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 He[a]

[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

Supplementary Figure

SUPPLEMENTAL TABLES

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

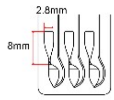
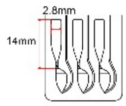
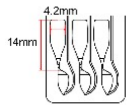
Pathogen	Sequence length (bp)	Description	Sequence (5′ to 3′)
FluA	135 bp	Forward Primer	5’-ATCGCGCAGAGACTTGAAGATGTCTTTGCTGG-3'
		Reverse Primer	5’-CACGGTGAGCGTGAACACAAACCCCAAAATC-3'
		Probe	5'-CTCTCATGGAATGGCTAAAGACAAGACCAATTC[FAM-dT][THF] [BHQ-dT]CACCTCTGACTAAG-3'SpC3
FluB	113 bp	Forward Primer	5’-CTATATGCTTTTTAAAACCCAAAGACCAAGA-3'
		Reverse Primer	5'-CTCAGCTAGAATTAGGCCTTTCTTCTTTGTTGCTG-3'
		Probe	5'-AAAGAAAAAGGAGATTCATCACAGAGCCCC[VIC-dT][THF][BHQ-dT]CAGGAATGGGAACAA-3'SpC3
RSV	116 bp	Forward Primer	5'- ATGGAAACATACGTGAACAAGCTTCACGAAGG-3'
		Reverse Primer	5'- AACATGGGCACCCATATTGTAAGTGATGCAGGGT-3'
		Probe	5'-CTCCACATACACAGCTGCTGTTCAATACAA[ROX-dT][THF][BHQ-dT]CCTAGAAAAAGACGAT-3'SpC3
AdV	125 bp	Forward Primer	5’-TTGCGTGGAGCGTTGGCGAGCGCCTCCGTACATC-3'
		Reverse Primer	5’-CGCGCGTCCTCTCGAGCCGCACCTTCTAAA-3'
		Probe	5’-TTGCTGGGCGCGTGCAAACCCCAACCGGTGTTAT[FAM-dT]A[THF][BHQ-dT]GGGCGAGATGAGAAT-3’SpC3
RhV	118 bp	Forward Primer	5’-ATCTGTTGAGGCTTGTGGCTATTCAGACAGGAT-3'
		Reverse Primer	5’- GTAATGCGGCCAGACCCCATACCCAACCACAG-3'
		Probe	5’- TATGCAAATAACCAGAGGAGATTCAACAATCACA[VIC-dT][THF][BHQ-dT] CAAGATGTAGCAAATG-3’SpC3
HPIV	134 bp	Forward Primer	5’- ATAACAACAAAGACAGAGCAATCTCAACGCCGACCC-3'
		Reverse Primer	5’- GTGTATACTTTGTTGATCAAGGAGTCTAGCATG-3'
		Probe	5’- CCAAGATCATAGATCAGGTGAGGAGAGTGGAATC[ROX-dT] [THF] [BHQ-dT] AGGAGAACAAGTGAGT-3’SpC3
Mpn	157 bp	Forward Primer	5’- TTTGGTGAAATAGCCCACCCCAAACCAATCGCC-3'
		Reverse Primer	5’- AAACTTTAGCTAAGTAATTGCGTGATTTGT-3'
		Probe	5'-TACGGTAGAGGTAGCGGAAACAGTTAAGGCAGC[FAM-dT][THF]C[BHQ-dT]AGCAACTTTGAAA-3'SpC3
Cpn	121 bp	Forward Primer	5’- TTTAAGGCAATGAAGCCTGCATTAGTGAACCACT-3'
		Reverse Primer	5’- CCAAGCCTACTGGATCCGCTGCTGCAAACTATAC-3'
		Probe	5'-CATCGTGTAAATGCTTATTGTAGGCCGGGT[VIC-dT]A[THF]G[BHQ-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

