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

Rapid detection of multiple respiratory viruses based on microfluidic isothermal amplification and a real-time colorimetric method

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Authors' contributions: Rongzhang Hao, Ruili Wang, and Hongbin Song designed the research plan; Ruili Wang, Yang Li, Wen Kong and Xudong Guo performed the experiments; Ruili Wang, Rongtao Zhao, Yi Yang, Feng Wu and Wanying Liu wrote the paper.

Contents

Table S1 Sequences of LAMP primers used for amplifying respiratory virus genes

Target gene (subtype)	primer	Sequence (5' to 3')
Flu A virus HA gene (H1N1)	F3	AAGCTCAGCAAATCCTACA
	B3	TCCCTCACTTTGGGTCTT
	FIP	GACTTTGTTGGTCAGCACTAGTAGAAAAGGGAAAGAAGTCCTCG
	BIP	TCTATCAGAATGCAGATGCATATGTTGCTATTTCCGGCTTGAA
	LF	GATGGTGAATGCCCCATAGC
	LB	TTTTGTGGGGTCATCAAGATACAG
Flu A virus HA gene (H3N2)	F3	CTACTGAGCTGGTTCAGAA
	B3	GGCATAATCCGGCACATC
	FIP	AGAGCATCTATTAGTGTGCAGTTTTCTCAATAGGTGAAATATGC GAC
	BIP	GAGACCCTCAGTGTGATGGCGTAACAGTTGCTGTAGGCT
	LF	CCATCAAGGATCTGATGAGGACT
	LB	GAAATGGGACCTTTTTGTTGAACG
Flu A virus HA gene (H5N1)	F3	GCAATCAACGACTCAACAA
	B3	ACAGTCCTCTTTTTCTTCTTCT
	FIP	AGTTAATCGCCCCCATCGGATGAAAAGTGAAGTGGAATATGGTA
	BIP	ACCATCGGAGAATGTCCCAAATACTCTCTCTTTGAGGACTATTTC TG
	LF	TGACACCTGGTGTTGCAGT
	LB	AAACAAATTAGTCCTTGCGACTGGG
Flu A virus HA gene (H7N9)	F3	TGAGAGGCGAGAAGGAAG
	B3	GAGCCATTTCATTTCTGCA
	FIP	GCCTGATTCTCTGAGAATTTGCCTTGATGTCTGTTATCCTGGGA
	BIP	TGACAAGGAAGCAATGGGATTCTCCTGATCTCCTACATGCA
	LF	CAGAGCTTCTTCATTCACGAATT
	LB	ACAGTGGAATAAGAACTAATGGAGC
Flu B virus HA gene	F3	TGATCTCAGAGCTGACACT
	B3	GCAGCTATCCTGTCTAAGC
	FIP	TAGATGCTCGTCTTCACTGTTTATTATAAGCTCACAAATAGAACT TGC
	BIP	AACTAAAGAAAATGCTGGGTCCCGCATTTGTGTTTGGTTTCGA
	LF	CCTTCGTTGGAAAGCAAGACT
	LB	TCT GCTGTAGACA TAGGAAACGG
Human adenovirus Hexon gene	F3	GTCTACTGGTCGCTCCCT
	B3	GAGGATCTGGTTCTCAGGGA
	FIP	ACCCACCACTGGGTAGTTGTTGCATGATGCAAGACCCAGTCA
	BIP	GAGCAAGCCGTGTACTCTCAGCCGGTTGAAGACGTGCGTG
	LF	TGTCTTGTGGAGCGGAAGG
	LB	AGCTCCGACAGGCCACTTC
Negative control	F3	GCGCAAGGTTACAACATCAC

