Electronic Supplementary Information

A Microbead Incorporated Centrifugal Sample Pretreatment Microdevice

Jae Hwan Jung,[a] Byung Hyun Park,[a] Young Ki Choi[b] and Tae Seok Seo*[a]

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Materials

Pure RNA and lysates of influenza A H3N2 virus were supplied from College of Medicine in Chungbuk National University. A PC sheet (1 mm thickness) was purchased from JinHung Steen & Lexan (Suwon, Korea), and a PC film (125 μm thickness) was kindly donated from Sejin T.S Co., Ltd. (Anseong, Korea). A pressure sensitive adhesive film (30 μm thickness) was obtained from Coretech (Ansan, Korea). Acid-washed glassbeads (150~212 μm diameter), TEOS, Gu-HCl, and ethanol were purchased from Sigma-Aldrich (Missouri). RNeasy Mini kit (Qiagen, Germany) was used for the off-chip based RNA purification. For the real-time PCR, primers and TaqMan probes were ordered from Neoprobe (Daejeon, Korea), and One-step PrimeScript™ RT-PCR kit was purchased from Takara Korea Biomedical Inc. (Daejeon, Korea).

Quantification of RNA capture yield

The initial concentration of the influenza A H3N2 virus was 2.14 × 10^5 $TCID_{50}$/mL. After lysis, the viral RNA was purified according to manufacturer's protocols, and the RNA copy number was calculated by a UV/Vis spectrometer (UV-2450, Shimadzu, Japan). A certain amount of virus lysates was used for the RNA capture experiment on the proposed centrifugal microdevice. We evaluated the capture yield by using real-time RT-PCR. To obtain the calibration curve for quantification, we performed the real-time RT-PCR with a serially diluted RNA template ranging from 10^6 to 10^3 copy number. 4 μL of the purified RNA solution was produced after RNA extraction on the centrifugal sample pretreatment microdevice as explained above. We took 2 μL from the purified RNA solution to prepare 20 μL of a real-time RT-PCR cocktail which consists of 5.6 μL of RNase-free water, 10 μL of 2× One Step RT-PCR buffer III, 0.4 μL of Takara Ex Taq HS (5U/ μL), 0.4 μL of PrimeScript RT enzyme Mix II, 0.4 μL of a forward primer (10 μM), 0.4 μL of a reverse primer (10 μM), and 0.8 μL of a TaqMan probe (10 μM). The sequence of TaqMan probe for detecting H1 gene was 5'-(FAM)–CAG AAT ATA CA CCR ATC ACA ATT GGA RAA–BHQ1-3'. The sequence of the forward primer was 5'-TGC TAT AAA CAC CAG CCT YC-3', and the sequence of the reverse primer was 5'-CGG GAT ATT CCT TAA TCC TGT G-3' which were designed for amplifying H1 gene (115 bp). The sequence of TaqMan probe for detecting H3 gene was 5'-(FAM)–CCC AGC ATT GAA CGT GAC TTA TGC CAA–BHQ1-3'. The sequence of the forward primer was 5'-TGG TTG ACC CAC TTA AAA TTC AAA-3', and the sequence of the reverse primer was 5'-GTG AAC CCC CCA AAT GTA CAA-3' which were designed for amplifying H3 gene (98 bp). The sequence of TaqMan probe for detecting M gene was 5'-(FAM)–CTC TCT ATC ATC CCG TCA GGC CCC C–BHQ1-3'. The sequence of the forward primer was 5'-AGT CTT CTA ACC GAG GTC GAA-3', and the sequence of the reverse primer was 5'-TGG ACA AAG CGT CTA CGC T-3' which were designed for amplifying M gene (239 bp). The thermal cycling protocol of the real-time RT-PCR was as follows: a reverse-transcription step at 42 ºC for 5 min, an initial heat inactivation step at 95 ºC for 10 sec, 45 cycles of denaturation at 94 ºC for 10 sec, annealing at 58 ºC for 30 sec, and extension at 72 ºC for 30 sec. The real-time RT-PCR was conducted by a CFX Connect™ Real-Time PCR System (Biorad, California).

Table S1 Sequence of the rotational operation[a]

Figure	Speed (RPM)	Time (sec)	Operation
2a	5000	10	Loading of the RNA sample
2b	5000	300	Loading of the washing solution and drying
2c	0	30	Siphon priming of the elution solution and removing the wastes
2d	2000	10	Loading of the elution solution
2e	5000	90	Complete recovery of the elution solution

[a] Total time : 440 sec

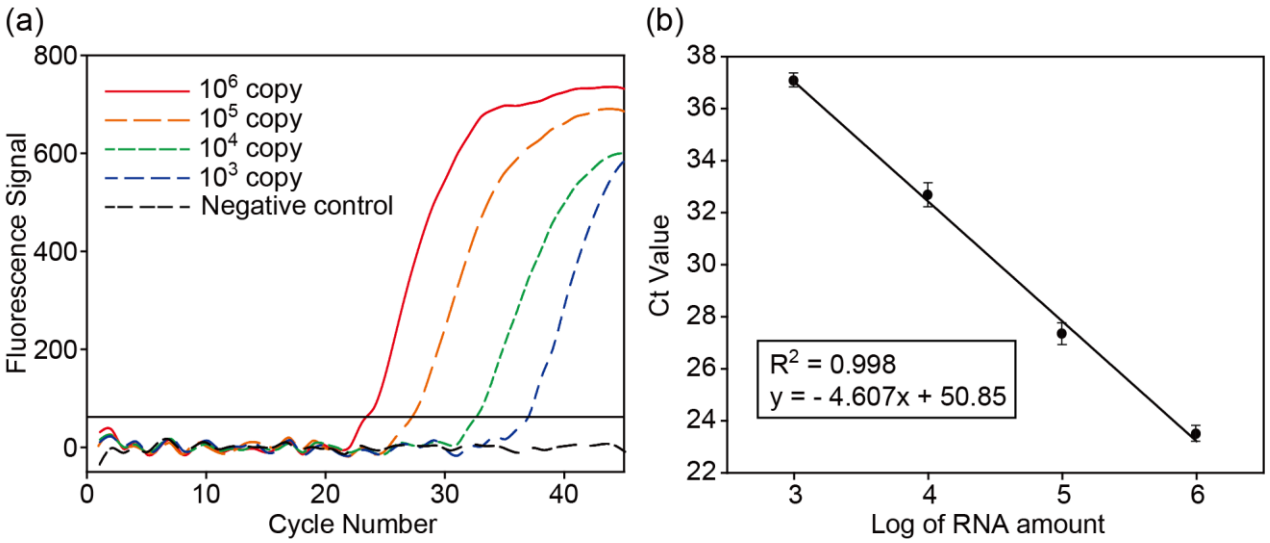

Fig. S1 (a) Real-time RT-PCR data using the purified serially diluted RNA templates of influenza A H3N2 virus. (b) A calibration curve (the Ct value *vs.* the logarithm of RNA copy number).