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

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

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

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

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

System	Method	Portability[a]	Pathogens	Targets	Sensitivity/ Specificity (%) (FluA)	Test time (min)	Test Signal	Sample to Answer	Ref.
Cepheid GeneXpert®	PCR	Portable	SARS-CoV-2/FluA/FluB/RSV	4	100/100	36	Fluorescence	Yes	1-3
Biofire Filmarray®	PCR	Portable	AdV/Coronavirus 229E/Coronavirus HKU1/Coronavirus NL63/Coronavirus OC43/ SARS-CoV-2/Human Metapneumovirus/ RhV /Enterovirus/FluA/FluA H1/ FluA H3/ FluA H1-2009/ FluB/ HPIV 1/ HPIV 2/ HPIV 3 /RSV/Bordetella parapertussis/Bordetella pertussis/ Mpn/Cpn	22	100/100	45	Fluorescence	Yes	4-6
QIAgen QIAstat-Dx®	PCR	Portable	FluA/FluA H1N1 2009/FluA H1/FluA H3/FluB/Coronavirus 229E/Coronavirus HKU1/Coronavirus NL63/Coronavirus OC43/SARS-CoV-2/HPIV1/HPIV2/HPIV3/HPIV4/RSV A/B/Humanmetapneumovirus A/B/Adv /Bocavirus/RhV/Enterovirus/Mpn/Legionella pneumophilia/Bordetella pertussis	22	99.2/99.5	60	Fluorescence	Yes	7-9

Visby Medical™	PCR	Ultraportable	SARS-CoV-2/FluA/FluB	3	100/99.1	30	Colorimetric (LFA)	Yes	3, 10-13
Cue™	Isothermal	Ultraportable	SARS-CoV-2/FluA/FluB	3	/	20	Electrochemical	Yes	3, 12
Abbott ID NOW™	NEAR	Portable	FluA/FluB	2	96.3/97.4	13	Fluorescence	No	3, 14, 15
Lucira™	LAMP	Ultraportable	SARS-CoV-2/FluA/FluB	3	91.4/99.8	30	Colorimetric (LFA)	Yes	3, 12, 13, 16, 17
DETECT™	LAMP	Ultraportable	SARS-CoV-2/FluA/FluB	3	/	30~60	Colorimetric (LFA)	No	11
Midge Medical	RPA	Ultraportable	FluA/FluB	1	/	28	Fluorescence	No	/
Our System	**RPA**	**Ultraportable**	**FluA, FluB, RSV, AdV, RhV,HPIV, Mpn, Cpn**	**8**	**100/100**	**25**	**Fluorescence**	**Yes**	**/**

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

References

1. 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.
2. 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.
3. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.

12. 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.

13. 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.

14. N. Kanwar, J. Michael, K. Doran, E. Montgomery and R. Selvarangan, *J. Clin. Microbiol.*, 2020, **58**, 01611-01619.

15. 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

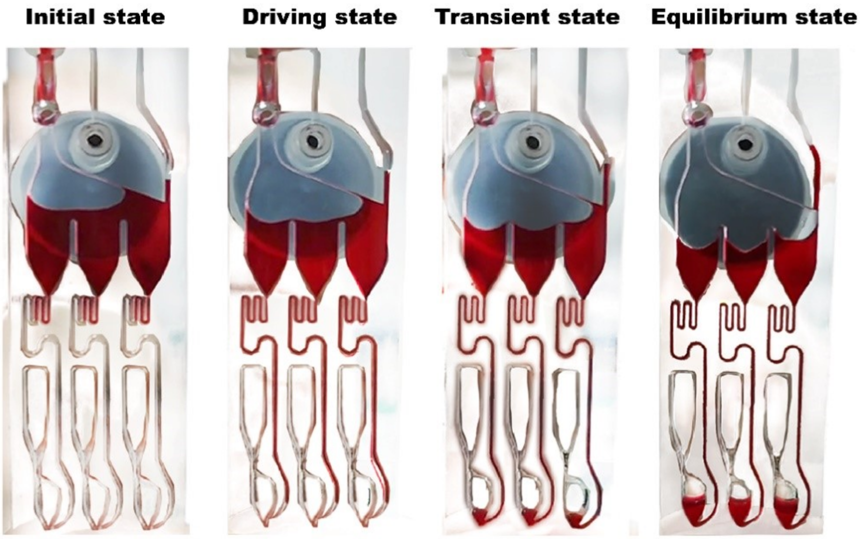


Fig. S1 Experimental snapshots demonstrating four synchronized fluid-flow states in three detection channels