Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

A fully integrated microdevice for a biobarcode assay based biological agent detection

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Materials

Three inactivated target bacteria (*Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*), one inactivated target virus (Vaccinia virus), and one inactivated toxin (Botulinum toxin A) were donated from the Korea Centers for Disease Control and Prevention (KCDC). Monoclonal antibodies of *B. anthracis*, *F. tularensis*, *Y. pestis*, and VV were also obtained from the KCDC and that of BoNT/A was purchased from Hytest Ltd. (3Cb20, Turku, Finland). Biotinylated rabbit polyclonal antibody of *B. anthracis* (3BA18), and biotinylated monoclonal antibodies of *F. tularensis* (3FT6) and *Y. pestis* (3YP8) were purchased from Hytest Ltd. (Turku, Finland). Biotinylated rabbit polyclonal antibody of VV was ordered from abcam (England), and polyclonal antibody of BoNT/A was obtained from the KCDC. Two pairs of bracket ladders and five pairs of biotinylated and fluorescein (FAM)-labeled barcode DNAs were ordered from Bioneer (Daejeon, Korea), and their sequence information is shown in Table S1.

Dynamic capillary array coating solution (DEH100) was purchased from The Gel Co. (San Francisco, CA, USA).

Table S1 Sequence information of the barcode DNAs and bracket ladders

Pathogen	Barcode DNA sequence	Length of barcode DNA (bp)
Short bracket ladder	5'- Amine - ACGTGGGGCACACAG - 3' 5'- FAM - CTGTGTGCCCCACGT - 3'	15
Bacillus anthracis	5'- Biotin - GAGGTACATCGAGGTAAGCA - 3' 5'- FAM - TGCTTACCTCGATGTACCTC - 3'	20
Francisella tularensis	5' - Biotin - ATGAGGTGACGTATATTTCTGAGTA - 3' 5' - FAM - TACTCAGAAATATACGTCACCTCAT - 3'	25
Yersinia pestis	5' - Biotin - ATAAGAAACAACTAATACCACATCATCCAT - 3' 5' - FAM - ATGGATGATGTGGTATTAGTTGTTTCTTAT - 3'	30
Vaccinia virus	5' - Biotin - AAAAAATATCATTCTAATTCAGGAGCTTCTGTTAT - 3' 5' - FAM - ATAACAGAAGCTCCTGAATTAGAATGATATTTTTT - 3'	35
Botulinum toxin A	5' - Biotin - AATATAGAAATTAATACCTACTACAAAATAATTAATCATA - 3' 5' - FAM -TATGATTAATTATTTTGTAGTAGGTATTAATTTCTATATT - 3'	40
Long bracket ladder	5' - Amine - TTAATTACAAATTCATACAATTTTTCAATACTAATTAACATATCG - 3' 5' - FAM - CGATATGTTAATTAGTATTGAAAAATTGTATGAATTTGTAATTAA - 3'	45

