Supplementary Information for

A paper-based device for performing loop-mediated isothermal amplification with real-time simultaneous detection of multiple DNA targets

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Table S1. LAMP primer set

Target Gene	Primer	Sequence
S.agalactiae **(Target 1)**	F3	GGAACTCTAGTGGCTGGT
	B3	CAATCACATCTGTTAAGGCT
	FIP	GCCATTTGCTGGGCTTGATTGCTGTATTAGAAGTACATGCTG
	BIP	TGAGGCTATTACTAGCGTGGAATCTACACGACTACCAATAGA
	LF	ACTTGTGGAGTTGTCACTTGA
	LB	AGACTTCATTGCGTGCCA

Target Gene	Primer	Sequence
S.pneumoniae **(Target 2)**	F3	AACTGATTGAAAGCCATTCA
	B3	GTCAACGTGGTCTGAGTG
	FIP	CCTGCTTCATCTGCTAGATTGCAAAGAAGAGTTCATGACGGAC
	BIP	TGCCGAAAACGCTTGATACATGTTTGGTTGGTTATTCGTG
	LF	GTAAGAGTTCGATATAAAGGCGGT
	LB	GGAGTTTAGCTGGAATTAAAACGCA

Target Gene	Primer	Sequence
S.aureus **(Target 3)**	F3	AGAAGTGATTCTGAAGATCCAAC
	B3	TATCAGTTCTTTGACCTTTGTCA
	FIP	TAACCGTATCACCATCAATCGAGTATACAGTGCAACTTCAACT
	BIP	GTCAAACAATGACATTCAGACTGGACCATATTTCTCTACACCTTT
	LF	TTAATTAATGTCGCAGGTTCTT
	LB	GATACACCTGAAACAAAGCATC

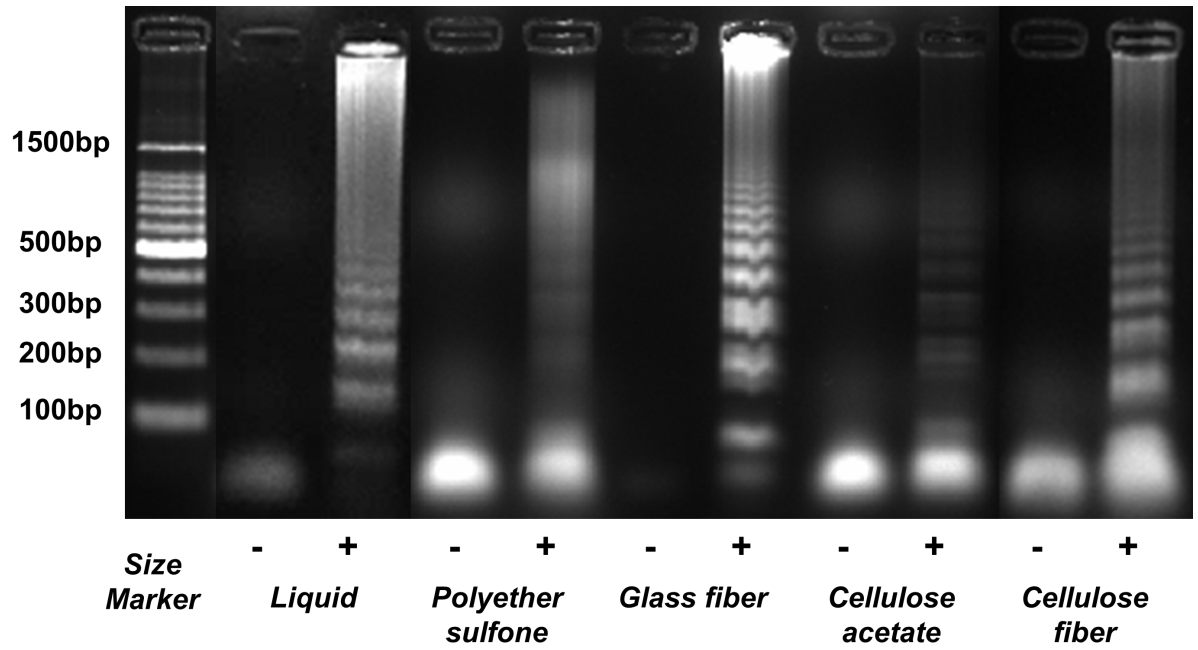
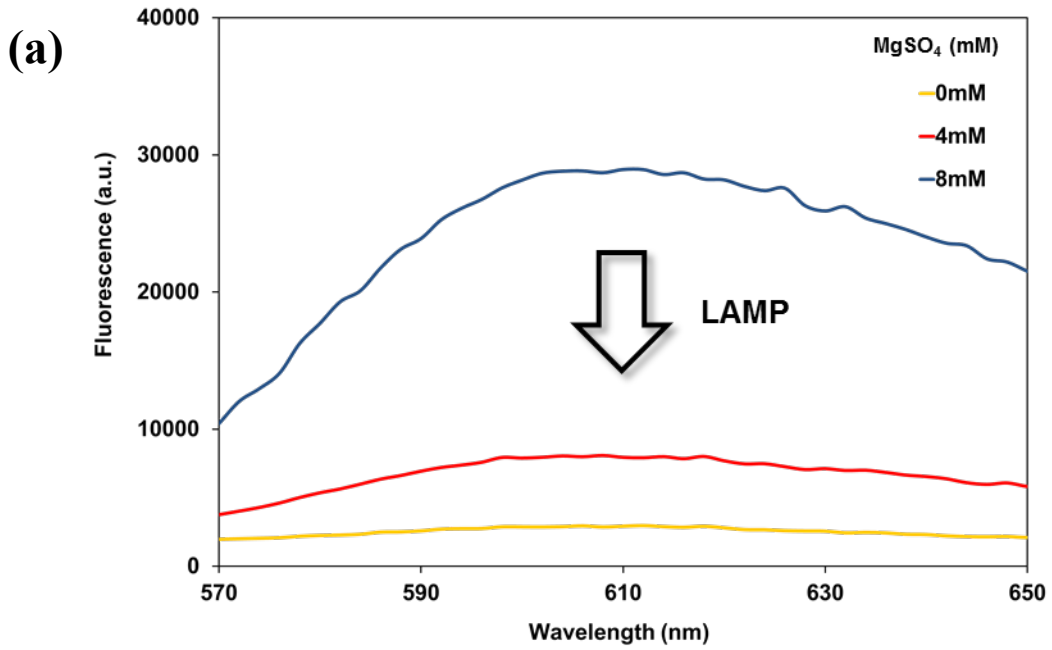


