Lab on a Chip

Supplementary Information

Title: Electrostatic Microfiltration (EM) Enriches and Recovers Viable Microorganisms at Low-abundance in Large-volume Samples and Enhances Downstream Detection

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The details of precise, efficient, rapid, flexible, easy, controllable, and thin (PERFECT) filter, homemade gadget for positive charge coating, and gadget of package for the electrostatic microfiltration (EM) processing, are shown in Fig. S1.

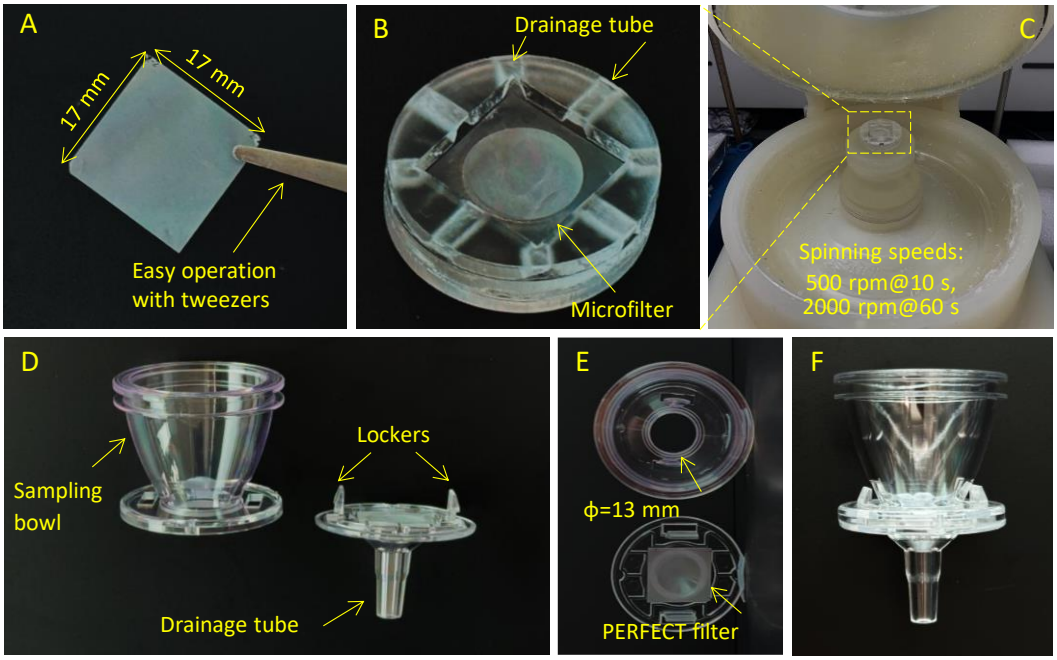

Fig. S1 PERFECT filter, spinning-based Ca-alginate gel coating and package gadget. A) Photo of PERFECT filter. B) Homemade holder with PERFECT filter for C) Spinning-based positive-charge (Ca-alginate gel) coating. Photos of filtration gadget from D) side and E) top-down views, and F) packaged PERFECT filter for EM processing.

The filter's hydrophilicity after the Ca-alginate gel coating was investigated by measuring the contact angles. As shown in Fig. S2, the reduction of contact angle from 76° to 68° demonstrates a slight increase in hydrophilicity, which is beneficial for electrostatic microfiltration.

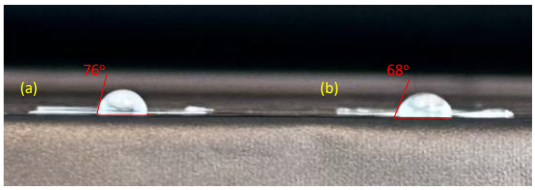

Fig. S2 The contact angles of H_2O on the PERFECT filters before (a) and after (b) the alginate-$CaCl_2$ coating.

The sample-to-result workflows of EM-based sample preparation, conventional centrifugation-based sample preparation, and an unconcentrated raw sampling approach (collecting 40 µL from initial raw samples) interfacing with digital amplification-based detection or post-release culture on lysogeny broth (LB) agar plates, were schematically shown in Fig. S3.

Fig. S4 presents the scatter plots of dLAMP signals (copies/µL on the Y-axis) for detection of bacteria in 10 mL LB spiked samples, in correlation with the abundances (CFU/mL on the X-axis) derived from the actual CFU counts obtained by the plating method.

