et al., 2024)
	P1-R	GGGTAGATCCACTTCCACTCC		
H. influenzae	BexA-F	TGCGGTAGTGTTAGAAAATGGTATTATG	151	(Wang et al., 2011)
	BexA-R	GGACAAACATCACAAGCGGTTA		
K. pneumoniae	WabG-F	TCTCAGCCAGACGCAGTTG	387	(Chen et al., 2014)
	WabG-R	GGAAGCACCAGGAACATCAC		
L. pneumophila	LEG-F	AAGATTAGCCTGCGTCCGAT	654	(Shen et al., 2015)
	LEG-R	GTCAACTTATCGCGTTTGCT		
B. pertussis	L-F	GTGATGGGGTGCAAGCTCTT	286	(Wang et al., 2015)
	L-R	ATCTACCCGCGGCTAGACAGG		
HMPV	HMPV-L-F	CATGCCCACTATAAAAGGTCAG	170	(Maertzdorf et al., 2004)
	HMPV-L-R	CACCCCAGTCTTTCTTGAAA		

Table S2 PCR condition for Meltarray-based qPCR assay.

Channel	Pathogen/Gene name			Tm value reference range (℃)
	Reaction tube A	Reaction tube B	Reaction tube C	
FAM	IBV (Victoria)	HMPV	Aspergillus	59–63
	IBV (Yamagata)	HCoV-NL63	*Chlamydia psittaci*	65–69
	IAV (H3N2)	HCoV-OC43	*Legionella*	70–74
	SARS-CoV-2 (ORF1ab)	/	Cryptococcus	75–79
CY5	SARS-CoV-2 (N)	HPIV-1	/	58–62
	IAV	HCoV-229E	*K. pneumoniae*	65–69.5
	RNase P	RNase P	*H. influenzae*	69.6–73.5
	/	HPIV-2	*M. pneumoniae*	74–78
ROX/Texas Red	IAV (H1N1)	Enterovirus	*C. pneumoniae*	60–63.5
	RSV (A)	/	*Group A Streptococcus*	64–68
	/	Adenovirus	*S. pneumoniae*	69–73
	RSV (B)	HCoV-HKU1	*B. pertussis*	74–78

VIC/ HEX	/	HPIV-4	/	62–66
	HPIV-3	/	/	66.5–70.5
	/	/	RNase P	71–74
	RhV	Bocavirus	/	76–80

Table S3 Primers for amplification of 7 housekeeping genes of *S. pneumoniae*.

Gene	Primer	Sequences (5′–3′)	Size of amplicon (bp)
aroE	aroE-F	GCC TTT GAG GCG ACA GC	405
	aroE-R	TGC AGT TCA (G/A)AA ACA T(A/T)T TCT AA	
gdh	gdh-F	ATG GAC AAA CCA GC(G/A/T/C) AG(C/T) TT	459
	gdh-F	GCT TGA GGT CCC AT(G/A) CT(G/A/T/C) CC	
gki	gki-F	GGC ATT GGA ATG GGA TCA CC	483
	gki-R	TCT CCC GCA GCT GAC AC	
recP	recP-F	GCC AAC TCA GGT CAT CCA GG	448
	recP-R	TGC AAC CGT AGC ATT GTA AC	
spi	spi-F	TTA TTC CTC CTG ATT CTG TC	472
	spi-R	GTG ATT GGC CAG AAG CGG AA	
xpt	xpt-F	TTA TTA GAA GAG CGC ATC CT	486
	xpt-R	AGA TCT GCC TCC TTA AAT AC	
ddl	ddl-F	TGC (C/T)CA AGT TCC TTA TGT GG	441
	ddl-R	CAC TGG GT(G/A) AAA CC(A/T) GGC AT	

Table S4 Primers for amplification of 7 housekeeping genes of *Hemophilus influenzae*.