Figure S1. Gel electrophoresis of LAMP products in different reaction pad. Gel lanes are labeled with the type of material tested. Each pad is displayed as two lanes that are negative (-) and positive (+) for the reaction product. Negative: no template DNA; positive: *Streptococcus pneumoniae* genomic DNA.

1



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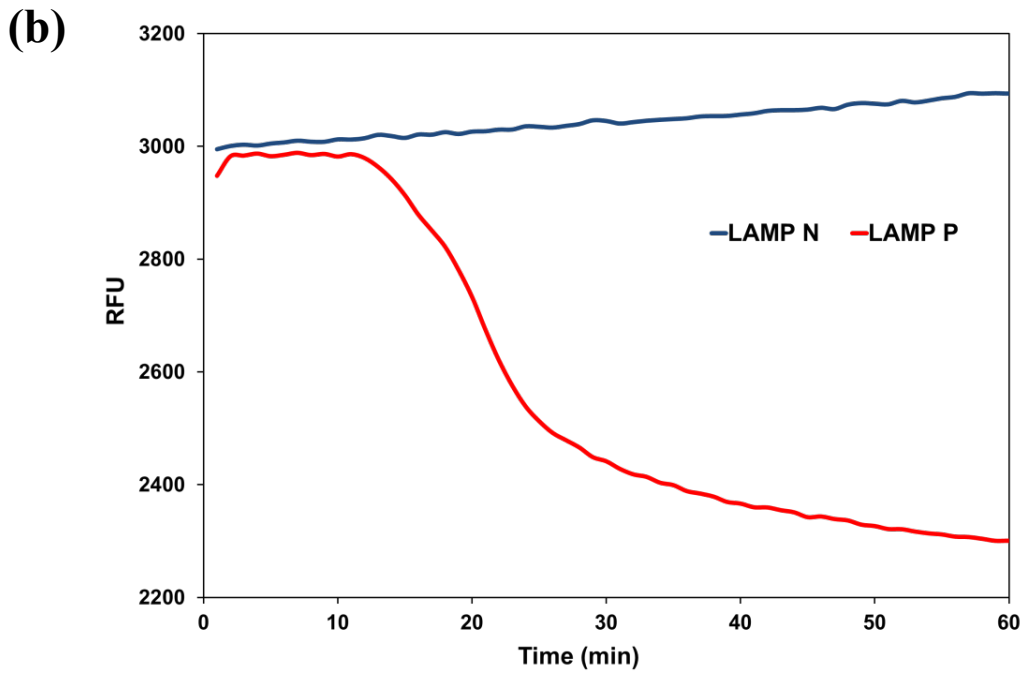