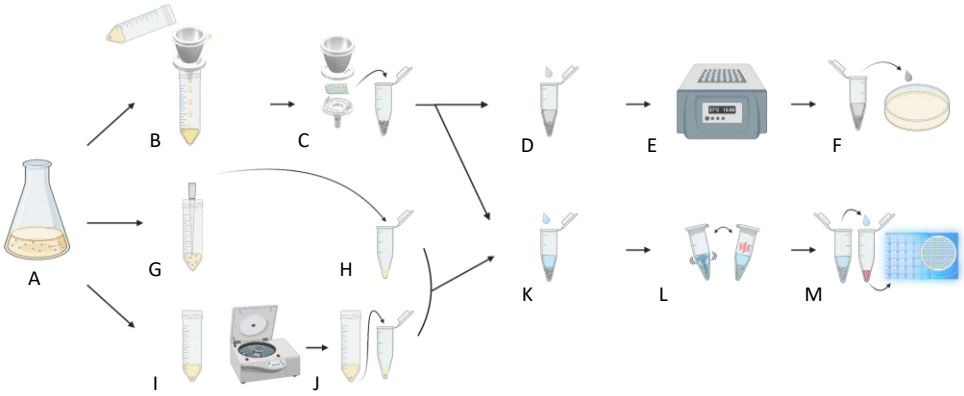

Fig. S3 Schematic of the workflow of electrostatic microfiltration (EM)-based, centrifugation-based sample preparation and an unconcentrated raw sampling approach, for interfacing with the digital amplification-based detection and culture of released pathogens on agar plates. A) Preparation of samples at desired abundance via spiking. B) Gravity-driven EM processing to capture the pathogens onto the EM-PERFECT filter. C) Transfer the EM-PERFECT filter containing pathogens into a microfuge tube for D–E) release, followed by F) plating and culture. G–H) Direct sampling of 40 µL from 10 mL raw sample. I–J) Centrifugation@10,000g for 10 min, dispose of the supernatant, and resuspension of the pellet into 54 µL nuclease-free H_2O. K) In-situ lysis via alternative L) heating and vortexing procedures (65°C@6 min, vortexing@1 min, 98°C@2 min, vortexing@1 min). M) Introduction of lysate into the Loop mediated isothermal AMPlification (LAMP) master mix, partition, and digital LAMP (dLAMP) running.

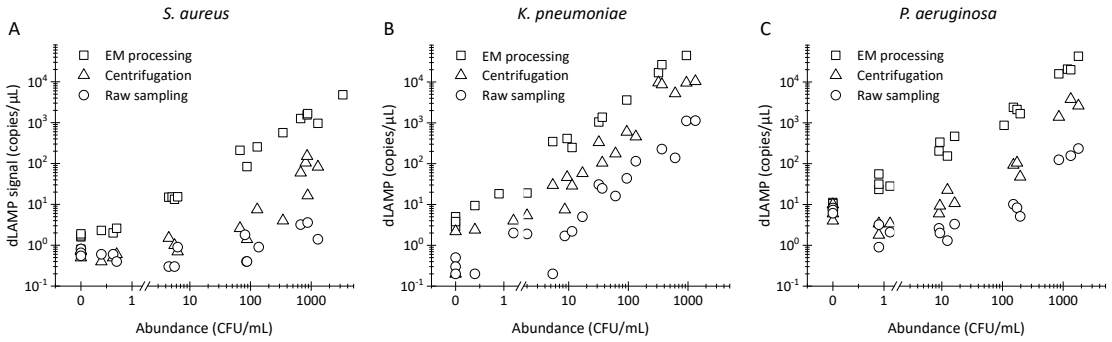

Fig. S4 The scatter plots of dLAMP signals in correlation with the abundances (derived from actual CFU counts based on the plating method), for detection of bacteria in 10 mL LB spiked samples: A) *S. aureus*, B) *K. pneumoniae*, and C) *P. aeruginosa*.

Fig. S5 presents the evident concentration increment achieved by the EM-based sample preparation (A, D, G), compared to centrifugation-based method (B, E, F) and unconcentrated raw sampling approach (C, F, I). The raw images and scatter plots of positive versus negative signal points/partitions from dLAMP detection are shown here with an abundance at the 100 CFU/mL level as the representative. Each dLAMP reaction consisted of more than 20,000 partitions. The displays presented here show images/data from around 5,000 partitions as the representative.

