Electronic Supplementary Information

Ultrafast rotary PCR system for multiple influenza viral RNA detection

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Rotary microchip fabrication

A SU-8 50 (MicroChem, MA, USA) photoresist was spun on a Si wafer and patterned by UV exposure through the mask layout designed by AutoCAD. PDMS pre-polymer and curing agent (Sylgard 184, Dow corning, MI, USA) were mixed with a 10:1 ratio and poured onto the master. After curing at 65 °C for 1 h, the PDMS replica was obtained, and bonded with a glass cover slip (18 mm × 18 mm, Marienfeld, Germany) by plasma treatment for 1 min.

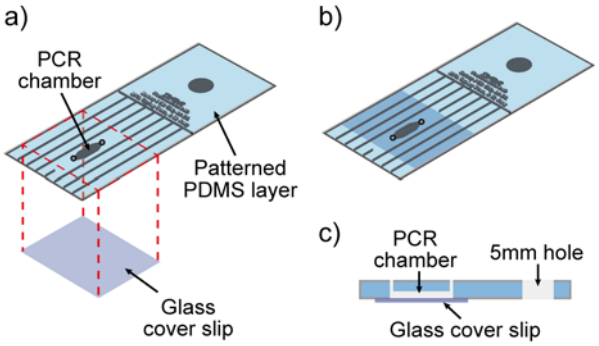

Fig. S1 A disposable Rotary microchip: a) A patterned PDMS layer and a glass cover slip, b) schematics for an assembled Rotary microchip, and c) side view of the Rotary microchip.

Design of the electrical circuits

A 4 mA current source was supplied to the RTD through the outer set of leads, and the measured voltage across the RTD was collected and processed using an active low-pass filter, whose signal was transferred to the DAQ board. Temperature control was accomplished through a proportion/integrator/differentiator (PID) module within a LabVIEW program, which outputs through the DAQ board to control the power supply of the film heaters within the thermal blocks.

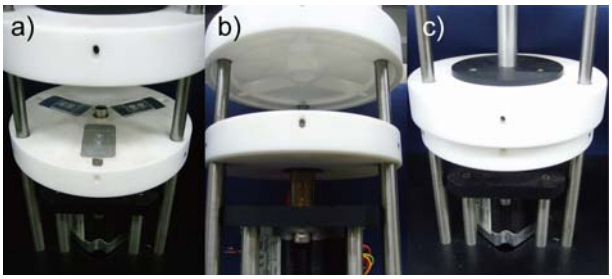

Fig. S2 Digital image for a Rotary Genetic Analyzer platform: a) Top view showing the Rotary stage which incorporates three thermal blocks, b) bottom view showing a upper teflon cover with block-shaped hollow, and c) a tightly sealed Rotary PCR system for thermal cycling.

