Supplementary Information (SI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2025

Supporting Information

Handheld RPA-Based Molecular POCT System for Rapid, Low-Cost 8-Plexed Detection of Respiratory Pathogens at Home

Yunfeng Zai,^{ab} Chao Min,^b Zunliang Wang,^{*a} Yongjun Ding,^b

Enben Su*ab and Nongyue Hea

^a State Key Laboratory of Digital Medical Engineering, School of Biological Science and Medical Engineering, Southeast University, Si Pai Lou 2, Nanjing 210096, China.

^b Getein Biotechnology Co., Ltd., Nanjing 210000, China.

*Correspondence and requests for materials should be addressed to E-mails: zlwang@seu.edu.cn (Zunliang Wang),

su.enben@getein.cn (Enben. Su)

Table of Content

Supplementary Tables

Table S1. Primers and probes used for RPA-based multiplex nucleic acids detection

Table S2. Cost analysis of the single-use microfluidic cartridge-based detection

 Table S3. Effect of volume ratio between air storage chamber and RPA chamber on

the real volume on fluid delivery

 Table S4. Comparative analysis of our system with representative commercially

 available POC NAAT devices

Supplementary Figure

Fig. S1 Experimental snapshots demonstrating four synchronized fluid-flow states in three detection channels

SUPPLEMENTAL TABLES

Table S1. Primers and probes used for RPA-based multiplex nucleic acids detection