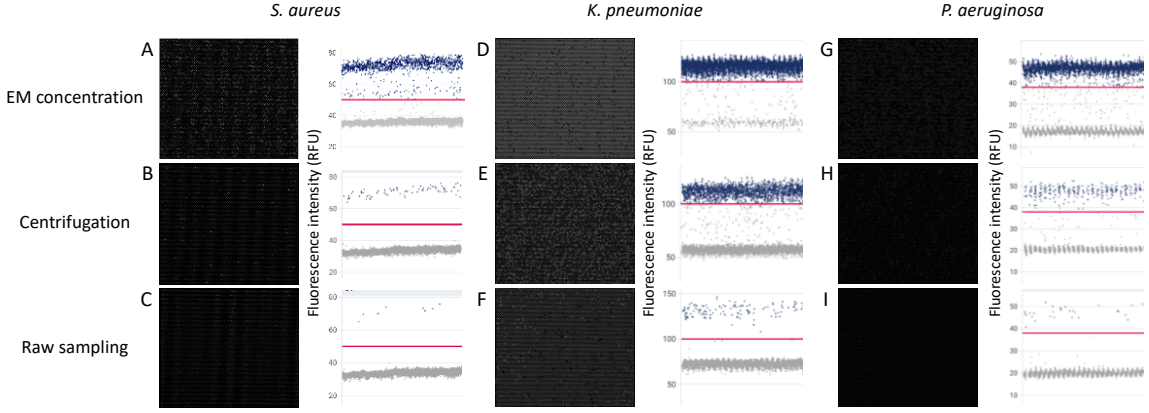

Fig. S5 The raw images and scatter plots of positive versus negative signal points/partitions from dLAMP detection of bacteria in 10 mL LB samples using different sample preparation methods, with the abundance at the 100 CFU/mL level as the representative: A, B, C) *S. aureus*, D, E, F) *K. pneumoniae*, and G, H, I) *P. aeruginosa*.

Table S1 dLAMP signals of *P. aeruginosa* detection obtained by EM-based sample preparation (Ca-alginate gel coating with Pluronic F-127 of different concentrations) and abundances derived from CFU counts, for 10 mL FBS spiked samples at the 100 CFU/mL level.

No.	Abundance (CFU/mL)	dLAMP signal (copies/μL)			
		Ca-Alginate	Plu(1)[a)]-Ca-Alginate	Plu(2)[a)]-Ca-Alginate	Plu(3)[a)]-Ca-Alginate
1	153	211.4	390.7	**557.8**	337.6
2	179	588.3	998.1	**1365.9**	965.8
3	66.5	226.2	365.8	**571.2**	399.6

[a)] Plu(1), (2), and (3): adding 1%, 2%, and 3% (w/v) Pluronic F-127 into 0.4% (w/v) alginic acid sodium salt in the preparation of positively charged coating.

Table S2 Capture efficiencies and actual colony-forming unit (CFU) counts, for 10 mL LB spiked samples.

No.	Species	Counted abundance (CFU/mL)	CFU counts (total No.)			Capture efficiency (%)	
			Input	Filtrate (EM)	Supernatant (centrifuge)	EM	Centrifuge
1	*S. aureus*	4.5	45	15	44	67	2.2
2		5.6	56	13	55	77	1.8
3		6.3	63	14	61	78	3.2
4		86.5	865	80	859	91	0.7
5		87.5	875	40	860	95	1.7
6		129	1290	120	1275	91	1.2
7		865	8650	260	8218	97	5.0
8		875	8750	1070	8374	88	4.3
9		1290	12900	1530	12380	88	4.0
1	*K. pneumoniae*	7.6	76	17	69	78	9.2
2		9.7	97	35	88	64	9.3
3		9.4	94	47	89	50	5.3
4		58.8	588	164	499	72	15
5		66.8	668	238	600	64	10
6		94	940	242	878	74	6.6
7		587.5	5875	1367	4930	77	16
8		667.5	6675	1448	5674	78	15
9		940	9400	3594	7144	72	24
1	*P. aeruginosa*	9	90	35	88	61	2.2
2		9.3	93	5	92	95	1.1
3		12.4	124	51	123	59	0.8
4		196	1960	1066	1912	46	2.5
5		93.3	933	106	905	89	3.0
6		173.7	1737	539	1686	69	2.9
7		1960	19600	6313	16660	68	15
8		1340	13400	4649	11792	65	12
9		1790	17900	2214	16468	88	8.0

According to the datasheet from Sigma-Aldrich for alginate lyase (Product No. A1603), the optimal PH for activity is PH6.3, thus viability and intactness of *K. pneumoniae* incubated in PH6.3 and PH7.0 phosphate buffered solution (PBS) were first tested. From the results listed in Table S11, PH6.3 had no obvious effect compared to PH7.0 at a certain concentration of alginate lyase. Subsequently, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* in PH 6.3 PBS of different alginate lyase concentrations were tested. The optimal release condition was set PH6.3 PBS of 0.1 unit/mL alginate lyase.