	B3	GCGTGACATTCCAGAACACA
	FIP	CGCGTTCACGAAACCGTGCTGATACTCACGCCTTGTTCGA
	BIP	TTGGACATCAACCGCTCATCGTGACGCTGCACACTCAGAG
	LF	TCGGGCGCAGAAGTTAGC
	LB	CTGTCGATTACATGTACACCCAC
Positive control	F3	CCGCGTGTATGAAGAAGG
	B3	AGACTCAAGCTGACCAGT
	FIP	TGCTGGCACGGAGTTAGCGAGGAAGGTGTTGTGGTTAA
	BIP	GCGGTAATACGGAGGGTGCACTTAATCAACCGCCTGC
	LF	GGTAACGTCAATTGCTGCG
	LB	AATCGGAATTACTGGGCGTAA

Table S2 Sequences of PCR primers used for amplifying respiratory virus genes

Target gene (Accession)	PCR primer	Sequence (5’ to 3’)
Influenza A (H1N1) virus	F	AAATCTAGTGGTACCGAGATATGCA
	R	GGGAGGCTGGTGTTTATAGCAC
Influenza A (H3N2) virus	F	ATCAGGGAGAGTCACAGTCTC
	R	ATGCTTCCATTTGGAGTGATGC
Influenza A (H5N1) virus	F	GGAGTTCTTCTGGACAA
	R	GTCGCAAGGACTAATCT
Influenza A (H7N9) virus	F	TTCCTGAGATTCCAAA
	R	GGTTGGTTTTTTCTATAAGCCG
Influenza B virus	F	GGGACATGAACAACAAAGATGC
	R	TGTCAGCTATTATGGAGCTG
Human adenovirus	F	TTCCCCATGGCICAYAACAC
	R	CCCTGGTAKCCRATRTTGTA
Negative control	F	AATGTTCACCTGGTT
	R	TTATGAATCCAATCA

