Electronic Supplementary Information (ESI)

Combination of a centrifugal microfluidic device with a solution-loading cartridge for fully automatic molecular diagnostics

Seung Jun Oh[a] and Tae Seok Seo[b,*]

[a]*Corporate R&D , LG Chem. Ltd., 30, Magokjungang 10-ro, Gangseo-gu, Seoul, 07796, Republic of Korea*

[b]*Department of Chemical Engineering, College of Engineering, Kyung Hee University, 1732, Deogyeong-daero, Giheung-gu, Yongin-si, Gyeonggi-do, 17140, Republic of Korea*

*Corresponding author. E-mail: seots@khu.ac.kr; Phone: +82-31-201-3676; Fax: +82-31-204-8114

Materials and methods

Preparation of real samples

Escherichia coli O157:H7 (*E. coli* O157:H7, KCTC 1039) and *Vibrio parahaemolyticus* (*V. parahaemolyticus,* KCTC 2729) were purchased from Korean Collection for Type Cultures. *Salmonella enterica subsp.* enterica serovar *typhimurium* (*S. typhimurium*, KCCM 11806) and *Listeria monocytogenes* (*L. monocytogenes*, KCCM 40307) were adopted from Korean Culture Center of Microorganisms. According to the manufacturers' instructions, those bacteria were cultured in designated media; *E. coli* O157:H7 and *S. typhimurium* in a Nutrient Broth (BD Biosciences, CA, USA), *V. parahaemolyticus* in a Nutrient Broth with 3% NaCl, and *L. monocytogenes* in a Brain Heart Infusion Broth (BD Biosciences, CA, USA). We rinsed and diluted the cultured bacteria using 1× PBS buffer (LPS solution, Korea). The diluted bacterial concentrations were measured based on spectrophotometer readings of optical density at 600 nm. Real samples were prepared to prove the feasibility of our system for practical applications. The bacteria were inoculated in milk with a concentration of 4×10^3 cells/μL for singleplex and multiplex detection experiments, and with concentrations of 4×10^2, 4×10 and 4 cells/μL for limit of detection tests.

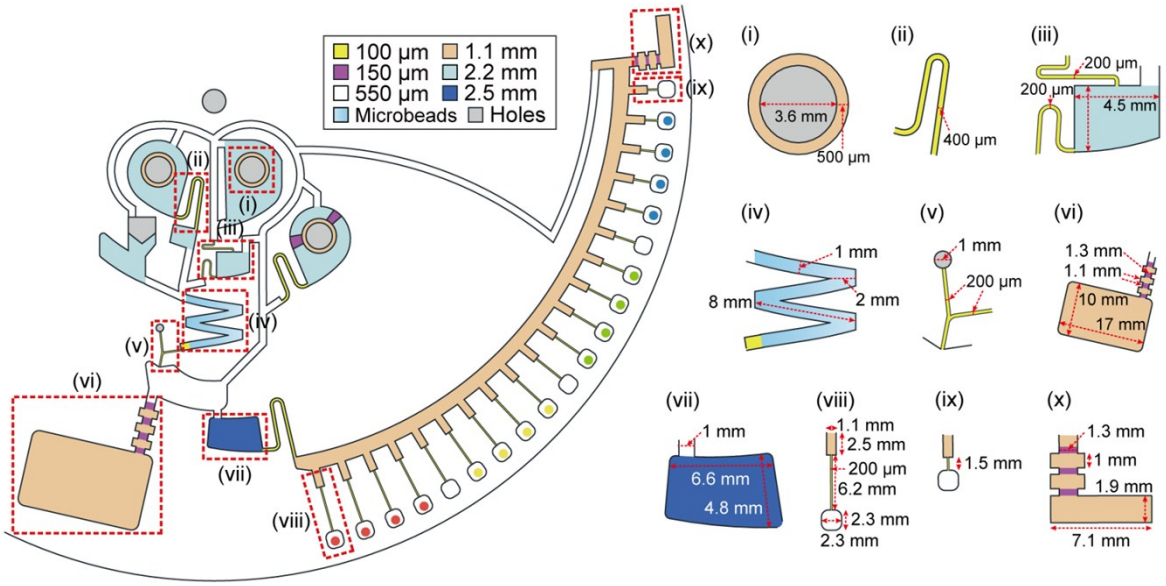

Figure S1. Detailed information about the dimensions of the microdevice structures (10 parts)