Table S3 Viabilities of released bacteria after alginate lyase reatment at different concentrations in PBS with different PHs.

No.	Species	Abundance (CFU/mL)	PH	Alginate lyase concentration	Viability[a)]	Intactness[b)]
1	*K. pneumoniae*	9.6e5	6.3, 10 min	0 (NC)	99.0%	99.8%
				0.1 unit/mL	98.9%	99.3%
				0.25 unit/mL	98.7%	88.0%
				0.5 unit/mL	98.4%	87.6%
			6.3, 30 min	0 (NC)	99.4%	99.8%
				0.1 unit/mL	95.1%	99.5%
				0.25 unit/mL	93.8%	93.3%
				0.5 unit/mL	92.6%	93.2%
1	*K. pneumoniae*	9.6e5	7.0, 10 min	0 (NC)	99.0%	99.6%
				0.1 unit/mL	98.8%	92.5%
				0.25 unit/mL	98.8%	60.3%
				0.5 unit/mL	98.7%	59.8%
			7.0, 30 min	0 (NC)	97.8%	99.8%
				0.1 unit/mL	96.2%	97.8%
				0.25 unit/mL	96.4%	80.8%
				0.5 unit/mL	95.6%	74.5%
1	*S. aureus*	6.73e5	6.3	0 (NC)	95.3%	98.4%
				0.1 unit/mL	95.3%	97.5%
				0.25 unit/mL	95.7%	64.4%
				0.5 unit/mL	93.1%	33.1%
2		1.05e6	6.3	0 (NC)	92.6%	94.7%
				0.1 unit/mL	93.7%	94.0%
				0.25 unit/mL	94.8%	58.4%
				0.5 unit/mL	93.3%	34.4%
3		1.08e6	6.3	0 (NC)	92.5%	97.0%
				0.1 unit/mL	92.9%	85.5%
				0.25 unit/mL	94.8%	57.6%
				0.5 unit/mL	94.5%	32.2%
2	*K. pneumonia*	2.3e6	6.3	0 (NC)	99.5%	99.6%
				0.1 unit/mL	99.7%	98.4%
				0.25 unit/mL	99.7%	94.0%
				0.5 unit/mL	99.6%	94.4%
3		8.3e5	6.3	0 (NC)	98.0%	99.8%
				0.1 unit/mL	98.6%	98.5%
				0.25 unit/mL	98.4%	95.0%
				0.5 unit/mL	99.8%	95.6%
4		7.2e5	6.3	0 (NC)	99.4%	99.8%
				0.1 unit/mL	95.1%	99.5%
				0.25 unit/mL	93.8%	93.2%
				0.5 unit/mL	92.6%	93.2%
1	*P. aeruginosa*	1.08e6	6.3	0 (NC)	96.8%	97.4%
				0.1 unit/mL	97.5%	98.2%
				0.25 unit/mL	96.0%	87.5%
				0.5 unit/mL	94.0%	61.2%
2		9.18e5	6.3	0 (NC)	94.5%	99.2%
				0.1 unit/mL	95.4%	95.2%
				0.25 unit/mL	95.4%	97.8%
				0.5 unit/mL	94.6%	60.3%
3		4.38e5	6.3	0 (NC)	83.4%	97.9%
				0.1 unit/mL	87.5%	86.9%
				0.25 unit/mL	87.4%	59.0%
				0.5 unit/mL	87.3%	51.2%

[a)] viability=percentage of SYTO9-positive singlets out of all singlets for live (w/o heat killing); [b)] intactness=percentage of PI-positive singlets out of SYTO9-positive singlets of dead (w/ heat killing); The heat killing condition: 95°C for 15 min with shaking of 1000 rpm on a dry bath/block heater (ThermoMixer C, Eppendorf, Germany)

Table S4 Sequences of primers/probes used in this study.