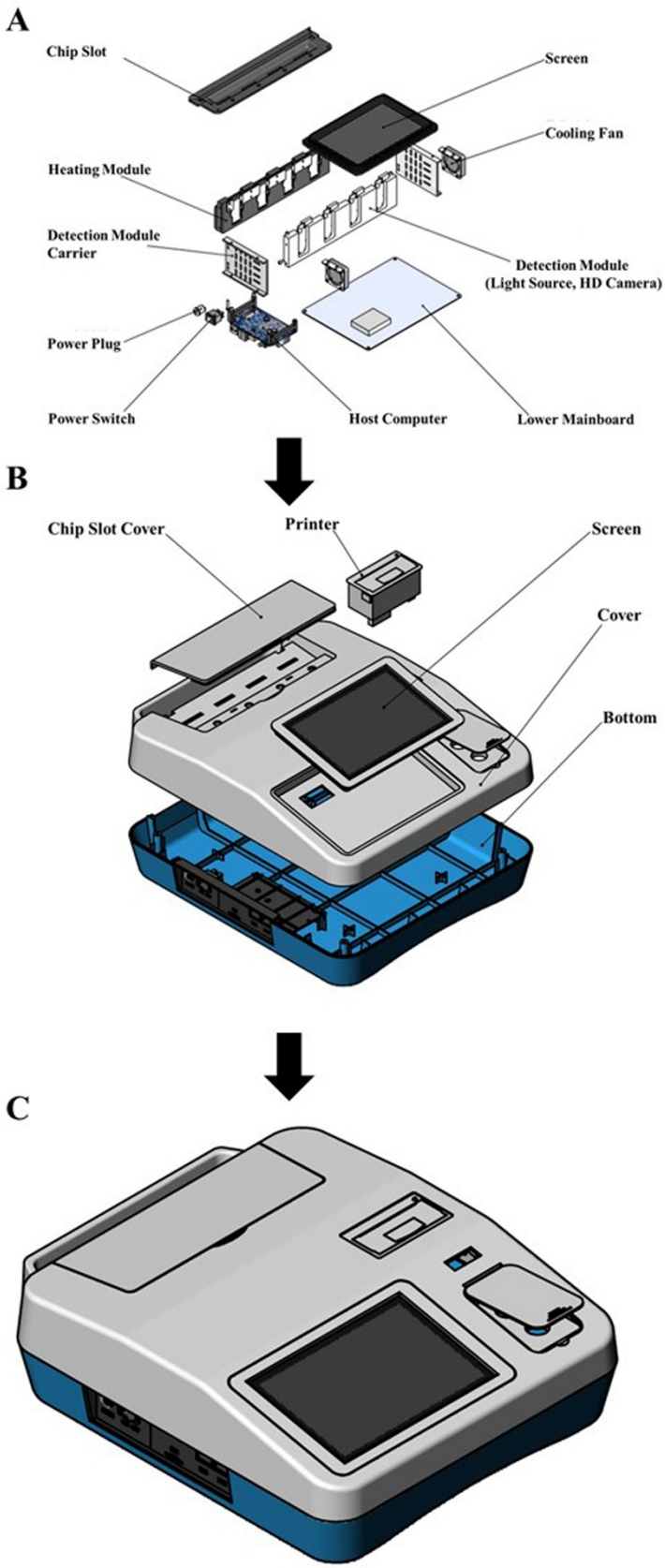


Fig. S1. The schematic drawing of the measurement instrument, A represent internal structure of the instrument, B represent external structure of the instrument, and C represent the detection instrument.