Gene	Primer	Sequences (5′–3′)	Size of amplicon (bp)
adk	adk-F	GGTGCACCGGGTGCAGGTAA	477
	adk-R	CCTAAGATTTTATCTAACTC	
atpG	atpG-F	ATGGCAGGTGCAAAAGAGAT	447
	atpG-R	TTGTACAACAGGCTTTTGCG	
frdB	frdB-F	CTTATCGTTGGTCTTGCCGT	489
	frdB-R	TTGGCACTTTCCACTTTTCC	
fucK	fucK-F	ACCACTTTCGGCGTGGATGG	345
	fucK-R	AAGATTTCCCAGGTGCCAGA	
mdh	mdh-F	TCATTGTATGATATTGCCCC	405
	mdh-R	ACTTCTGTACCTGCATTTTG	
pgi	pgi-F	GGTGAAAAAATCAATCGTAC	468
	pgi-R	ATTGAAAGACCAATAGCTGA	
recA	recA-F	ATGGCAACTCAAGAAGAAAA	426
	recA-R	TTACCAAACATCACGCCTAT	

Table S5 Primers for amplification of 8 housekeeping genes of *M. pneumoniae*.

Name		Primer sequence (5′–3′)	Amplicon (bp)	Tm (℃)
ppa	F	CGCTGACCAAGCCTTTCTAC	256	60
	R	CACTCCAAACTTTGCACTCCC		
pgm	F	AGCACCTTGCACGATGAAGA	1072	60
	R	CCTGCGCCTTCGTTAATTGG		
gyrB	F	TTGTCCCGGACTTTACCGTG	429	60
	R	TGTTTTCGACAGCAAAGCGG		
gmk	F	GAGCGGTGTTGGCAAAAGTA	394	60
	R	TGCATCCTCGTCATTACGCTT		
glyA	F	CAGAGAACTATGTGAGTAGGGACA	676	60
	R	TGACAACCCGGAAAGACACC		
atpA	F	GTCGCTGATGGCATTGCTAAG	796	60
	R	CCAGTAAACGCGAGTGCAAG		
arcC	F	CCCCATCAAGCCGTGTACTT	570	60
	R	TTGGGCAATAATGGCCGTCT		
adk	F	GTAGCCAACACCACCGGATT	473	60
	R	ACGGTGTCTTCGTAAAGCGT		

Table S6 Standard curve of tested 14 pathogens.

Virus	Standard curve	R^2	Efficiency (%)	LOD (copies/reaction)	LOD (copies/L)
SARS-CoV-2	y = −3.4559x + 33.465	0.9997	96.2	3.2	1280
IAV	y = −3.06x + 41.03	0.9992	99.3	15.85	6340
RSV	y = −2.902x + 41.805	0.9837	98.7	5	2000
IBV	y = −3.07x + 41.245	0.9995	99.7	15.85	6340
HMPV	y = −2.838x + 39.195	0.989	98.4	3.2	1280
AdV	y = −3.42x + 43.494	0.9935	101.2	31.6	12640
HCOV-OC43	y = −3.546x + 36.958	0.996	101.4	1.33	532
HCOV-NL63	y = −3.739x + 37.119	0.998	101.3	1.4	560
B. pertusis	y = −3.03x + 42.345	0.9911	99.7	12.6	5040
M. pneumoniae	y = −3.02x + 42.35	0.9936	99.5	15.85	6340
S. pneumoniae	y = −3.08x + 39.14	0.9991	99.8	2.0	800
H. influenzae	y = −3.111x + 40.66	0.995	99.7	2.0	800
L. pneumophila	y=-3.345x + 43.25	0.991	99.2	16.28	6512
K. pneumoniae	y=-3.343x + 43.26	0.992	99.2	4.0	1600

Supplementary text

The bacterial adsorption capacity of mica particles was assessed by passing 12 L of sterile deionized water, inoculated with $(1.2 \pm 0.5) \times 10^9$ CFU/L of *Klebsiella pneumoniae*, through the mica filter. The abundance of *K. pneumoniae* cells in the collected effluent was evaluated utilizing chromogenic media plates. The processes of bacterial adsorption and recovery from the water samples were quantified usinspectively.

g Eqs. (S1) and (S2), re

$$Bacterial\ adsorption(\%) = \frac{C_0 - C_i}{C_0} \times 100\% \quad , \qquad (S1)$$

$$Bacterial\ recovery(\%) = \frac{C_r}{C_0} \times 100\% \quad , \qquad (S2)$$

The variable Co (CFU) represents the quantity of bacteria introduced into the water samples. The concentration of microorganisms present in the natural water samples prior to concentration is denoted as Ci (CFU). The quantity of bacteria presented in the effluent following concentration, expressed as Cr (CFU).

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