Pathogen	Primer/Probe ID	Sequences
S. aureus	SA F3-6	ACAAAGGGCAGCGAAACC
	SA B3-6	ACGGCTAGCTCCTAAAAGGT
	SA FIP-6	GCTTCATGTAGTCGAGTTGCAGACTGGTCAAGCAAATCCCATAAAGTTG
	SA BIP-6	GCTACGGTGAATACGTTCCCGGTACTCCACCGGCTTCGG
	SA LF-6	CAATCCGAACTGAGAA
	SA LB-6	TCTTGTACACACCGCCCGTCA
	SA-BIP-6-HEX	/5HEX/TGCTACGGTGAATACGTTCCCGGTACTCCACCGGCTTCGG
	SA-Bd-6-FQ	CCGGGAACGTATTCACCGTAGCA/3IABkFQ/
K. pneumoniae	KP F3-2	GCAACTCGACTCCATGAA
	KP B3-2	CGACTTCACCCCAGTCAT
	KP FIP-2	CAAGGCCCGGGAACGTATTCGGAATCGCTAGTAATCGTAG
	KP BIP-2	ACACCGCCCGTCACACCATAAAGTGGTAAGCGCCCTCC
	KP LF-2	CACCGTAGCATTCTGA
	KP LB-2	GGGAGTGGGTTGCAAAAGAAG
	KP-BIP-2-Cy5	/5Cy5/ACACCGCCCGTCACACCATAAAGTGGTAAGCGCCCTCC
	KP-Bd-2-RQ	ATGGTGTGACGGGCGGTGT/3IAbRQSp/
P. aeruginosa	PA F3-8	AAGCGGTGGAGCATGTG
	PA B3-8	GTGCTGGTAACTAAGGACAAGG
	PA FIP-8	AGGCACCAATCCATCTCTGGAAAGGAAGCAACGCGAAGAACCT
	PA BIP-8	GGAACTCAGACACAGGTGCTGCTTGCGCTCGTTACGGGA
	PA LF-8	AGCATGTCAAGGCCAGGTA
	PA LB-8	CTGTCGTCAGCTCGTGTCGTG
	PA-8-FIP-FAM	/56FAM/AGGCACCAATCCATCTCTGGAAAGGAAGCAACGCGAAGAACCT
	PA-8-Fd-FQ	CTTTCCAGAGATGGATTGGTGCCT/3IABkFQ/
C. albicans	CA F3-1	GGTCCAGACACAATAAGGATT
	CAB3-1	CTCAAACTTCCATCGACTTG
	CA FIP-1	CCAACTAAGAACGGCCATGCGACAGATTGAGAGCTCTTTCTTGA
	CA BIP-1	GGAGTGATTTGTCTGCTTAATTGCGTGACTATACCAGCAAATGC
	CA LF-1	CCACCACCCACAAAA
	CA LB-1	ACGAACGAGACCTTAACCTACT
	CA-1-FIP-Cy5	/5Cy5/CCAACTAAGAACGGCCATGCGACAGATTGAGAGCTCTTTCTTGA
	CA-1-F1c-RQ	GCATGGCCGTTCTTAGTTGG/3IAbRQSp/
Herpes Simplex Virus	HSV-F	CATCACCGACCCGGAGAGGGAC
	HSV-R	GGGCCAGGCGCTTGTTGGTGTA
	HSV-P	/56FAM/CCGCCGAACTGAGCAGACACCCGCGC/3IABkFQ/

Table S5 dLAMP signals of bacterial detection obtained by EM-, centrifugation-based sample preparation (EM, C), unconcentrated raw sampling approach (R), and abundances derived from actual CFU counts, for 10 mL LB spiked samples.