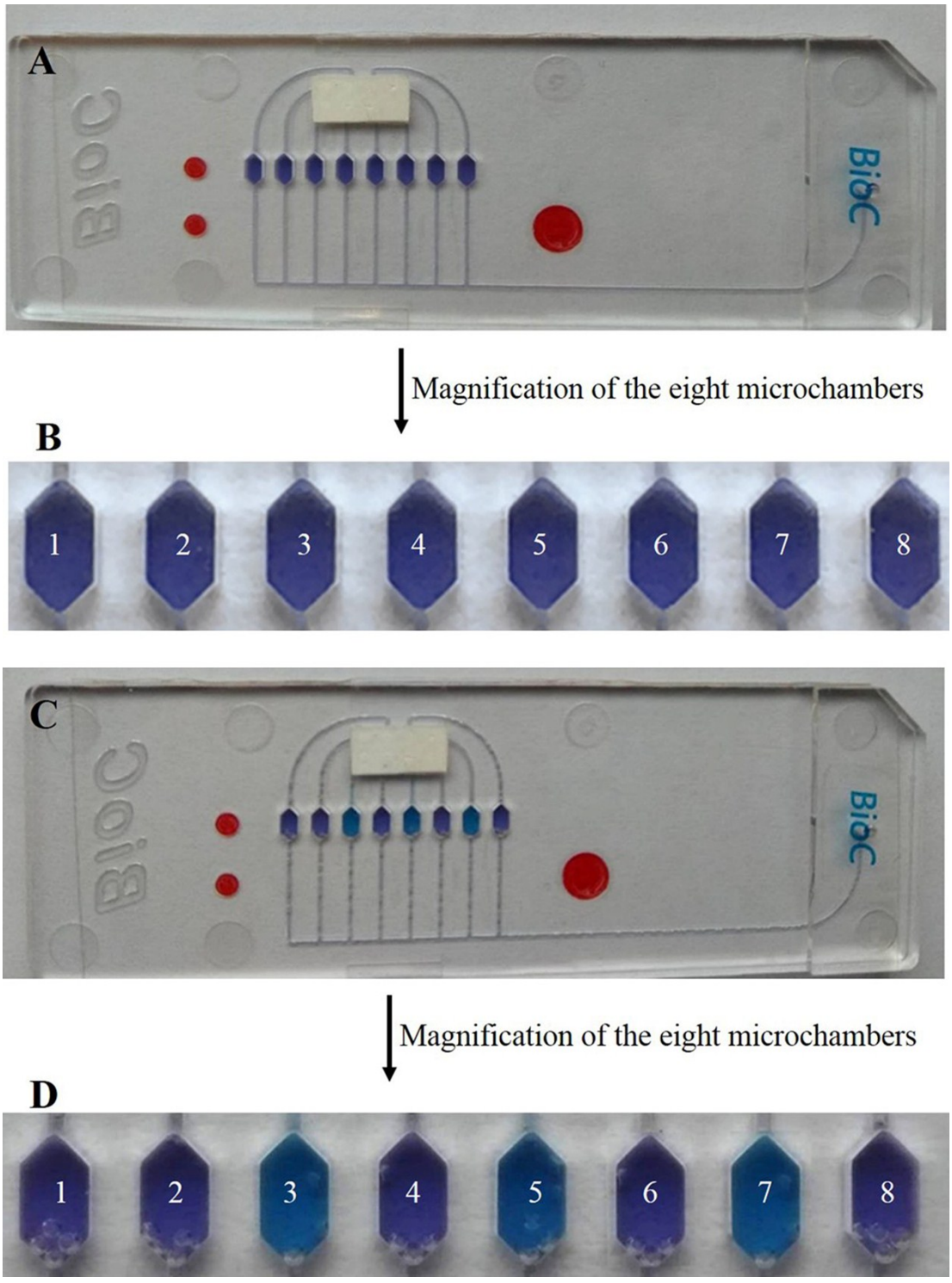
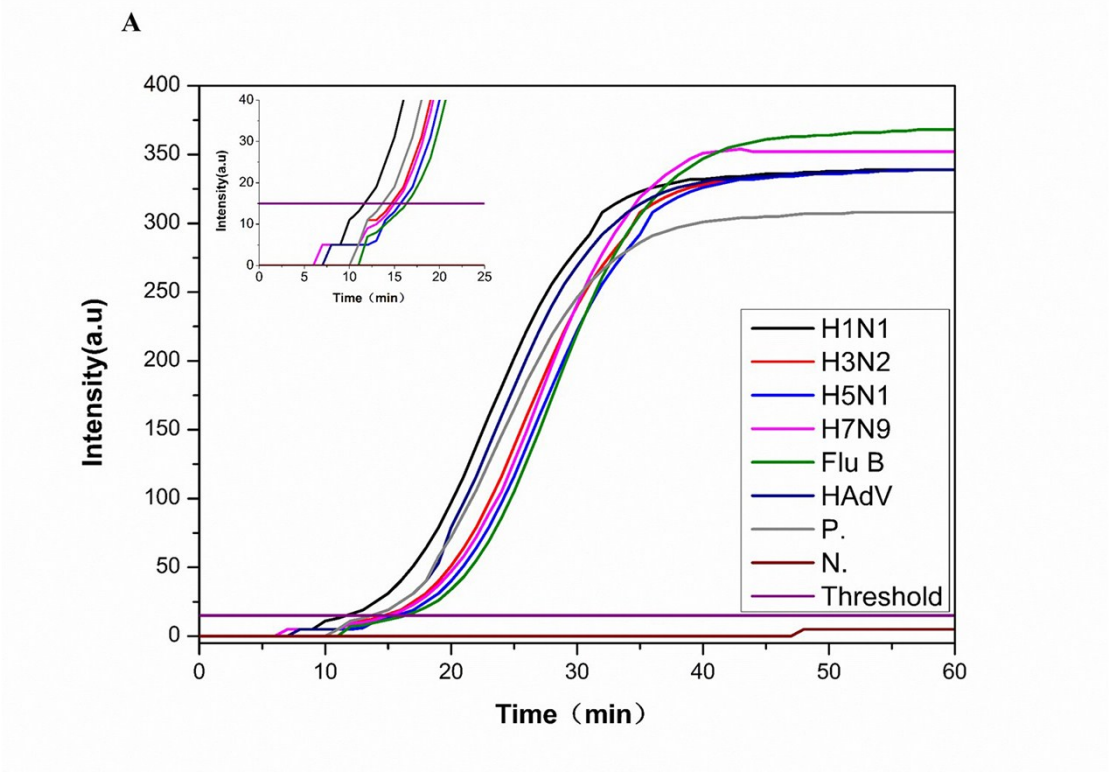