3

4 **Figure S2.** LAMP detection by HNB fluorescence. (a) Fluorescence spectrum of HNB in LAMP
5 buffer solution. HNB was excited at 530 nm, and emission was scanned from 570 nm to 650 nm. (b)
6 Real-time PCR analysis graph at 575 nm excitation and 602 nm emission (ROX setting in Bio-Rad
7 CFX96 real-time system).

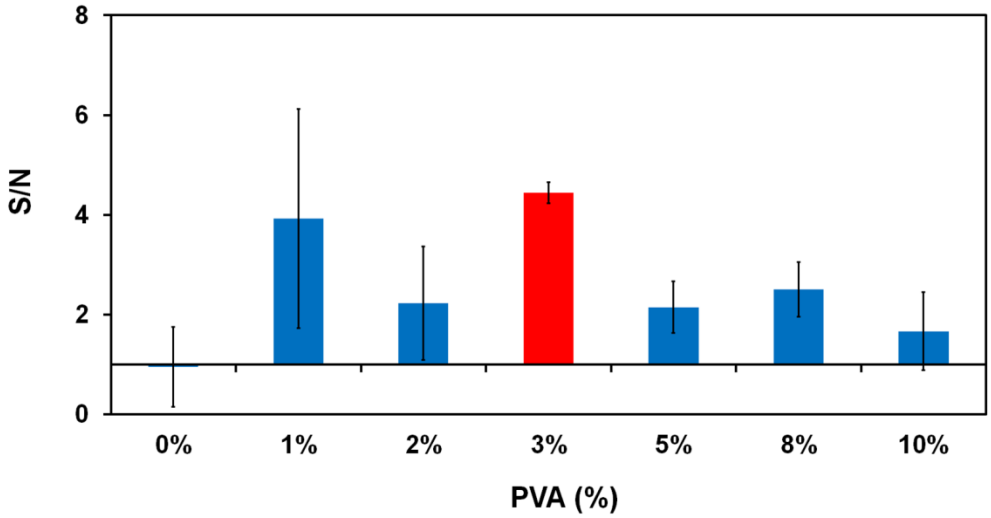

Figure S3. Optimization of PVA concentration. 3% PVA was selected as the optimal condition for the drying solution. (S/N: fluorescence intensity ratio between positive (S) and negative signal (N) after the end of LAMP)

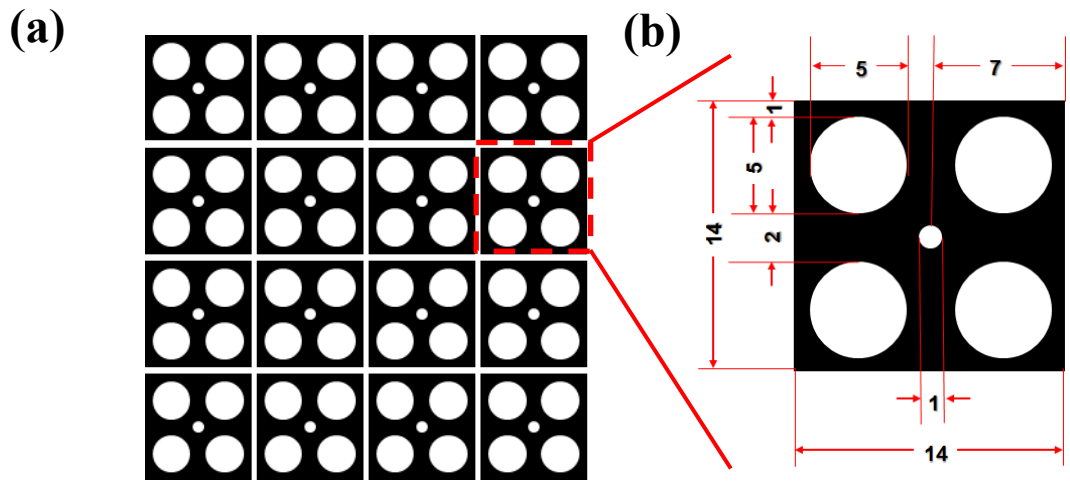

Figure S4. Patterning shape of the fluidic channel. (a) Real size of the pattern. The PES membrane was patterned using this image with a wax printer (Xerox, ColorQube8570). (b) Enlarged image for labeling the size of each part (unit: mm). A 14mm × 14mm chip contained a 1-mm injection hole and four 5-mm reaction holes.