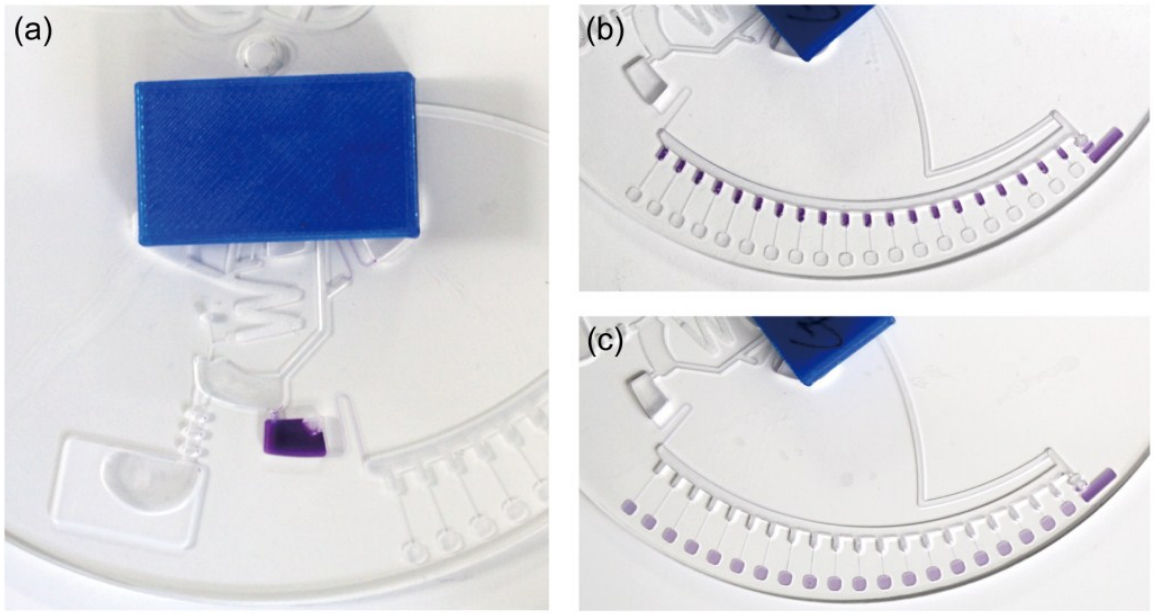

Figure S2. Real pictures showing the operation of the microdevice. (a) After the collection of the LAMP cocktail/EBT and eluted DNA (corresponding to Fig. 3f) (b) After the division of the LAMP mixture into 20 aliquots (corresponding to Fig. 3g) (c) After the filling of the 20 reaction chambers with the LAMP mixture (corresponding to Fig. 3h)

Table S1. Primer sequence information for the multiplexed LAMP reaction

Target gene / Accession #	Primer	Sequence (5' to 3')	Length [bp]
E. coli O157:H7 fliC gene (CP002967.1 : 2147323-2149017)	FIP	CTACCAACCTGTCTGAAGCGCATCGACATGTTGGACACTTCG	42
	BIP	GGTGACTGCGGAATCCAGACGTGAGGCAATTGCATCCATCG	41
	F3	GCCTGCTGGATGATCTGC	18
	B3	CGGCTCTGCAACCAAAGA	18
S. typhimurium invA gene (M90846.1)	FIP	TCCGCGACACGTTCTGAACCCTCTATTGTCACCGTGGTCC	40
	BIP	TGCCGATTTGAAGGCCGGTACAGTACGCTTCGCCGTTC	38
	F3	GCGGTGGGTTTTGTTGTCT	19
	B3	CGTAAAGCTGGCTTTCCCTT	20
V. parahaemolyticus toxR gene (L11929.1)	FIP	TCGACTCCACATTCACTCGATTACTGATAACTTGCCAGACG	41
	BIP	CCTGCCGAATGGCGATTACAATTAATTGTCGATTCAGCCG	40
	F3	CAACCATGGTGACTGTGA	18
	B3	CCAGCGACCTTTCTCTGA	18
L. monocytogenes iap gene (JQ015300.1)	FIP	ATCAAATGTAGTTGGTCCGTTACCAGCTGAAGCTCAAAAACACC	44
	BIP	ATGTATTTGCTAAAGCGGGAATCTGTAGTGCTAGCGTATTGTGC	44
	F3	AATTCAAGTGCAAGTGCTAT	20
	B3	GTTTTGCTTGAGATTCAGAGA	21

Table S2. Rotational speed control protocol for device operation

Step	Speed [RPM]	Time [s]	Operation
1	5000	60	DNA binding and bead washing
2	0	10	Siphon priming
3	-5000	20	Elution and collection
4	0	10	Siphon priming
5	1000~-1000	10	Mixing of the LAMP mixture by a shake-mode
6	-1000	30	Aliquoting of the LAMP mixture
7	-5000	20	Transfer of the LAMP mixture aliquots to reaction chambers