No.	Species	Counted abundance (CFU/mL)	dLAMP signal (copies/µL)			Normalized dLAMP signal (copies/µL)		
			EM	C	R	EM	C	R
10		0 (NC)	1.6	0.7	0.8	-	-	-
11		0 (NC)	1.9	0.5	0.7	-	-	-
12		0 (NC)	1.7	0.7	0.6	-	-	-
13		0.63	2.0	0.5	0.6	3.17	0.79	0.95
14		0.4	2.3	0.4	0.6	5.75	1.00	1.50
15		0.7	2.6	0.6	0.4	3.71	0.86	0.57
16		5.6	13.3	1.0	0.3	23.75	1.79	0.54
17		6.3	15.3	0.7	0.9	24.29	1.11	1.43
18		4.5	15.0	1.5	0.3	33.33	3.33	0.67
19		5.1	15.1	-	-	29.61	-	-
20		87	210.6	2.6	-	243.47	3.88	-
21	*S. aureus*	88	83.8	1.4	-	95.77	1.60	-
22		129	255.6	7.5	-	198.14	5.81	-
23		140	-	-	0.9	-	-	0.64
24		85.8	-	-	0.4	-	-	0.47
25		81	-	-	1.8	-	-	2.22
26		3330	4803.2	-	-	1442.40	-	-
27		342	567.6	4.0	-	1659.65	11.70	-
28		865	1261.2	59.5	3.2	1458.03	88.81	3.70
29		875	1663.4	16.5	3.6	1901.03	18.86	4.11
30		1290	963.3	83.0	1.4	746.74	64.34	1.09
31		858	1544.6	151.6	-	1800.23	176.69	-
32		811	-	104.4	-	-	128.73	-
10		0 (NC)	2.9	2.2	0.5	-	-	-
11		0 (NC)	4.95	0.19	0.3	-	-	-
12		0 (NC)	3.75	0.19	0.2	-	-	-
13		0.4	9.4	2.4	0.2	23.43	6.00	0.50
14		2	19.1	5.4	1.9	9.55	2.70	0.95
15		1.7	-	-	2.8	-	-	1.65
16		1.2	-	4	2.0	-	3.33	1.67
17		0.9	18.3	-	-	20.33	-	-
18		5.4	347.5	29.8	0.2	643.52	55.19	0.37
19		11.4	248.0	28.8	2.2	217.54	25.26	1.93
20		9.4	412.0	46.6	-	438.30	49.57	-
21	*K. pneumoniae*	17	-	58.2	5.0	-	34.34	2.94
22		8.6	-	7.5	1.7	-	8.72	1.98
23		34	1046.3	333.9	30.7	3077.35	982.06	90.29
24		36.5	1368.3	105.1	24.8	3748.77	287.95	67.95
25		94	3610.0	599.4	43.7	3840.43	637.66	46.49
26		133	-	460.6	114.5	-	346.32	86.09
27		60.8	-	176.5	16.1	-	290.30	26.48
28		340	16877.0	9684.5	-	49638.24	28483.82	-
29		365	26481.0	8680.7	227.6	72550.68	23782.74	623.56
30		940	44507.3	9554.7	1112.0	47348.19	10164.57	1182.98
31		1330	-	10416.8	1129.2	-	7832.18	849.02
32		608	-	5247.7	138.4	-	8631.09	227.63
10		0 (NC)	10.5	3.9	3.4	-	-	-
11		0 (NC)	8.0	1.9	2.4	-	-	-
12		0 (NC)	6.9	1.3	1.1	-	-	-
13		0.9	23.4	3.4	3.2	26.00	3.78	3.56
14		0.9	55.8	1.8	0.9	62.00	2.00	1.00
15		1.1	28.2	3.4	2.1	25.18	3.04	1.88
16		0.9	31.5	-	-	35.00	-	-
17		9	204.4	6.0	2.6	227.11	6.67	2.89
18		9.3	335.7	9.3	2.0	360.97	10.00	2.15
19	*P. aeruginosa*	16.3	466.1	10.7	3.3	285.95	6.56	2.02
20		12.4	153.4	22.6	1.3	123.71	18.23	1.05
21		196	1676.5	47.7	5.1	855.36	24.34	2.60
22		174	2121.0	104.9	8.3	1218.97	60.29	4.77
23		151	2392.1	93.0	10.1	1584.83	61.59	6.69
24		107	866.7	-	-	810.00	-	-
25		1337	19947.0	3794.3	156.4	14922.57	2838.56	117.00
26		1787	42785.3	2631.4	234.5	23946.55	1472.77	131.25
27		850	15814.0	1393.4	124.3	18604.71	1639.29	146.24
28		1183	20686.0	-	-	17486.05	-	-

Table S6 dLAMP signals of bacteria detection obtained by EM-based sample preparation and abundances derived from actual CFU counts, for 10 mL fetal bovine serum (FBS) spiked samples.

No.	Species	Counted abundance (CFU/mL)	dLAMP signal (copies/μL)	Normalized dLAMP signal (copies/μL)
33		0 (NC)	0.3	-
34		0 (NC)	0.2	-
35		0 (NC)	0.6	-
36		13.9	3.6	2.59
37	*S. aureus*	18.2	7.7	4.23
38		14.1	2.6	1.84
39		141	17.8	12.62
40		149	36.7	24.63
41		153	36.7	23.99
33		0 (NC)	0.5	-
34		0 (NC)	0.7	-
35		0 (NC)	0.7	-
36		23.1	108.0	46.73
37	*K. pneumoniae*	26.4	68.7	26.01
38		26.2	216.9	82.77
39		215	595.9	277.16
40		234	945.1	403.89
41		209	309.9	148.25
29		0 (NC)	0.9	-
30		0 (NC)	0.5	-
31		0 (NC)	0.7	-
32		18.6	41.1	22.10
33	*P. aeruginosa*	6.9	12.6	18.26
34		4.8	17.6	36.67
35		30.8	74.9	243.02
36		235	238.9	101.66
37		224	314.6	140.45
38		169	473.9	280.41

Table S7 dLAMP signals of detection of fungi obtained by EM-based sample preparation and abundances derived from actual CFU counts of *C. albicans*, for 10 mL brain heart infusion broth (BHIB) spiked samples.

