**Electronic Supplementary Information**

# Integration of reverse transcriptase loop-mediated isothermal amplification with an immunochromatographic strip on a centrifugal microdevice for influenza A virus identification

**J. H. Jung, B. H. Park, S. J. Oh, G. Choi, and T. S. Seo***

***Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX***
**DOI: 10.1039/b000000x**

**Fabrication of the ICS**

The ICS made of a buffer loading pad (3.6 mm x 18 mm), a conjugate pad (3.6 mm x 0.8 mm), an absorbent pad (3.6 mm x 19 mm ) that were purchased from Ahlstrom(Helsinki Finland). A nitrocellulose membrane (3.6 mm x 25 mm x 12 um) was purchased from M.D.I (India). The nitrocellulose membrane was attached on the polystyrene plate and then the absorbent pad was adhesive on the nitrocellulose membrane at the left side. The buffer loading pad and conjugate pad were attached on the nitrocellulose membrane at the right side. Briefly, colloidal Au NPs (avg. diameter:48.9 nm) were synthesized by adding 15 ml of chloroauric acid (2%, w/v) to 1.5 L of a de-ionized water and heating at 95 ℃ followed by boiling for 5 min. Then 5.1 ml of trisodium citrate (10%, w/v) was added and heated to 95℃ for 5 min. Streptavidin (0.05 mg) and mouse IgG-AuNPs were mixed with 10 ml of colloidal AuNPs solution at pH 6.5. The Au NP conjugate streptavidin and mouse IgG were sprayed onto a conjugate pad. The Goat anti-mouse IgG (1 mg/ml), Texas Red IgG (1 mg/ml) and Digoxigenin IgG (2.5 mg/ml) were jetted linearly onto the nitrocellulose membrane using a dispensing platform to form a test and control line (0.1 uL/mm$^2$). The nitrocellulose membrane, the conjugate pad, the buffer loading pad and the absorbent pad were assembled with an adhesive card.[1,2]

**Table S1. Primer designs for multiplex RT-LAMP reaction to identify influenza A virus**

| Target gene | Primer | Sequence (5' to 3') | Length (bp) |
|---|---|---|---|
| H1 | F3 | AAGCTCAGCAAATCCTACA | 19 |
| | B3 | TCCCTCACTTTGGGTCTT | 18 |
| | FIP | GACTTTGTTGGTCAGCACTAGTAGA-AAAGGGAAAGAAGTCCTCG | 44 |
| | BIP | ATCAGAATGCAGATGCATATG-GCTATTTCCGGCTTGAACT | 40 |
| | LF | **(Texas Red)** GATGGTGAATGCCCCATAGC | 20 |
| | LB | **(Texas Red)** TTTTGTGGGGACATCAAGATACAG | 24 |

| | | | |
|---|---|---|---|
| **H3** | F3 | GAGCTGGTTCAGAGTTCCT | 19 |
| | B3 | GCATAATCCGGCACATC | 18 |
| | FIP | AGAGCATCTATTAGTGTGCAGTTTTC-AACAGGTGAAATATGCGAC | 45 |
| | BIP | GAGACCCTCAGTGTGATGGC-GTAACAGTTGCTGTAGGCT | 39 |
| | LF | **(Texas Red)** CCATCAAGGATCTGATGAGGACT | 23 |
| | LB | **(Texas Red)** AATGGGACCTTTTTGTTGAACG | 22 |
| **H5** | F3 | GAACTCTAGACTTTCATGACTCAA | 24 |
| | B3 | TTACTCCACTTATTTCCTCTCTT | 23 |
| | FIP | CGTTACCAAGCTCCTTTGC-ATGTCAAGAACCTTTACGACA | 40 |
| | BIP | GTTTCGAGTTCTATCACAGAT-TGATTCTTCTGAATACTGCGGGTAGT | 47 |
| | LF | **(Texas Red)** CCCTAAGCTGTAGTCGGACC | 20 |
| | LB | **(Texas Red)** TGGAAAGTGTAAGAAACGGAACG | 23 |
| **M** | F3 | TTCTAACCGAGGTCGAAAC | 19 |
| | B3 | GGACAAAGCGTCTACGC | 17 |
| | FIP | TGTTCTT**Y***CCTGCAAAGACA-TCTATCATCCCGTCAG | 39 |
| | BIP | CTAAAGACAAGACCAATC-ACTGGGCACGGTGAGCG | 40 |
| | LF | **(Digoxigenin)** TCTGYGCGATCTCGGCT | 17 |