Fig. S2. Colorimetric change of microfluidic chip after reaction. (A) The whole microfluidic chip before the reaction. (B) Magnification of the eight microchambers before the reaction. (C) The whole microfluidic chip after the reaction. (D) Magnification of eight microchambers after the reaction and the color changes observed after reaction on the chip. Positive reactions occurred in microchambers 3, 5 and 7 (microchambers 1, 2, 4, 6, and 8 all are embedded negative controls, while microchambers 3, 5, and 7 are all embedded H1N1 primer, the detection samples are FluA H1N1 virus).



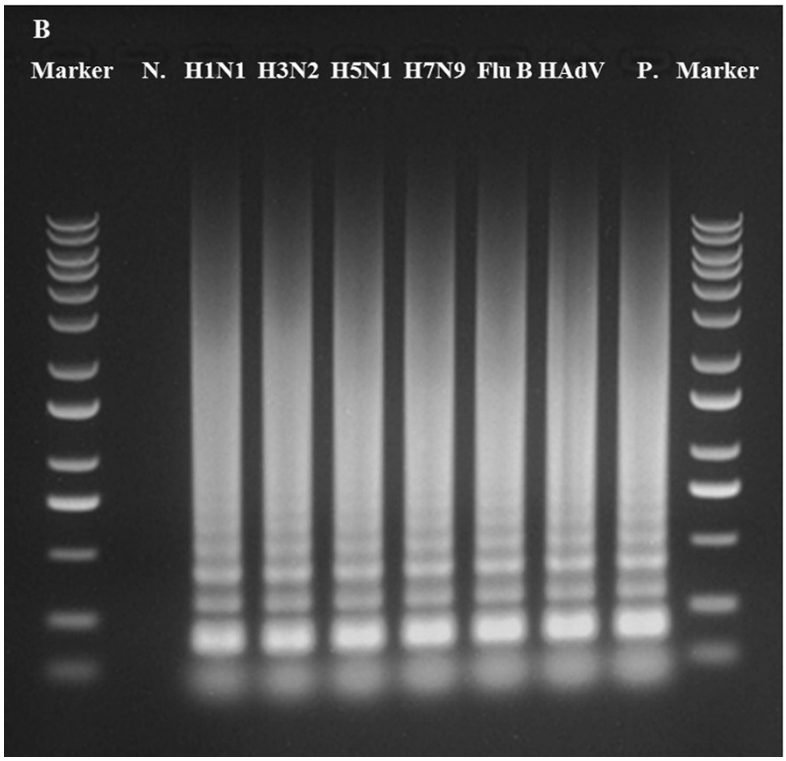


Fig. S3. Detection results of multiple respiratory viruses. A show the real-time colorimetric detection results of FluA H1N1, FluA H3N2, FluA H5N1, FluA H7N9, FluB, HAdV, positive control, and negative control, respectively. B show the gel electrophoresis detection result after real-time colorimetric detection of FluA H1N1, FluA H3N2, FluA H5N1, FluA H7N9, FluB, HAdV, positive control, and negative control, respectively.