No.	Abundance (CFU/mL)	dLAMP signal (copies/μL)	No.	Abundance (CFU/mL)	dLAMP signal (copies/μL)	Normalized dLAMP signal (copies/μL)
1	0 (NC)	2.0	11	0.35	5.3	15.14
2	0 (NC)	1.9	12	0.6	10.2	17.00
3	0 (NC)	2.0	13	0.35	8.2	23.43
4	0 (NC)	1.0	14	0.55	7.3	13.27
5	0 (NC)	0.6	15	13.4	5339.0	3984.33
6	0 (NC)	0.5	16	6.4	6451.5	10079.84
7	0 (NC)	1.6	17	4.9	3386.0	6910.20
8	0 (NC)	0.5	18	8.3	816.5	983.73
9	0 (NC)	1.0	19	6.8	7392.3	10871.03
10	0 (NC)	1.2				

Table S8 dLAMP signals of bacteria detection obtained by EM-based sample preparation and CFU counts of spiked bacteria, for 100 mL or 500 mL bottled drinking water spiked samples.

No.	Species	CFU counts (total No.)	dLAMP signal (copies/μL)	
			Volume @100 mL	Volume @ 500 mL
42		0 (NC)		0.1
43		0 (NC)		0.2
44		0 (NC)		0.4
45	*S. aureus*	9	0.9	0.8
46		16.9	1.4	0.8
47		21	2.4	1.8
48		11.8	2.0	1.1
49		8.8	1.1	0.5
42		0 (NC)		1.0
43		0 (NC)		1.2
44		0 (NC)		1.4
45	*K. pneumonia*	21	57.2	3.6
46		24.9	12.7	5.3
47		18.1	9.5	36.8
48		9.9	3.1	1.9
49		9	1.2	1.1
39		0 (NC)		0.8
40		0 (NC)		1.2
41		0 (NC)		1.1
42	*P. aeruginosa*	23	3.0	9.3
43		23.2	4.2	8.2
44		19.6	10.5	2.9
45		13.5	1.0	6.1
46		9.1	15.6	4.4

Table S9 dLAMP signals of multiplex detection of bacteria obtained by EM-based sample preparation and abundances derived from actual CFU counts, for 10 mL LB spiked samples.

No.	Abundances (CFU/mL)			dLAMP signals (copies/μL)		
	S. aureus	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*K. pneumonia*	*P. aeruginosa*
1	0 (NC)	0 (NC)	0 (NC)	0.8	3.3	2.8
2	0 (NC)	0 (NC)	0 (NC)	0.3	1.7	0.5
3	0 (NC)	0 (NC)	0 (NC)	1.4	5.4	5.1
4	21	151	46	9.5	3202.8	395.1
5	8	72	65	5.0	1479.9	388.0
6	13	150	62	6.6	4715.1	580.2
7	20	129	43	6.5	2747.6	566.8
8	162	151	46	96.5	2814.3	351.1
9	87	72	65	108.7	1565.6	657.4
10	101	150	62	82.7	4526.8	599.1
11	156	129	43	105.0	2455.9	661.9

Table S10 dLAMP signals of multiplex detection of bacteria obtained by EM-based sample preparation and abundances derived from actual CFU counts, for 10 mL FBS spiked samples.

No.	Abundances (CFU/mL)			dLAMP signals (copies/μL)		
	S. aureus	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*K. pneumonia*	*P. aeruginosa*
12	0 (NC)	0 (NC)	0 (NC)	0.1	0.3	1.1
13	0 (NC)	0 (NC)	0 (NC)	0.1	0.2	0.0
14	0 (NC)	0 (NC)	0 (NC)	0.0	0.0	0.2
15	13	151	62	2.6	395.1	413.2
16	13	150	143	6.9	988.5	727.8
17	20	129	43	2.3	607.1	60.9
18	101	151	62	7.2	270.4	277.1
19	159	150	143	17.0	943.4	673.0
20	156	129	43	10.6	867.5	71.7

Table S11 dLAMP signals of multiplex detection of bacteria obtained by EM-based sample preparation and abundances derived from actual CFU counts, for 100 mL or 500 mL bottled drinking water spiked samples.