Synthesis of particle probes

First, two types of the bracket ladder labeled magnetic particles were prepared. The lengths of the bracket ladders were 15 bp and 45 bp. One single stranded DNA (ssDNA) was modified with an amino-functional group at 5' end, and the complementary DNA was labeled with a fluorescein (FAM) dye at 5' end. After hybridization, 500 pmol of the amino- and FAM-labeled double stranded bracket ladder was reacted with 100 μL of tosyl-activated Dynabeads® M-280 (2.8 μm diameter, 30 mg/mL) magnetic beads at 37 °C for 48 hr in a shaking incubator. By an amine linkage between tosyl and amino group, the bracket ladders were conjugated on the surface of the magnetic beads. Then, the bracket ladder labeled particle probes were washed by a phosphate buffered saline (PBS) buffer, and the surface was blocked by a 0.5 % (w/v) bovine serum albumin (BSA) solution to prevent any non-specific bindings of proteins or cells. The final product was washed and resuspended in 1 mL of a PBS buffer and stored at 4 °C prior to use. The loaded DNA amount of the 15 bp and 45 bp ladder probes was 0.846 μg and 2.724 μg in 3 mg of magnetic particles, respectively. The antibody-labeled magnetic particle probes were prepared through biotin-streptavidin interaction. Dynabeads® MyOne™ Streptavidin T1 (1 μm diameter, 10 mg/mL) were used for making the magnetic microparticle (MM) probes. 100 μL of a bead solution were washed with a PBS buffer and gently mixed with 1 mL of a biotinylated monoclonal antibody solution (20 μg/mL) at room temperature for 30 min. The biotin-labeled antibodies were linked on the streptavidin coated MM. After washing the antibody labeled MMs, 1 mL of a hydroxyl polyethylene glycol biotin (Biotin-PEG-OH) solution (2k Da, 32 mg/L) was added to block the surface to prevent non-specific binding of biomolecules. The immobilized quantity of the antibody was calculated by subtracting the amount of the unreacted antibodies from the input amount of antibodies. Almost all of the added antibodies (20 μg) were immobilized on the MM probes due to rapid and efficient reaction between the biotin and the streptavidin.

Lastly, five types of the barcode DNA and antibody labeled polystyrene microparticle (PM) probes were prepared. 10 μg of biotinylated antibodies were added to 200 μL of a streptavidin coated polystyrene microparticle solution (1 μm diameter, 10 mg/mL), and was gently incubated at room temperature for 30 min. After antibody immobilization, the barcode DNAs whose size was 20, 25, 30, 35, and 40 bp were conjugated on the surface of the PM. The double stranded barcode DNAs were produced by hybridization between the

biotin labeled ssDNA and the complementary FAM labeled ssDNA as shown in Table S1. After washing the antibody labeled PMs with 1 mL of a 0.01 M PBS buffer under centrifugation at 13,000 rpm, 500 pmole of the double stranded biotin and FAM labeled barcode DNAs were added to the washed PMs in 200 μL of a binding/wash buffer. Finally, the PMs were blocked by incubating them in 1 mL of a 32 mg/L Biotin-PEG-OH solution at room temperature for 1 hr. After purification, the final PM product was resuspended in 1 mL of a PBS buffer and stored at 4 °C prior to use. The amount of the immobilized antibodies were 10 μg in all the PM probes (2 mg), and the loaded barcode DNA amount for targeting *B. anthracis*, *F. tularensis*, *Y. pestis*, VV, BoNT/A was 6.422 μg, 6.904 μg, 8.696 μg, 6.626 μg, and 12.304 μg for 2 mg PM probes, respectively.

The fabrication process of an integrated microdevice

The microfabrication was performed on a borofloat glass wafer (4-in diameter and 1.1 mm thickness). Micropump channel dimension was 640 μm width × 70 μm height. The passive mixer contains micropillars inside the channel, and its dimension was 11.3 cm length × 640 μm width × 70 μm height. Capillary electrophoretic channel was 7-cm long to separate the barcode DNAs and bracket ladders.