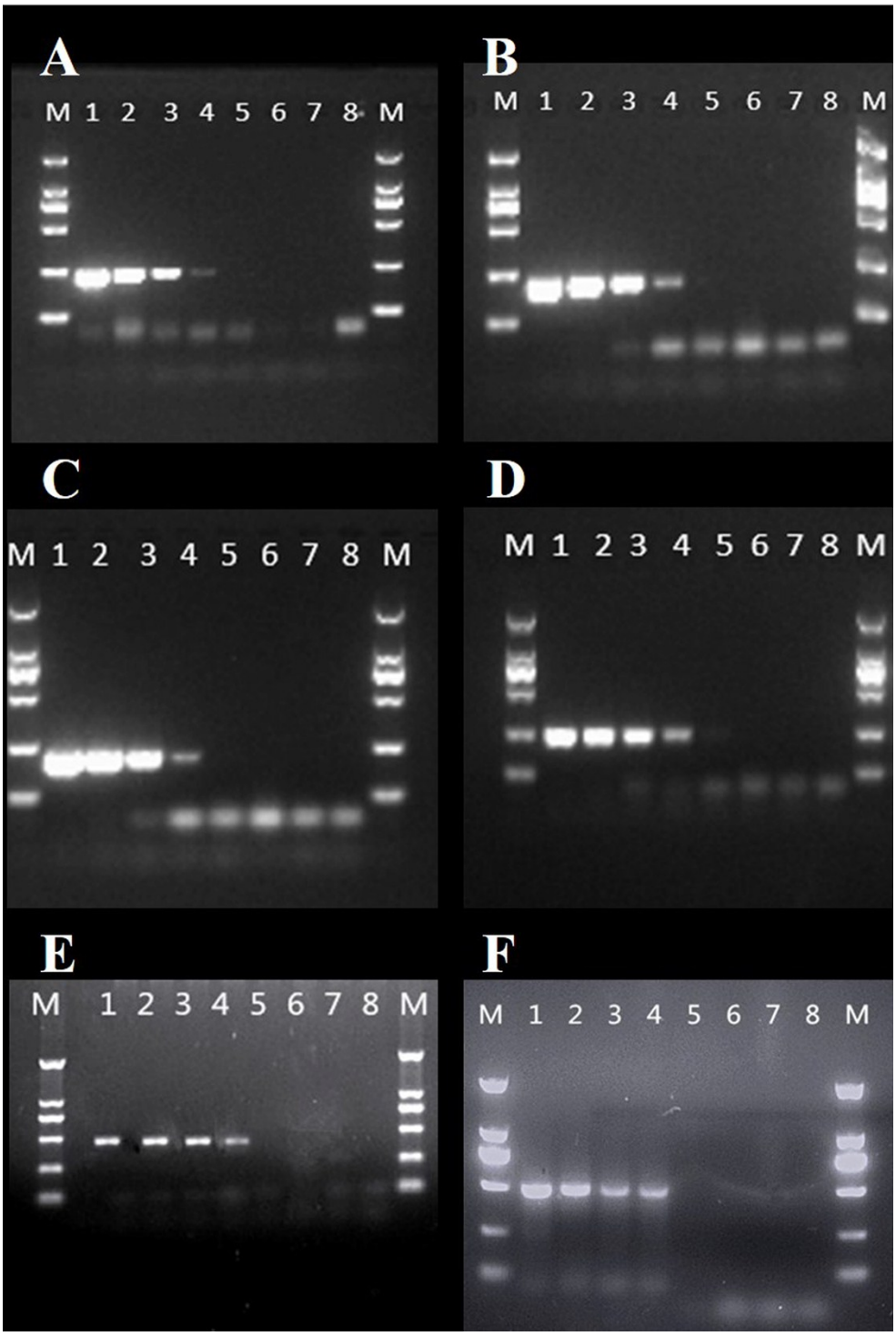


Fig. S4. Gel electrophoresis patterns. Sensitivity evaluation using 10-fold serial dilutions of standards: sensitivity of the system for different viruses. Panels A–F show PCR results for FluA H1N1, FluA H3N2, FluA H5N1, FluA H7N9, FluB, and HAdV, respectively. Within each panel, lanes 1–8 represent test results with 1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 10 fg/μl, and the negative control, respectively.