***Y = C (50%) + T (50%)**

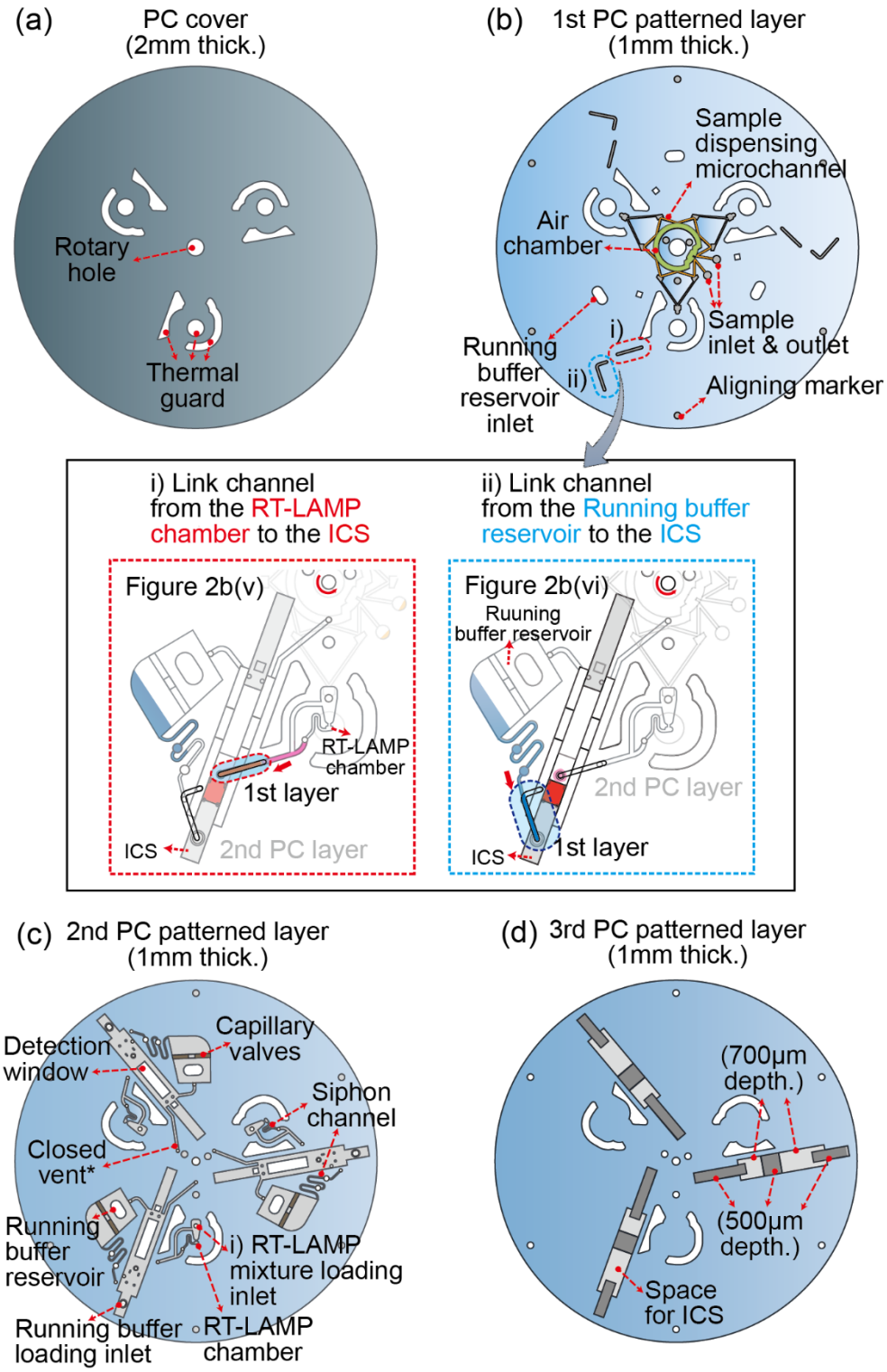


**Figure S1** (a) Schematic illustration of the PC cover. (b) Schematic design of the 1st PC patterned layer. (i) A link channel between the RT-LAMP chamber and the ICS, and (ii) a link channel between the running buffer reservoir and the ICS. (c) Schematic illustration of the 2nd PC patterned layer. (d) Schematic illustration of the 3rd PC patterned layer.

5

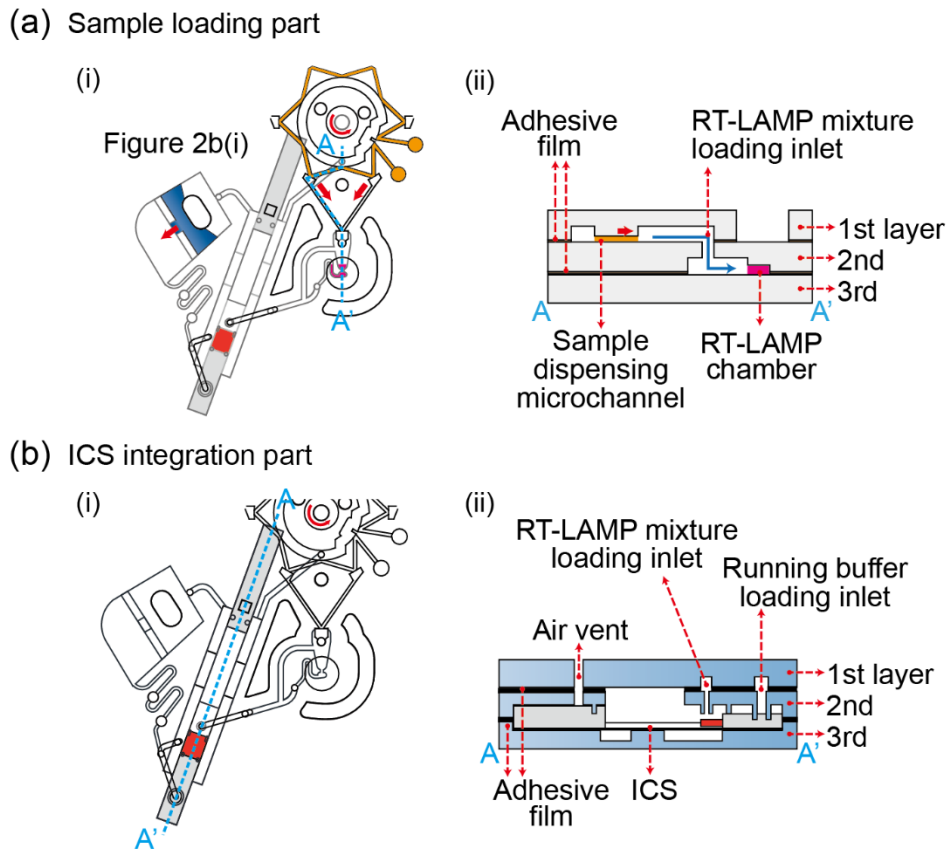


**Figure S2.** (a) Schematic view of the sample loading part, (i) a floor plan and (ii) a cross-section view of the sample loading from the sample dispensing microchannel to the RT-LAMP chamber. (b) Schematic illustration of the ICS integration part, (i) A floor plan and (ii) a cross-section view of the ICS integration part.

## References

1 Y. T. Kim, J. H. Jung, Y. K. Choi and T. S. Seo, *Biosens. Bioelectron.*, 2014, **61**, 485-490.

2 J. H. Jung, S. J. Oh, Y. T. Kim, S. Y. Kim, W.-J. Kim, J. Jung and T. S. Seo, *Anal. Chim. Acta*, 2014, DOI: 10.1016/j.aca.2014.10.020.