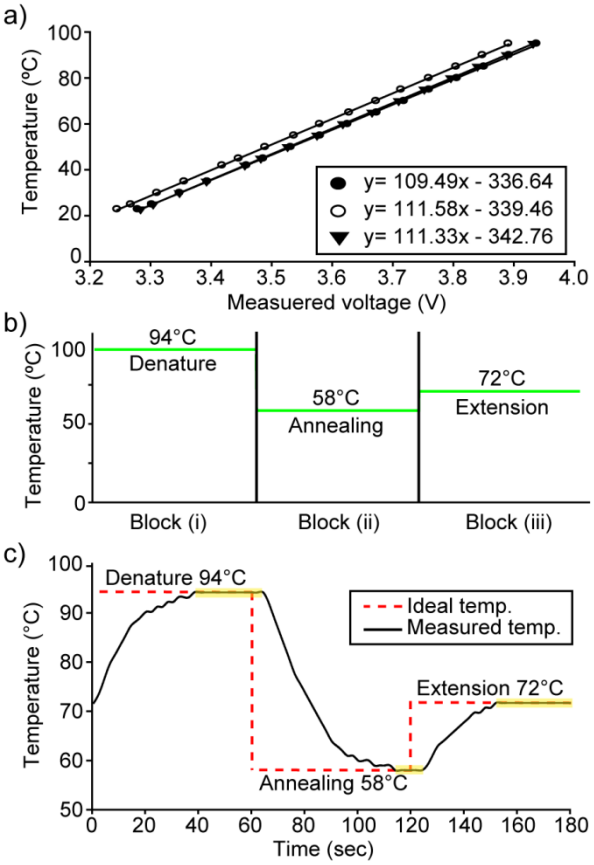

Fig. S3 a) RTD calibration curves for three thermal blocks, b) temperature profiles of the three thermal blocks, and c) temperature profile of a Rotary microchip during thermal cycling with 94˚C denature for 60 sec, 58 ˚C annealing for 60 sec, and 72 ˚C extension for 60 sec.

Table S1 Information of primer sequences for genotyping Influenza A H1N1, H3N2, and H5N1

Sub-type	*Target genes*	*Primers sequences(5'→3')*	*Amplicon (bp)*	*Melting temperature*
H1N1	H1	F: FAM-GTG CTA TAA ACA CCA GCC TYC CA R: CGG GAT ATT CCT TAA TCC TGT RGC	102	58.4 °C 59.6 °C
H3N2	H3	F: FAM-AAT GAC CAA ATC TTC CCG TAT G R: TGC TTA TTC TGC TGG GGA TAT T	150	54.3 °C 54.4 °C
H5N1	H5	F: FAM-AAG CTC TAT CAA AAC CCA ACC A R: CAA AAG AAC TCC ATC CTT CCA C	172	54.3 °C 54.1 °C
Influenza A	M	F: FAM-ATC AGG CAT GAA AAC AGA ATG GT R: TGG AGC TAG GAT GAG TCC CAA TA	160	56.2 °C 56.4 °C

RT-PCR cocktail preparation

A 20 μL of RT-PCR mixture consisted of 4 μL of 5 X RT-PCR buffer, 0.8 μL of 10 mM dNTP mix, 2 μL of the enzyme mix, 1.2 μL of each primer sets with 10 μM for forward and reverse primers, and 2 μL of RNA templates with a final concentration of 12 pg/μL except for the limit of detection study. From the RT-PCR cocktail, 1 μL of the mixture was loaded into the PCR chamber

RT-PCR operation in Rotary system

Firstly, reverse transcription was carried out at 50 °C for 15 min on the block (ii) in the Fig. 1, and an initial activation step was conducted at 95 °C for 5 min on the block (i). After adjusting the temperature of block (i) to 94 °C for denature, block (ii) to 58 °C for annealing, and block (iii) to 72 °C for extension, the microchip was spun on the thermal block (i), (ii), and (iii) successively for PCR amplification. The RTD calibration of each heat block was obtained as shown in Fig. S1a and the low thermal mass of PCR chamber was rapidly heated and naturally cooled on the Rotary system. Total cycle number was 34, and the final extension was performed for 7 min at 72 °C.

Table S2 Representative thermal cycling scheme of the RT-PCR in the Rotary system

Process		*Temper-ature*	*Process time*	*Block No.* [1)]
Reverse transcription		50 °C	15 min	(ii)
Initial activation		95 °C	5 min	(i)
Thermocycling (34 cycles)	Denature	94 °C	1 min	(i)
	Annealing	58 °C	1 min	(ii)
	Extension	72 °C	1 min	(iii)
Final extension		72 °C	7 min	(iii)

[1)] The block number (i), (ii), (iii) is referred from Fig. 1

Table S3 Control of the thermal block temperature according to the reduced thermocycling time for rapid Rotary RT-PCR reaction

Thermocycling process	*Denature*	*Annealing*	*Extension*
60/60/60 sec	94 °C	58 °C	72 °C
30/60/30 sec	94 °C	58 °C	72 °C
30/30/30 sec	94 °C	52 °C	72 °C
15/30/15 sec	96 °C	50 °C	76 °C
15/15/15 sec	96 °C	47 °C	76 °C