FuAForward Prime5-ATCGCGAGAGACTTGAAGATGTCTTGCTGG-5'FuAAeverse Prime5-CACGGTGAACACAAACCCCAAAATC-3' CaTTGTGTGGAATGGCTAAAGACAAAGACCAAATC[FAM] CaTTGTGTGGAATGGCATGAGAGAGACAAAGACCAATC[FAM] CaTTGTGTGTGACGAAGACCAAGACCAAGACCAAGA-3' S-CTCAGCAGAATGGGAATTAGGCCTTTCTTGTGTGGCG-3'FuBAreverse Prime5-CTCAGCATAGAGAGACTACAGAGACCCC[VIC] CaTTGTGTGAGAGAGAGAGAGACAA3Spc3FuBForward Prime5-AAAGAAAAGGAGATTCAGAGAGACAAGAGAGAGAGAGAGA	Pathogen	Sequence length (bp)	Description	Sequence (5' to 3')					
FluA135 bpReverse Prime5*CACGGTGAACACACACACACACACACACACACACACACAC	FluA		Forward Primer	5'-ATCGCGCAGAGACTTGAAGATGTCTTTGCTGG-3'					
ProbeProbeProtect CTCATGGAATGGCTAAAGACAAGACCAATTC[FAMB CTTTTIBRQ-dTCACCAAGACCAAGACCAATTC[FAMB CTTTTIBRQ-dTCACAAGACCAAGACCAAGACCAATTC]FuBBForward Prime5-CTCAGCTAGAATAGGCACTTCTTCTTGTGCGCACAAGACCAAGACCACAGACCACAAGACCACAAGACCACAAGACCACAAGACCACAAGACCACAAGACCACAAGACCACAAGACCACAAGACAA		135 bp	Reverse Primer	5'-CACGGTGAGCGTGAACACAAACCCCAAAATC-3'					
Frobe Π [ITHF][BHQ-dT]CACCTCTGACTAAG-3'SpC3FluBForward Primer5'-CTATATGCTTTTAAAACCCAAAGACCAAGA-3'FluB113 bpReverse Primer5'-CTAGGAAAAAGGAGATTCATCACAGAGCCCC[VIC- dT](THF][BHQ-dT]CAGGAATGGGAACAA-3'SpC3ProbeForward Primer5'-AAGAAAAAGGAGATTCATCACAGAGCCCC[VIC- dT](THF][BHQ-dT]CAGGAATGGGAACAA-3'SpC3RSV116 bpReverse Primer5'-AACATGGGCACCATATTGTAAGTGAGGGGA-3'Probe5'-AACATGGGCACCCATATTGTAAGTGAAGGAGGT-3'RSV116 bpS'-CTCCACATACAGGGCACGTGTCAATACAA[ROX- dT](THF][BHQ-dT]CCTAGAAAAAGACGAT-3'SpC3AdV125 bpReverse Primer5'-CGCGCGTCTCTGAGCGCCCCTCTAAA-3'				5'-CTCTCATGGAATGGCTAAAGACAAGACCAATTC[FAM-					
FuBForward Prime5-CTATATGCTTTTAAAACCAAAGACCAAGA-5'FuB113 bpReverse Prime5-CTAGAGAAAAGGAGATTCATGAGAGCCCCTVC-5ProbeProbeS-AAAGAAAAGGAGATTCATGAGAGACCCCTVC-5ProbeForward Prime5-ATGGAACAAGGAGACAAGGAGACAAGAGAGAGAGAGAGAG			Probe	dT][THF] [BHQ-dT]CACCTCTGACTAAG-3'SpC3					
FluB113 bpReverse Prime5-CTCAGCTAGAATGGGCCCTTCTTGTGGCGGGGGGGGGGG			Forward Primer	5'-CTATATGCTTTTTAAAACCCAAAGACCAAGA-3'					
ProbeS-AAAGAAAAGGAGATTCATCAGAGAGCCCUS CITHFIBHQ-dTCAGGAAACAA-3'SpC3PRSVProbeForward PrimeP16 bpReverse PrimeS-AACATGGGAACAAAGCTTCAGGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG	FluB	113 bp	Reverse Primer	5'-CTCAGCTAGAATTAGGCCTTTCTTCTTTGTTGCTG-3'					
$Proce \qquad \arrow \arrow$			Ducho	5'-AAAGAAAAAGGAGATTCATCACAGAGCCCC[VIC-					
$ \begin{array}{llllllllllllllllllllllllllllllllllll$			Probe	dT][THF][BHQ-dT]CAGGAATGGGAACAA-3'SpC3					
RSV $116 bp$ Reverse Primer5'-AACATGGGCACCCATATTGTAAGTGATGCAGGGT-3' $Probe$ Pr			Forward Primer	5'- ATGGAAACATACGTGAACAAGCTTCACGAAGG-3'					