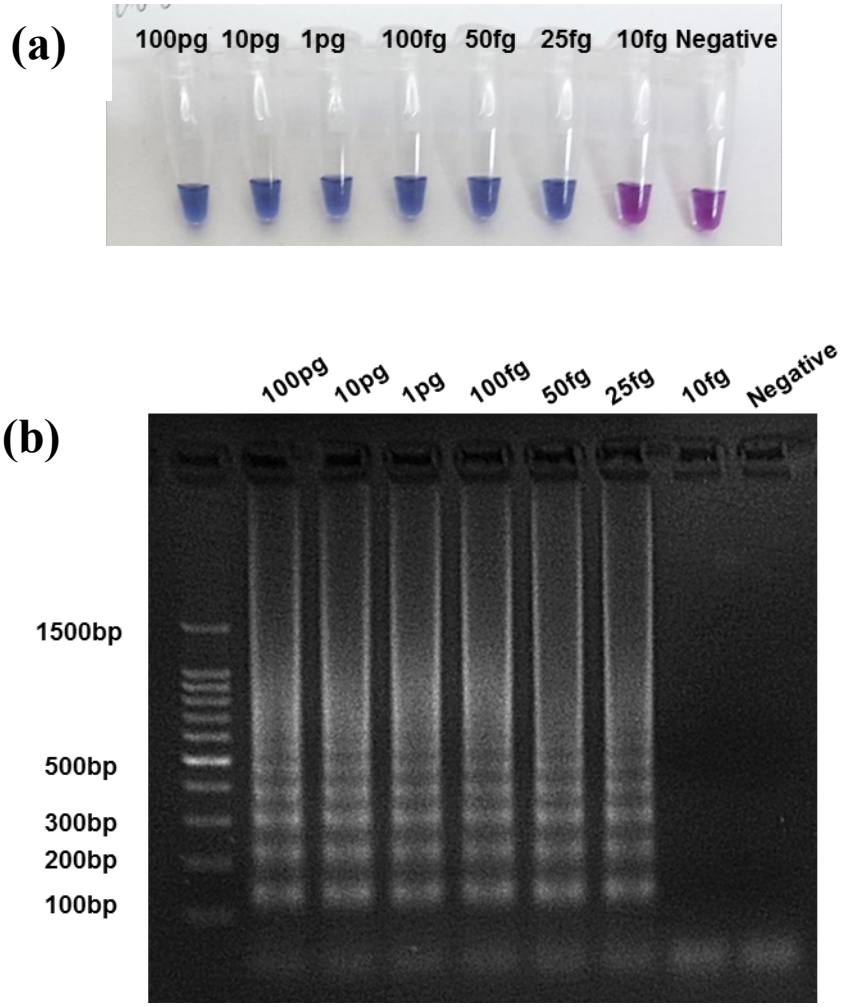

Figure S5. Quantitative assay of *Streptococcus pneumoniae* genomic DNA in liquid solution. From 10 fg to 100 pg genomic DNA of *Streptococcus pneumoniae* genomic DNA was amplified by LAMP. (a) Colorimetric image of LAMP solutions after amplification. (b) Gel electrophoresis of LAMP solutions. The limit of detection (LOD) for *Streptococcus pneumoniae* DNA was 25 fg.