No.	Abundances (CFU/mL)			dLAMP signals (copies/μL)		
	S. aureus	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*K. pneumonia*	*P. aeruginosa*
21	0 (NC)	0 (NC)	0 (NC)	0.8	0.8	0.2
22	0 (NC)	0 (NC)	0 (NC)	0.9	0.5	0.0
23	0 (NC)	0 (NC)	0 (NC)	0.6	0.6	0.8
			Volume @ 100 mL			
24	12.3	19.3	6.1	0.7	2.4	1.4
25	10.7	10.3	8.7	3.9	15.0	10.3
26	9.6	19.4	8.5	0.3	37.8	7.7
27	12.5	4.3	4.2	0.8	8.7	22.5
28	7.6	4.4	6	0.3	6.6	11.5
			Volume @ 500 mL			
29	12.3	19.3	6.1	1.6	2.9	1.8
30	10.7	10.3	8.7	3.9	17.8	14.2
31	9.6	19.4	8.5	2.4	6.8	10.5
32	12.5	4.3	4.2	0.6	6.1	4.2
33	8.8	10.1	9.5	1.9	9.2	4.8

Table S12 dPCR signals of HSV detection obtained by EM-, ultracentrifugation-based sample preparation (EM, UC), unconcentrated raw sampling approach (R), and abundances derived from plaque-forming unit (PFU) counts combining serial dilution factors, for 10 mL viral spent media samples.

No.	Abundance (PFU/mL)	dPCR signal (copies/µL)		
		EM	UC	R
1	0 (NC)	0.1	0.1	0.1
2	0 (NC)	0.0	0.0	0.1
3	0 (NC)	0.0	0.0	0.1
4	1	0.5	0.2	0.3
5	1	0.5	0.0	0.2
6	1	0.2	0.1	0.0
7	10	18.5	0.2	0.0
8	10	56.3	0.5	0.1
9	10	21.8	0.0	0.1
10	100	165.4	0.6	0.2
11	100	152.5	0.5	0.1
12	100	160.2	0.2	0.1
13	1000	2740.5	0.0	0.2
14	1000	1859.6	0.7	0.0
15	1000	2439.6	0.0	0.0
16	10000	29493.1	429.3	172.5
17	10000	21860.3	286.9	219.5
18	10000	28681.1	401.9	165.8

Table S13 dLAMP signals of detecting multiplex spiking samples containing *S. aureus*, *K. Pneumoniae*, and *P. aeruginosa*, using EM-PERFECT filters stored for different durations.

No.	**Day of storage**	**Abundance (CFU/mL)**			**dLAMP signals (copies/µL)**			**Normalized dLAMP signals (copies/µL)**		
		S. aureus	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*	*K. pneumonia*	*P. aeruginosa*
1	7				111.3	1808.9	920.2	46.5	2871.3	724.6
2	6				103.3	2225.9	986.1	43.2	3533.2	776.5
3	5				135.9	1746.8	1049.0	56.8	2772.7	826.0
4	4	239	63	127	70.1	3028.2	927.0	29.3	4806.7	729.9
5	3				102.0	2534.5	658.1	42.7	4023.0	518.2
6	2				127.1	1264.5	852.8	53.2	2165.9	671.5
7	1				80.0	2597.7	1111.5	33.5	4123.3	875.2
8	7				63.7	1769.6	509.2	37.0	876.0	337.2
9	6				63.3	1774.4	600.2	36.8	878.4	397.5
10	5				61.3	2133.9	801.3	35.6	1056.4	530.7
11	4	172	202	151	34.3	1729.4	873.6	19.9	856.1	578.5
12	3				27.2	2185.9	860.5	15.8	1082.1	569.9
13	2				72.6	1888.3	765.9	42.2	934.8	507.2
14	1				56.7	2032.0	848.1	32.9	1005.9	561.7
15	7				30.8	704.3	701.1	18.1	323.0	369.0
16	6				30.1	523.2	802.3	17.6	240.0	422.3
17	5				65.2	1332.6	1155.8	38.1	611.3	608.3
18	4	171	218	190	33.2	1030.7	1122.3	19.4	472.8	590.7
19	3				73.8	1522.6	1372.5	43.2	698.4	722.4
20	2				41.2	803.9	1017.9	24.1	368.8	535.7
21	1				46.2	758.6	1154.7	27.0	348.0	607.7