Probe5'-CTCCACATACAGCTGCTGTTCAATACAA[ROX- dT][THF][BHQ-dT]CCTAGAAAAGACGAT-3'SpC3AdV125 bpReverse Primer5'-CTCCACATACAGCGCGCCCCCGTACAA-3'	RSV	116 bp	Reverse Primer	5'- AACATGGGCACCCATATTGTAAGTGATGCAGGGT-3'					
AdV 125 bp Reverse Primer dT][THF][BHQ-dT]CCTAGAAAAAGACGAT-3'SpC3			Probe	5'-CTCCACATACACAGCTGCTGTTCAATACAA[ROX-					
AdV125 bpReverse Primer5'-TTGCGTGGAGCGTTGGCGAGCGCCTCCGTACATC-3'				dT][THF][BHQ-dT]CCTAGAAAAAGACGAT-3'SpC3					
AdV 125 bp Reverse Primer 5'-CGCGCGTCCTCTCGAGCCGCACCTTCTAAA-3'	AdV	125 bp	Forward Primer	5'-TTGCGTGGAGCGTTGGCGAGCGCCTCCGTACATC-3'					
			Reverse Primer	5'-CGCGCGTCCTCTCGAGCCGCACCTTCTAAA-3'					
5'-TTGCTGGGCGCGTGCAAACCCCAACCGGTGTTAT[FAM-			Droha	5'-TTGCTGGGCGCGTGCAAACCCCAACCGGTGTTAT[FAM-					
dT]A[THF][BHQ-dT]GGGCGAGATGAGAAT-3'SpC3			Probe	dT]A[THF][BHQ-dT]GGGCGAGATGAGAAT-3'SpC3					
Forward Primer 5'-ATCTGTTGAGGCTTGTGGCTATTCAGACAGGAT-3'	RhV		Forward Primer	5'-ATCTGTTGAGGCTTGTGGCTATTCAGACAGGAT-3'					
RhV 118 bp Reverse Primer 5'- GTAATGCGGCCAGACCCCATACCCAACCACG-3'		118 bp	Reverse Primer	5'- GTAATGCGGCCAGACCCCATACCCAACCACAG-3'					
5'- TATGCAAATAACCAGAGGAGATTCAACAATCACA[VIC-			Droho	5'- TATGCAAATAACCAGAGGAGATTCAACAATCACA[VIC-					
dT][THF][BHQ-dT] CAAGATGTAGCAAATG-3'SpC3			PIODE	dT][THF][BHQ-dT] CAAGATGTAGCAAATG-3'SpC3					
Forward Primer 5'- ATAACAACAAAGACAGAGCAATCTCAACGCCGACCC-3'		134 bp	Forward Primer	5'- ATAACAACAAAGACAGAGCAATCTCAACGCCGACCC-3'					
HPIV 134 bn Reverse Primer 5'- GTGTATACTTTGTTGATCAAGGAGTCTAGCATG-3'	HPIV		Reverse Primer	5'- GTGTATACTTTGTTGATCAAGGAGTCTAGCATG-3'					
5'- CCAAGATCATAGATCAGGTGAGGAGAGTGGAATC[ROX-	1111 /		Probe	5'- CCAAGATCATAGATCAGGTGAGGAGAGTGGAATC[ROX-					
dT] [THF] [BHQ-dT] AGGAGAACAAGTGAGT-3'SpC3			11000	dT] [THF] [BHQ-dT] AGGAGAACAAGTGAGT-3'SpC3					
Forward Primer 5'- TTTGGTGAAATAGCCCACCCCAAACCAATCGCC-3'			Forward Primer	5'- TTTGGTGAAATAGCCCACCCCAAACCAATCGCC-3'					
Mpn 157 bp Reverse Primer 5'- AAACTTTAGCTAAGTAATTGCGTGATTTGT-3'	Mpn	157 bp	Reverse Primer	5'- AAACTTTAGCTAAGTAATTGCGTGATTTGT-3'					
Probe 5'-TACGGTAGAGGTAGCGGAAACAGTTAAGGCAGC[FAM-	1	Ĩ	Probe	5'-TACGGTAGAGGTAGCGGAAACAGTTAAGGCAGC[FAM-					
dT][THF]C[BHQ-dT]AGCAACTTTGAAA-3'SpC3				d1][1HF]C[BHQ-d1]AGCAACTTTGAAA-3'SpC3					
Forward Primer 5^{-111} AGGUAAIGAAGUUIGUAIIAGIGAAUUAUI-3' Revenue Primer 5^{2} CCAACCETACTCCATCCCCTCCAAACTATAC 2'	Cpn		Forward Primer	3 - 111AAGGCAATGAAGCCTGCTACTACAACCACT-3'					
Cpn 121 bp 5- CCAAGULTACTOGATCCGCTGCAAACTATAC-5		121 bp	Kevelse Primer						
Probe dT]A[THF]G[BHO-dT]CTATCTACGGCAGTAG-3'SpC3			Probe	dT]A[THF]G[BHO-dT]CTATCTACGGCAGTAG-3'SpC3					

