Supporting Information

Portable and simultaneous detection of four respiratory pathogens through microfluidic LAMP and real-time fluorescent assay

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S1 Primer selection for *Mycoplasma pneumoniae*

The target sequences and LAMP primer sequences used are shown in Table S1 and S2, respectively. The fluorescence detection results of four LAMP primer sets for MP detection are shown in Fig. S1A, where the No. 1 primer set peaked earlier and took the shortest time to reach the detection plateau period. Therefore, the No. 1 MP primer set was selected for subsequent studies. Loop primers were absent in the stem-loop structure, and hence, a set of four primers including F3, B3, FIP, BIP was used to amplify the target sequence.

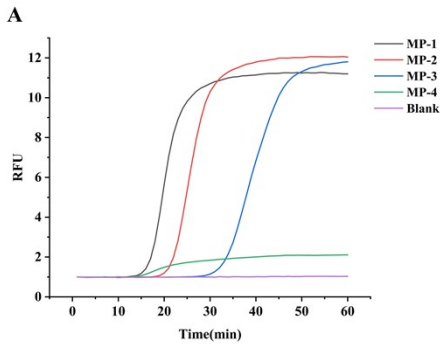

Fig. S1 (A) Fluorescence detection plot of LAMP primer screening for *Mycoplasma pneumoniae*.

S2 Primer selection for respiratory syncytial virus type A

The target sequences and LAMP primer sequences used are shown in Table S1 and Table S2, respectively. The basic LAMP primers (F3, B3, FIP and BIP) were first screened. As shown in Fig. S2A, No. 2 basic primer set had an earlier peak onset and reached the highest fluorescence value faster, indicating that this primer set had the highest amplification efficiency. Loop primers were further designed and validated using the No. 2 basic primer set. As shown in Fig. S2B, the designed loop primers contained only LB, and the detection efficiency of the five primer sets were not statistically different. Based on the parallelism of the assays, No. 4 loop primer was chosen to accelerate the LAMP reaction.

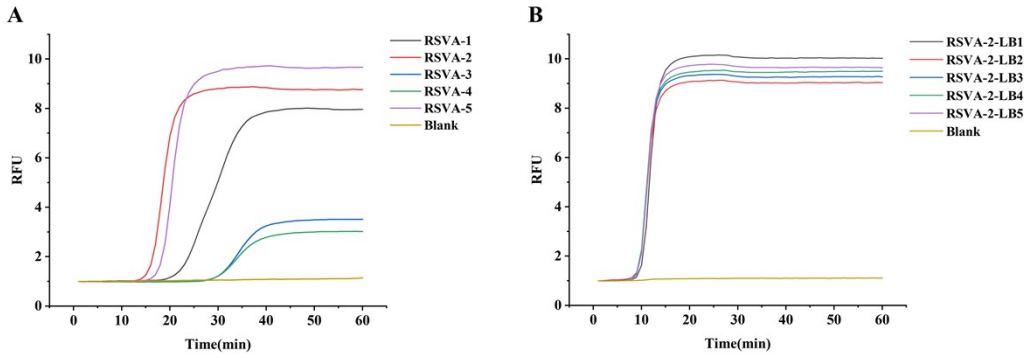

Fig. S2 (A) Fluorescence detection plot of the respiratory syncytial virus type A LAMP primer screen. (B) Fluorescence detection plot of a LAMP loop primer screen for respiratory syncytial virus type A.