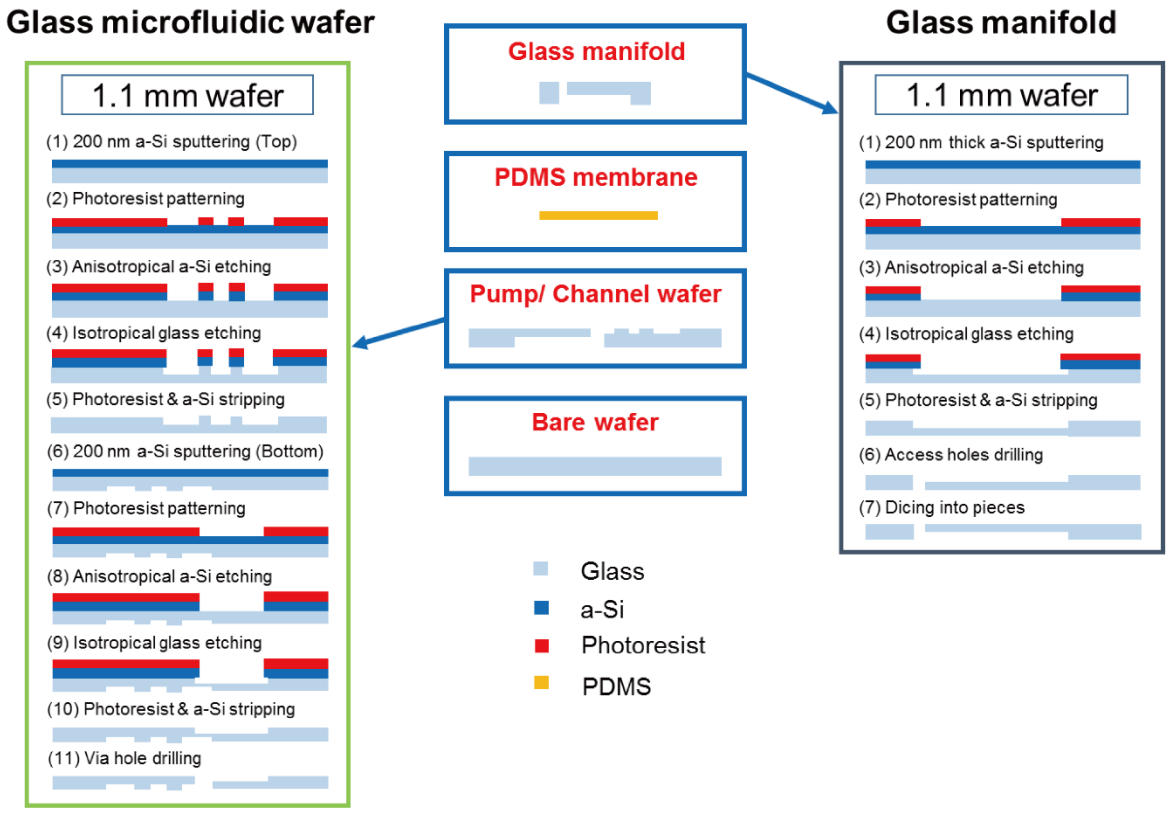

Fig. S1 Fabrication process of the integrated microdevice for multiplex biological agent detection. The integrated microdevice was composed of four layers; a glass manifold and a PDMS monolithic membrane, a glass wafer which included a micropump channel on the top, and a passive mixer, a magnetic separation chamber and a μCE channel on the bottom, and a bare glass wafer.

The calculation of the relative elution time ratio of barcode DNAs

The relative elution time ratio in the CE data was calculated by the following equation. We obtained the absolute t values of the bracket ladders and the target barcode DNA from the on-chip μCE data, and then calculated the relative elution time ratio to match the assigned peak with the biological agent.

$$Relative\ elution\ time\ ratio\ (R_{elu}) = \frac{(t_{target} - t_{15})}{(t_{45} - t_{15})} \quad (where\ t_n = elution\ time\ of\ n\ bp\ DNA)$$

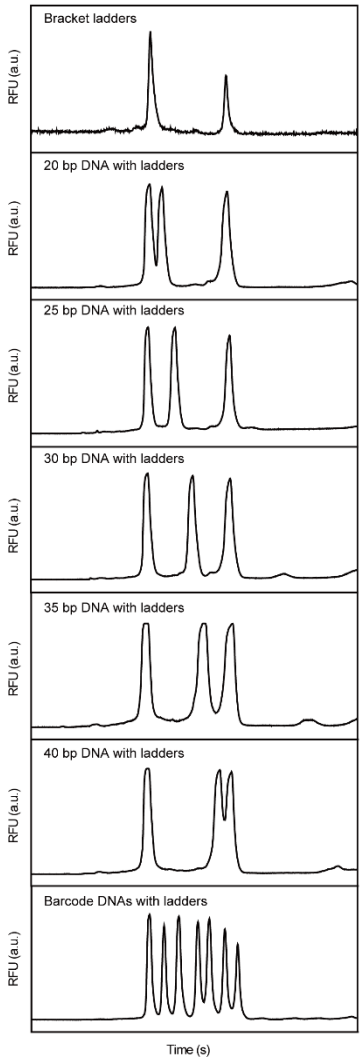

Fig. S2 Electropherogram showing the separation of all the biobarcode DNAs and bracket ladders.[1]