IC	139 bp	Forward Primer	5'- CCAAGCACAGTGGTGGCAAAAGCTTATTTGTGT-3'			
		Reverse Primer	5'- AACGGACAGTGTAACCCATGGAGCTAACTTCGG-3'			
		Probe	5'-CACTGGCTTCATACTTCCAGAGAAGATCTG[ROX-dT]			
			[THF] [BHQ-dT]CCTATTGGAACATC-3'SpC3			

Microfluidic cartridge device	Cost (\$) per test	Note (Single use)
Cartridge substrate	0.10	PP injection molding
Bladder	0.15	Silicone
Thermo-bonded composite film	0.05	PET + PP
Push-to-seal lid + Sealing ring	0.10	Negotiated price
Test reagents	1.00	Lysis + RPA
Total cost per multiplex test	1.40	

Table S2. Cost analysis of the single-use microfluidic cartridge-based detection

Volume Ratio Between air	Design	Measured R	Volume			
storage chamber and RPA chamber	schematic	Chamber A	Chamber B	Chamber C	Deviation	
	2.8mm	16.0	15.6	17.2	18.65%	
1:1	8mm	15.8	14.5	15.4	23.83%	
		17.0	16.2	15.8	18.33%	
	2.8mm	20.2	19.7	20.6	0.85%	
2:1	14mm	20.6	21.5	19.0	1.83%	
		19.0	18.7	19.6	4.50%	
3:1	4.2mm	21.5	22.0	21.2	7.85%	
		22.5	23.3	21.7	12.5%	
	000	21.9	22.0	22.6	10.8%	

Table S3. Effect of volume ratio between air storage chamber and RPA chamber on the real volume on fluid delivery

Note: The target RPA reaction volume is 20 μ L; this table reports the measured liquid volumes delivered to each RPA chamber (A, B, and C).

System	Method	Portability ^a	Pathogens	Targets	Sensitivity/ Specificity (%) (FluA)	Test time (min)	Test Signal	Sam ple to Ans wer	Ref.
Cepheid	PCR	Portable	SARS-CoV-	4	100/100	36	Fluorescenc	Yes	1-3
GeneXpert®			2/FluA/FluB/RSV				e		
			AdV/Coronavirus						
			229E/Coronavirus						
			HKU1/Coronavirus						
			NL63/Coronavirus						
			OC43/ SARS-Cov-						
			2/Human						
			Metapneumovirus/						
Biofire	DCD	D (11	Knv	22	100/100	45	Fluorescenc	v	1.6
Filmarray®	PCK	Portable	/Enterovirus/FluA/F	22	100/100	45	e	Yes	4-6
			Elera H1 2000/						
			FluA H1-2009/						
			/DSV/Dordetelle						
			parapartussis/Borda						
			tella pertussis/Borde						
			Mnn/Cnn						
			FluA/FluA H1N1						
			2009/FluA H1/FluA						
			H3/FluB/Coronavir						
			us						
			229E/Coronavirus						
			HKU1/Coronavirus						
			NL63/Coronavirus						
			OC43/SARS-CoV-						
QIAgen	PCR	Portable	2/HPIV1/HPIV2/H	22	99.2/99.5	60	Fluorescenc	Yes	7-9
QIAstat-Dx [®]			PIV3/HPIV4/RSV				e		
			A/B/Humanmetapn						
			eumovirusA/B/Adv						
			/Bocavirus/RhV/En						
			terovirus/Mpn/Legi						
			onella						
			pneumophilia/Bord						
			etella pertussis						

Table S4. Comparative analysis of our system with representative commercially available POC NAAT devices

			Mpn, Cpn				u		
Our System	RPA	Ultraportable	AdV, RhV,HPIV,	8	100/100	25	ce	Yes	1
			FluA, FluB, RSV,				Fluorescen		
Medical	MA	Onapolitable		1	1	28	e	INO	/
Midge	RPA	Ultraportable	Flu4/FluB	1	/	28	Fluorescenc	No	/
DETECT	LAMI	Ultraportable	2/FluA/FluB	5	/	30~60	c (LFA)	No	11
DETECTIM	LAMP		SARS-CoV-	3			Colorimetri		
Lucira TM LAM	LAMI	Ultraportable	2/FluA/FluB	5	91.4/99.8	30	c (LFA)	Yes	16, 17
	ΙΑΜΡ		SARS-CoV-	3			Colorimetri		3, 12, 13,
NOWTM	NEAK	Foltable	FluA/FluB	2	90.3/97.4	13	e	No	5, 14, 15
Abbott ID	NEAD	Portable	Flu A/FluD	2	96 3/97 4	12	Fluorescenc	No	2 14 15
Cue™ Isothe	Isotnermai	Ultraportable	2/FluA/FluB	3	/	20	mical	Yes	3, 12
	I		SARS-CoV-	2			Electroche		
Medical TM	PCK	Ultraportable	2/FluA/FluB	3	100/99.1	30	c (LFA)	Yes	3, 10-13
Visby	DCD		SARS-CoV-	2			Colorimetri		2 10 12

^a Ultraportable systems are handheld, whereas portable systems require a desktop setup.