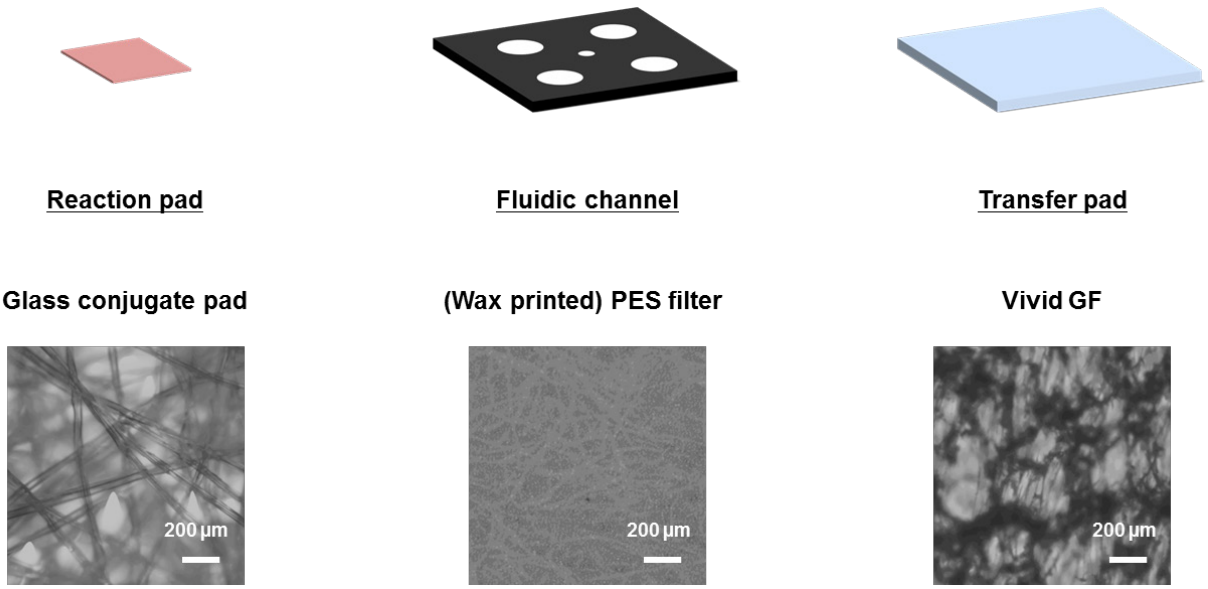

Figure S6. Microscopic image of paper materials. The pore size of each material is related to its functions in the devised structure.

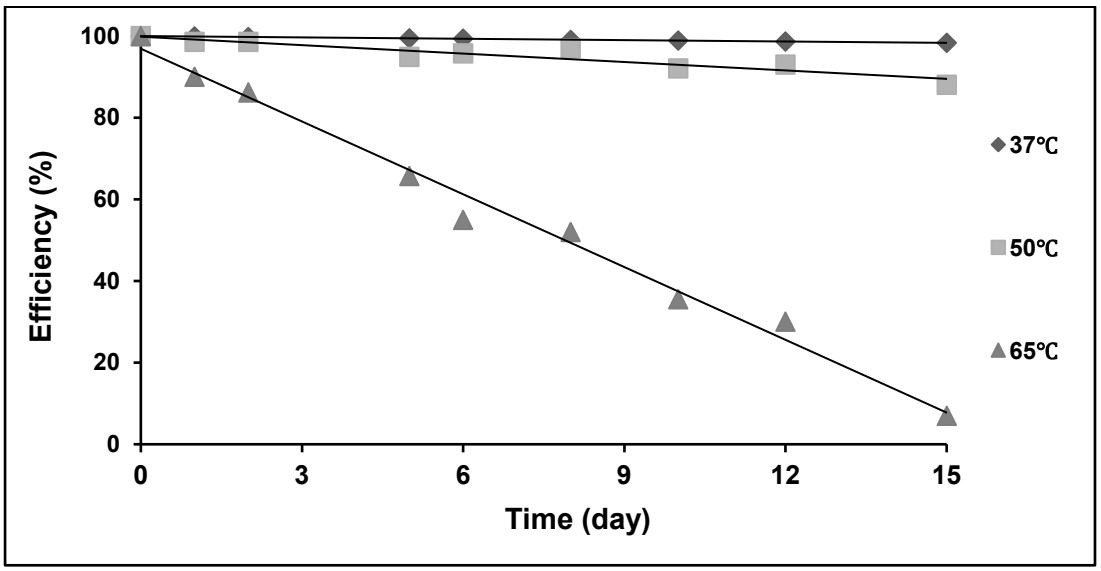

Figure S7. The result of acceleration test for 15 days. LAMP reaction activity of paper devices were tested in 37 ℃, 50 ℃, and 65 ℃ temperature over time for evaluation of stability in storage condition. (Efficiency %: Ratio of the normalized intensity value between the first day and measured day. LAMP reaction was performed by 700 pg of S.pneumoniae DNA each day.)