Table S2 The relative elution time ratio (R_{elu}) of barcode DNAs. [1]

Pathogen	Relative elution time ratio
Short bracket ladder	0
Bacillus anthracis	0.1651
Francisella tularensis	0.3331
Yersinia pestis	0.5499
Vaccinia virus	0.6789
Botulinum toxin A	0.8547
Long bracket ladder	1

1 M. Cho, S. Chung, J. H. Jung, G. Rhie, J. H. Jeon and T. S. Seo, *Biosens. Bioelectron.*, 2014, **61**, 172-176.

Correlation between the pathogen concentration and the relative CE peak intensity

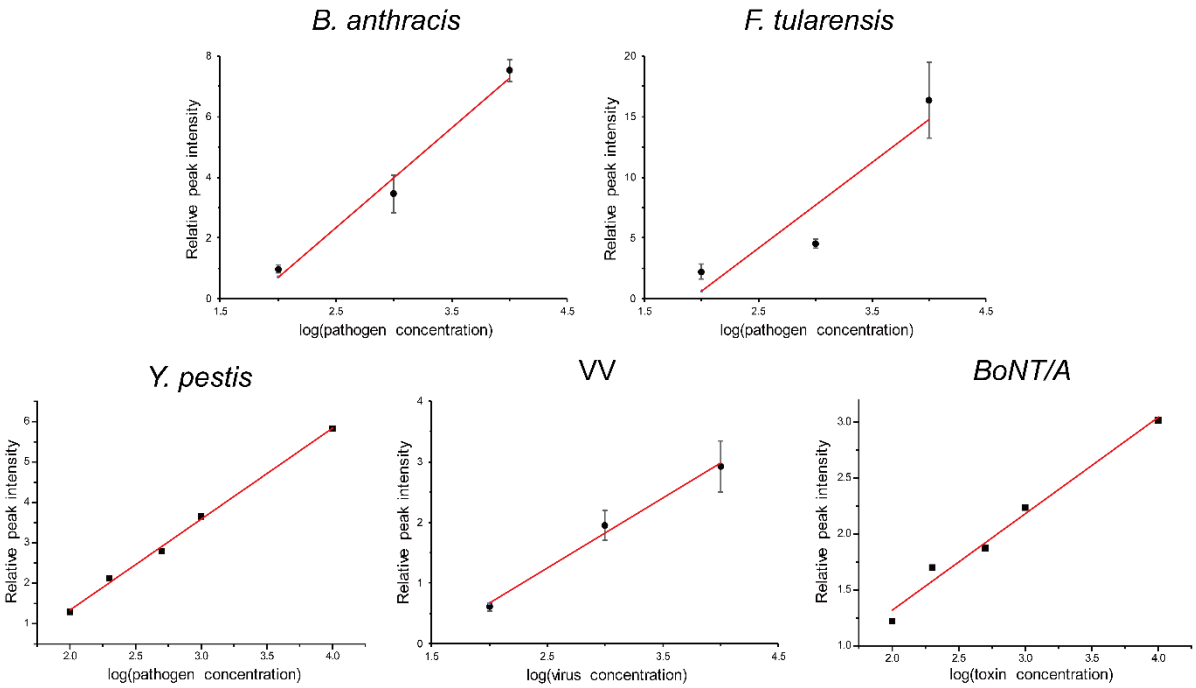

Fig. S3 Correlation between the log (pathogen concentration) and the relative peak intensity.