References

- E. C.-m. Leung, V. C.-y. Chow, M. K.-p. Lee, K. P.-s. Tang, D. K.-c. Li and R. W.-m. Lai, J. Clin. Microbiol., 2021, 59, 02965-02920.
- C. B. Jensen, U. V. Schneider, T. V. Madsen, X. C. Nielsen, C. M. G. Ma, J. K. Severinsen,
 A. M. Hoegh, A. B. Botnen, R. Trebbien and J. G. Lisby, *J. Clin. Virol.*, 2024, 172, 105674.
- H. Harpaldas, S. Arumugam, C. C. Rodriguez, B. A. Kumar, V. Shi and S. K. Sia, *Lab Chip*, 2021, 21, 4517-4548.
- 4. P. Upadhyay, F. Surur and V. Singh, *Diagnostics*, 2024, 14, 2350.
- T. Y. Kim, J.-Y. Kim, H. J. Shim, S. A. Yun, J.-H. Jang, H. J. Huh, J.-W. Kim and N. Y. Lee, *J. Virol. Methods*, 2021, 298, 114304.
- C. Ranadheera, G. J. German, L. Steven, D. Eung, D. Lyubashenko, J. C. Pepin, M. Zivcec,
 K. Antonation and C. R. Corbett, *Sci. Rep.*, 2022, **12**, 4947.
- A. L. Leber, J. G. Lisby, G. Hansen, R. F. Relich, U. V. Schneider, P. Granato, S. Young, J. Pareja and I. Hannet, *J. Clin. Microbiol.*, 2020, 58, 00155-00120.
- L. Peñarrubia, S. N. Rao, R. Porco, M. Varo, P. Muñoz-Torrero, F. Ortiz-Martinez, J. Pareja,
 M. López-Fontanals and D. Manissero, *Sci. Rep.*, 2023, 13, 2833.
- M. Caza, J. Hayman, A. Jassem and A. Wilmer, *Diagn. Microbiol. Infect. Dis.*, 2024, 110, 116368.

- 10. J. A. Otoo and T. S. Schlappi, *Biosensors*, 2022, **12**, 124.
- 11. B. Bruijns, L. Folkertsma and R. Tiggelaar, *Biosens. Bioelectron.:X*, 2022, **11**, 100158.
- V. Narasimhan, H. Kim, S. H. Lee, H. Kang, R. H. Siddique, H. Park, Y. M. Wang, H. Choo,
 Y. Kim and S. Kumar, *Adv. Mater. Technol.*, 2023, 8, 2300230.
- L. M. Holtgrewe, S. Jain, R. Dekova, T. Broger, C. Isaacs, G. Theron, P. Nahid, A. Cattamanchi, C. M. Denkinger and S. Yerlikaya, *J. Clin. Med.*, 2024, 13, 5894.
- N. Kanwar, J. Michael, K. Doran, E. Montgomery and R. Selvarangan, J. Clin. Microbiol., 2020, 58, 01611-01619.
- K. Mitamura, H. Shimizu, M. Yamazaki, M. Ichikawa, T. Abe, Y. Yasumi, Y. Ichikawa, T.
 Shibata, M. Yoshihara and K. Shiozaki, *J. Infect. Chemother.*, 2020, 26, 216-221.
- 16. L. Smy, N. A. Ledeboer and M. G. Wood, J. Clin. Microbiol., 2024, 62, e00312-23.
- 17. M. Zahavi, H. Rohana, M. Azrad, B. Shinberg and A. Peretz, *Diagnostics*, 2022, 12, 1877.

SUPPLEMENTAL FIGURE



Fig. S1 Experimental snapshots demonstrating four synchronized fluid-flow states in three detection channels