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Electronic Supplementary Information

A self-contained all-in-one cartridge for sample preparation and real-time PCR in rapid influenza diagnosis

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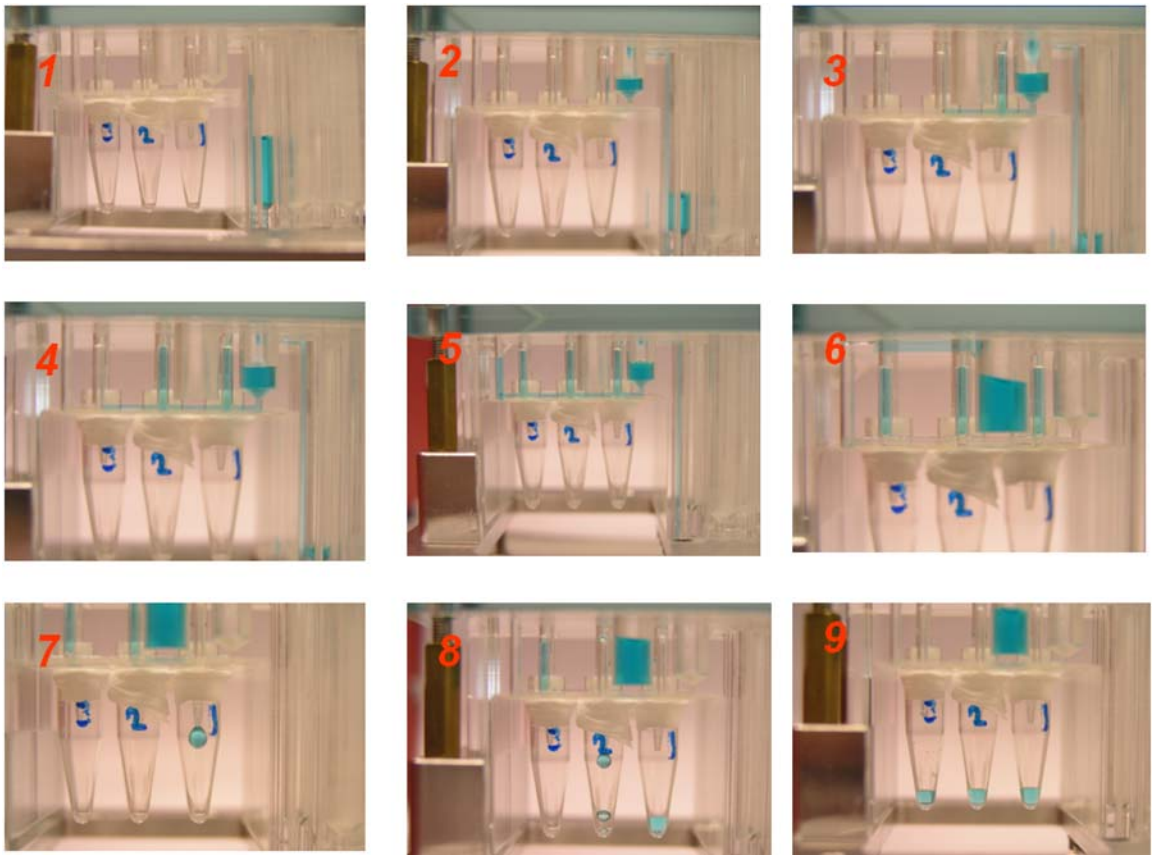

Fig. S1 Time sequence photographs of aliquot dispensing of RNA eluent, demonstrated with blue food dye. (1) The eluent passed through the silica membrane, and (2) began to fill up the eluent chamber. (3–5) As the eluent chamber was filled, passive valves were sequentially filled up to the constriction. (6) Excess eluent was directed to the excess eluent chamber. Note that the fluid within each aliquot reservoir was isolated. (7–9) Precise dispensing of eluent aliquots into the respective PCR tubes 1–3.

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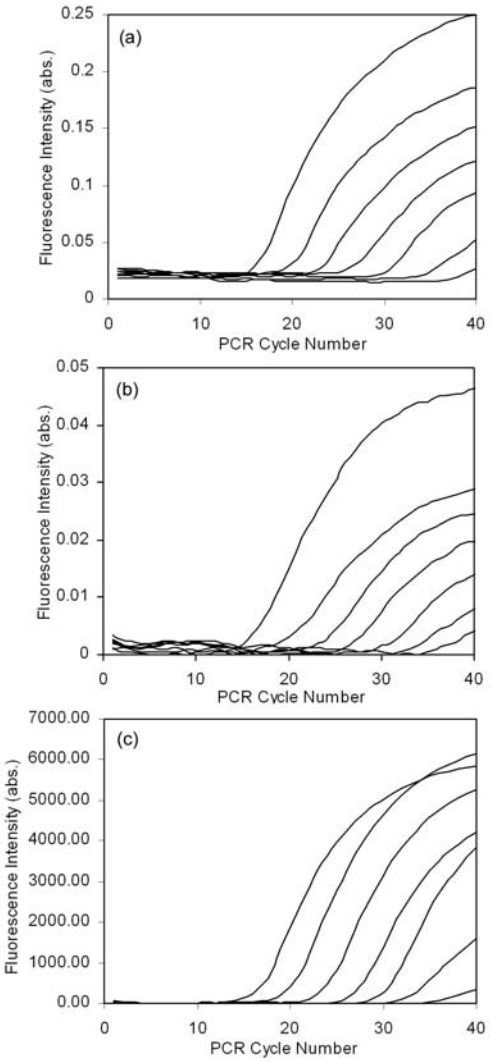

