

Supporting Information

A Sample-to-Answer, Quantitative Real-time PCR System with Low-Cost, Gravity-Driven Microfluidic Cartridge for Rapid Detection of SARS-CoV-2, Influenza A/B, and Human Papillomavirus 16/18

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SUPPLEMENTAL FIGURES

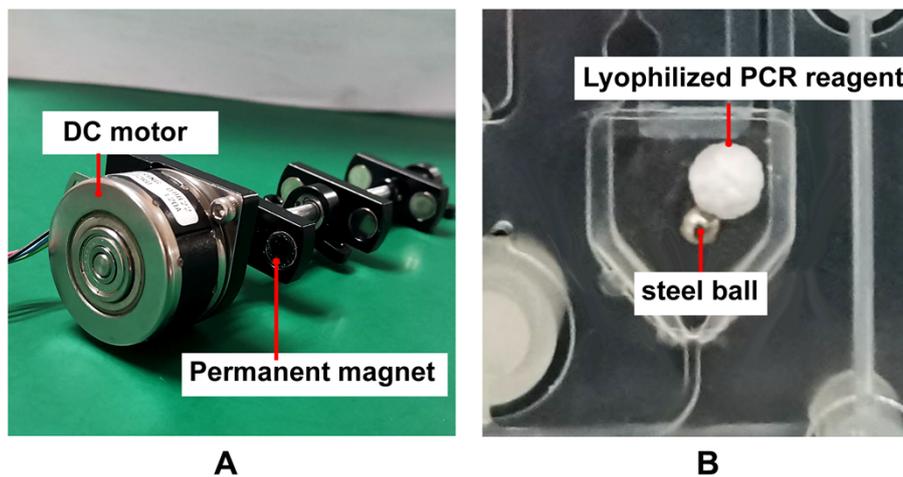


Fig. S1 Structural diagram of magnetic mixing module: (A) a DC motor and a permanent magnet; (B) a steel ball and a spherical lyophilized PCR reagent pre-stored in the mixing chamber.

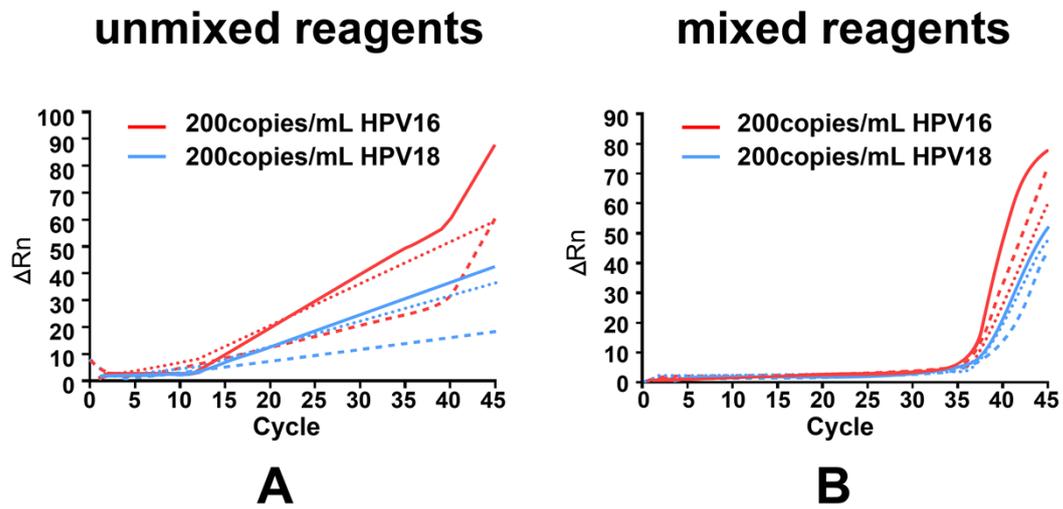


Fig. S2 qPCR amplification curves of the HPV16/18 cartridge tests with (A) unmixed reagents and (B) reagents mixed by our magnetic mixing module, respectively.

SUPPLEMENTAL TABLES

Table S1. Verification of the limit of detection of SARS-CoV-2, Influenza A and Influenza B cartridge test

Rep#	Ct value of SARS-CoV-2 test			Ct value of Influenza A test			Ct value of Influenza B test		
	500 copies/ml	200 copies/ml	100 copies/ml	500 copies/ml	200 copies/ml	100 copies/ml	500 copies/ml	200 copies/ml	100 copies/ml
1	33.63	35.18	36.27	34.08	35.91	38.3	33.81	34.25	35.81
2	33.22	34.39	36.25	33.35	36.38	37.63	33.85	35.21	36.65
3	33.59	34.87	N/A	33.87	34.77	N/A	33.46	35.23	N/A
4	33.68	34.28	36.11	33.64	35.32	37.07	34.26	34.33	36.31
5	33.03	34.83	35.84	34.24	36.04	38.03	34.32	34.92	36.92
6	33.79	35.07	N/A	33.92	35.18	N/A	33.81	35.05	36.65
7	34.05	35.24	36.58	33.88	34.85	37.4	33.82	34.35	36.97
8	33.01	35.23	35.72	34.37	36.32	N/A	33.43	35.18	N/A
9	33.42	34.34	36.49	33.38	35.38	N/A	34.03	34.95	37.09
10	33.49	34.37	36.91	34.12	36.05	36.9	33.79	34.29	38.03
11	33.98	34.24	N/A	33.65	N/A	36.66	34.32	35.72	36.51
12	34.05	35.12	37.04	34.29	36.25	38.37	33.78	35.09	N/A
13	34.16	34.70	N/A	34.35	34.85	37.82	33.53	34.45	36.84
14	33.69	34.22	36.8	33.93	36.14	N/A	33.97	34.81	37.21
15	33.77	34.71	36.74	33.61	35.81	37.83	33.55	35.02	36.98
16	33.53	34.35	36.73	33.69	35.86	36.88	33.75	34.94	36.26
17	33.35	34.72	36.96	33.59	34.5	N/A	33.77	34.74	36.76
18	33.52	34.32	36.96	33.69	36.11	38.21	34.17	35.24	N/A
19	33.58	35.32	36.41	33.58	34.92	N/A	34.22	34.47	36.62
20	34.31	34.98	37.12	34.24	34.59	38.08	33.92	35.18	N/A
Positivity Rate	100.00%	100.00%	80.00%	100.00%	95.00%	65.00%	100.00%	100.00%	75.00%