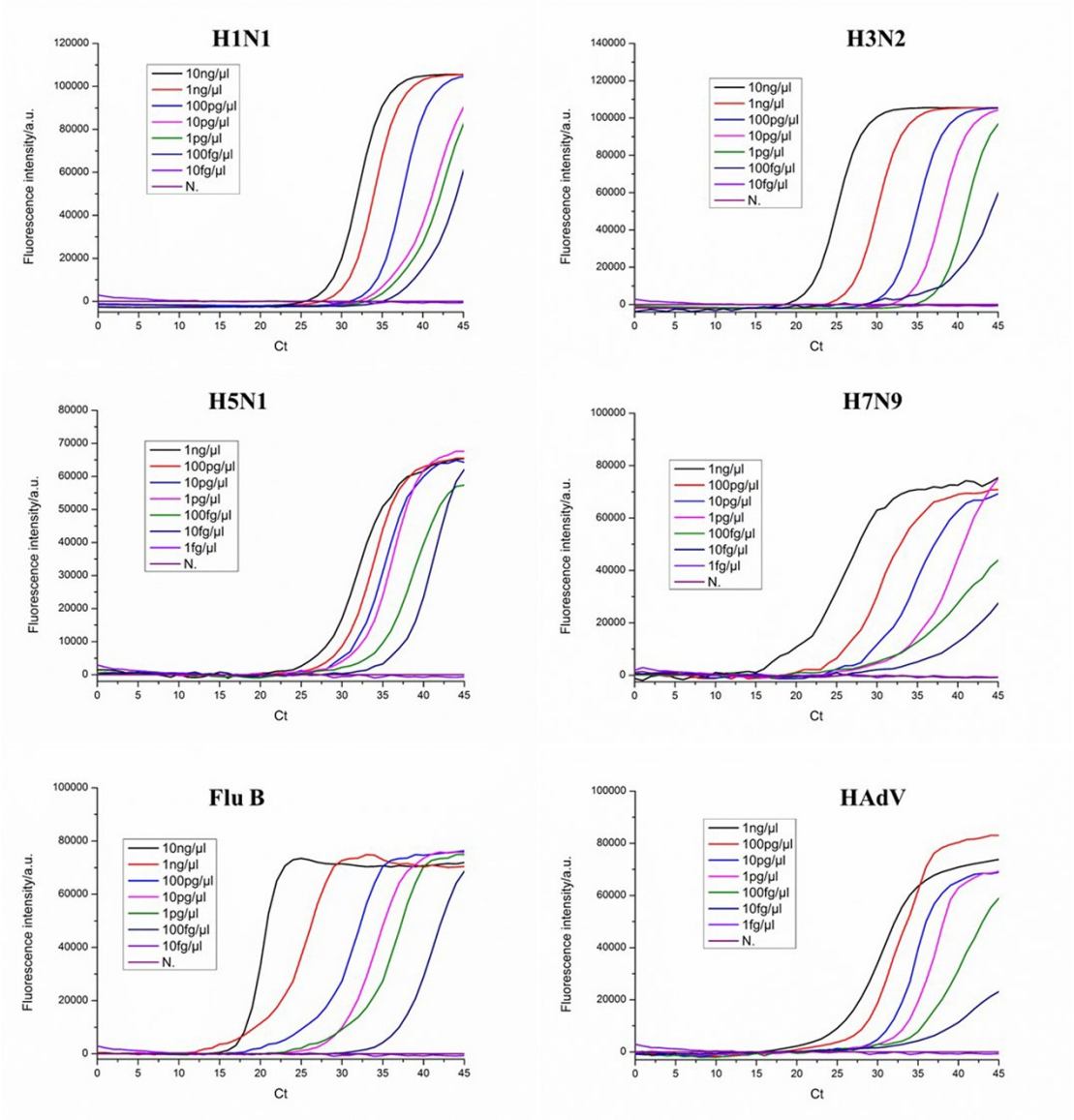


Fig. S5. Results for Real-time PCR detection. Evaluation of sensitivity using 10-fold serial dilutions of standards: sensitivity of the system for different viruses (FluA H1N1, FluA H3N2, FluA H5N1, FluA H7N9, FluB, and HAdV, respectively).