Fig. S2 On-cartridge real-time PCR. The real-time fluorescence curves of serial diluted (1–10^6 folds) GAPDH cDNA, amplified and measured with (a) the custom-built thermal cycler, (b) the MJ Research Opticon system, and (c) the Bio-Rad CFX96 system. The custom-built thermal cycler utilized LEDs as light source and a PMT for detection, the MJ Research Opticon system employed LEDs plus PMTs, and the Bio-Rad CFX96 system used LEDs and photodiodes.

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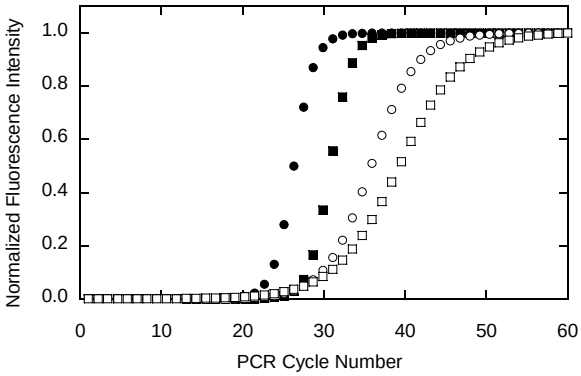

Fig. S3 On-cartridge real-time PCR. The real-time fluorescence curves of serial diluted ((●) 0-fold, (■) 10-fold, (○) 10^2-fold and (□) 10^3-fold) influenza A, amplified and measured with the custom-built thermal cycler and detection system.

Table S1 C_T values of real-time RT-PCR of mouse GAPDH spiked with various amounts of reagents of QIAamp Viral RNA Mini Kit. RT-PCR was performed with Taqman RNA-to-C_T 1-Step Kit (4392938, Applied Biosystems) in a Bio-Rad CFX-96 system with 20 µl of reaction mixture containing 0.5 µl of TaqMan RT Enzyme Mix, 10 µl of TaqMan RT-PCR Mix, 1 µl of Taqman Assays-by-Design for GAPDH (Mm99999915_g1), 5.5 µl (0.05 ng/µl) of mouse liver total RNA (7810, Ambion), and 3 µl of serial diluted reagents ($1-10^4$ folds diluted in nuclease-free water) of QIAamp kit. The RRT-PCR was conducted at 48°C for 15 min and 95°C for 10 min, with 40 cycles of 95°C for 15 s and 60°C for 60 s. The C_T value of control experiment with nuclease-free water was 21.52.

Spiked Chemicals	Vol% of Spiked Chemicals*			
	15%	1.5%	0.15%	0.015%
Wash Buffer AW1	—[†]	—[†]	—[†]	22.23
Wash Buffer AW 2	—[†]	—[†]	20.99	22.82
Lysis Buffer	—[†]	—[†]	22.81	—[#]
Ethanol	—[†]	—[†]	22.13	21.93

* For example, 15 vol% was represented by 3 µl of reagent in 20 µl of reaction mixture, while 1.5 vol% was represented by 3 µl of 10× diluted reagent in 20 µl of reaction mixture.
[†] The RT-PCR experiment failed.
[#] This experiment was not conducted.

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Table S2 C_T values of real-time RT-PCR of RNA extracted by the all-in-one system vs. the Qiagen spin column, and the original unpurified sample.

Total Liver RNA Loaded (ng/μl)	Unpurified Liver RNA (C_T)	Qiagen Spin Column (C_T)	All-in-One Extraction (C_T)
1000	18.4	19	19.5
100	20.9	23.2	22.5
10	25.3	27.2	26
1	28.7	29	30.6
0.1	31.9	—[†]	—[†]

[†] The RT-PCR experiment failed.

Table S3 C_T values of the real-time fluorescence curves of serial diluted (1–10^4 folds) influenza A patient samples.

Influenza A (Dilution Folds)	Qiagen Spin Column + Bio-Rad CFX96 System (C_T)	All-in-One Extraction + Bio-Rad CFX96 System (C_T)	All-in-One System (C_T)
1	23.03	24.70	25.74
10^1	27.23	28.57	30.26
10^2	30.14	32.40	33.03
10^3	33.96	35.20	37.68
10^4	36.49	38.24	—[†]

[†] The RT-PCR experiment failed.