Table S2. Verification of the limit of detection of HPV16 and HPV18 cartridge test

Rep#	Ct value of HPV16 test			Ct value of HPV18 test		
	500 copies/ml	200 copies/ml	100 copies/ml	500 copies/ml	200 copies/ml	100 copies/ml
	1	34.01	35.26	35.78	34.11	34.79
2	33.52	34.91	36.42	33.88	35.08	35.81
3	33.75	35.40	N/A	34.28	34.82	N/A
4	34.04	35.02	N/A	33.75	34.60	36.18
5	33.64	35.54	36.48	33.82	35.10	35.56
6	33.77	34.98	N/A	33.67	35.23	35.93
7	34.38	35.28	36.26	33.74	34.69	35.95
8	34.23	35.48	36.47	33.50	35.30	36.14
9	34.14	35.28	N/A	34.21	35.22	35.98
10	33.76	35.12	35.60	33.74	35.25	N/A
11	33.84	35.25	36.26	33.68	35.31	N/A
12	34.09	35.02	N/A	33.97	35.46	36.56
13	33.90	35.04	35.54	33.78	34.97	37.14
14	33.58	35.03	N/A	33.82	34.95	35.62
15	34.37	35.34	36.34	33.73	34.90	N/A
16	34.26	35.20	37.04	33.89	34.85	36.29
17	33.74	35.54	36.26	33.34	35.08	36.23
18	33.97	35.29	N/A	33.74	35.09	35.61
19	34.03	35.41	35.97	33.61	35.28	36.17
20	33.78	34.95	N/A	34.18	35.14	N/A
Positivity Rate	100.00%	100.00%	60.00%	100.00%	100.00%	75.00%

Table S3. Relationship between mixing effects and the resulting limits of detection (LOD) of HPV18 test under different mixing settings of rotation speed and mixing time

No.	Rotation speed (r/min)	Mixing time (s)	Mixing effect (turbidity)	LOD (copies/mL)
1	/	/	Very turbid	10000
2	100	10	Turbid	2000
3	100	20	Slightly turbid	500
4	100	30	Slightly turbid	500
5	150	10	Slightly turbid	500
6	150	20	Transparent	200
7	150	30	Transparent	200
8	200	10	Air bubbles	5000
9	200	20	Air bubbles	5000

Table S4. Comparison of the detection specificity and sensitivity for nucleic acid detection of respiratory pathogen using our gravity-driven POC cartridge system and 5 popular commercially available POC platforms afforded with active microfluidic pumping techniques

POC platform	Microfluidics pumping (Active/Passive)	SARS-CoV-2	Influenza A	Influenza B
		Sensitivity/ Specificity	Sensitivity/ Specificity	Sensitivity/ Specificity
Our system	Passive	97.6% /	100% /	100% /
	Gravity-driven	100%	100%	100%
Cepheid Xpert Xpress ¹	Active	100% /	100% /	100% /
	Pneumatic	100%	100%	100%
BioFire [®] Filmarray ²	Active	98.4% /	100% /	100% /
	Pneumatic	98.9%	100%	99.9%
QIAstat-Dx ^{®3}	Active	100% /	99.2% /	94.6% /
	Pneumatic	100%	99.5%	99.9%
Cobas Liat ⁴	Active	100% /	98.4% /	97.9% /
	Pneumatic	100%	96.5%	99.4%
GenePOC Revogene ⁵	Active Centrifugal-driven	97.7% / 97.7%	/	/

Reference

- [1] Cepheid Xpert Xpress CoV-2/Flu/RSV plus - Instructions for Use(fda.gov), <https://www.fda.gov/media/152163/download>, (accessed October 2021)
- [2] BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ) - Instructions for Use (fda.gov). <https://www.fda.gov/media/142696/download> (accessed April 2022)
- [3] QIAstat-Dx Respiratory SARS-CoV-2 Panel - Instructions for Use (fda.gov), <https://www.fda.gov/media/136571/download> (accessed July 2021)
- [4] cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test for use on the cobas Liat System - Instructions for Use (fda.gov), <https://www.fda.gov/media/142193/download>, (accessed March 2022)
- [5] Revogene SARS-CoV-2 - Instructions for Use (fda.gov), <https://www.fda.gov/media/154060/download>, (accessed November 2021)