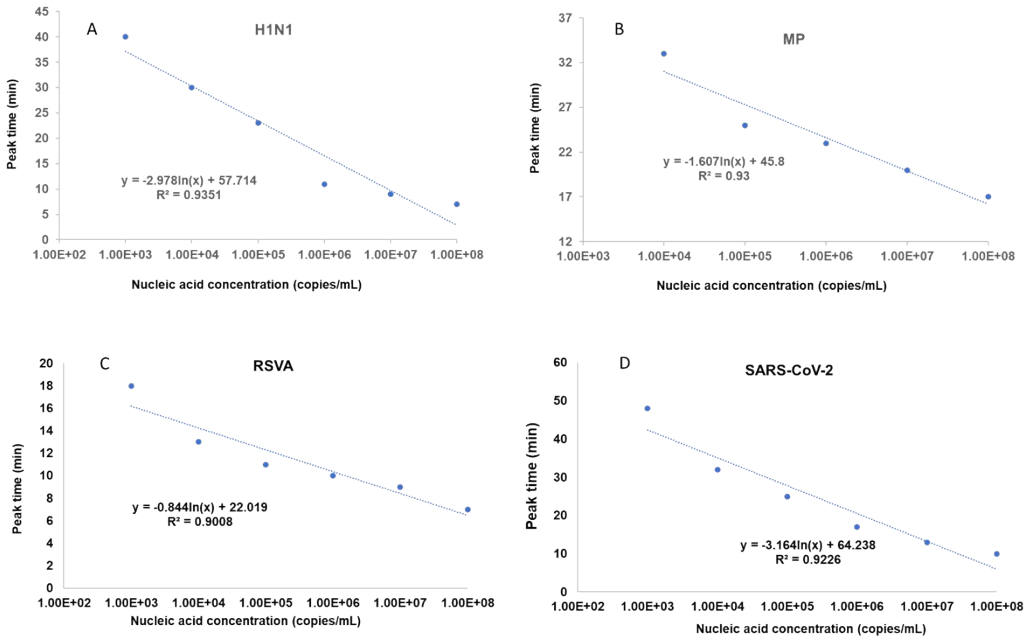

Fig. S3 The relationship between the LAMP peak time in tube and the concentration of target nucleic acid for the four raspatory pathogens.

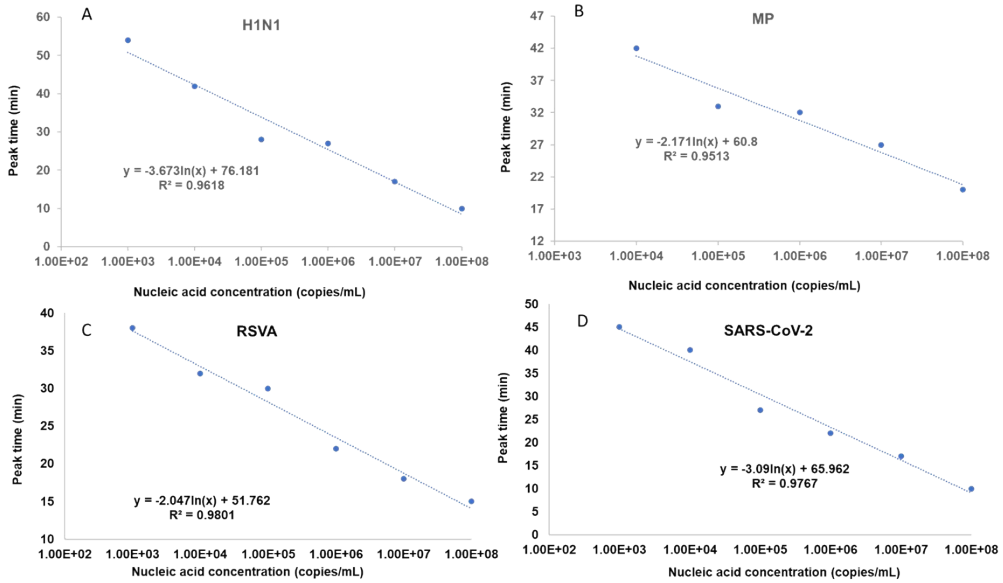

Fig. S4 The relationship between the peak time of microfluidic LAMP assay and the concentration of target nucleic acid for the four raspatory pathogens.

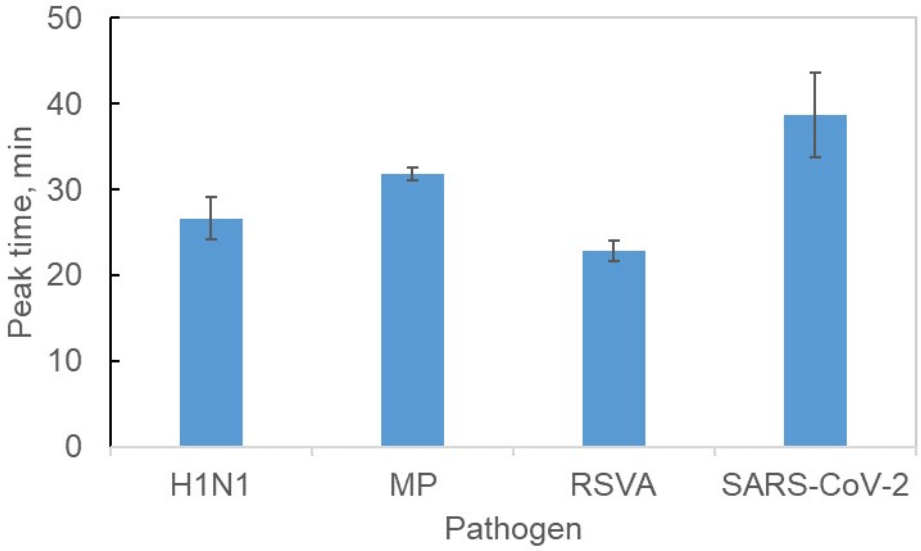

Fig. S5 The peak time of the microfluidic LAMP assay for the four respiratory pathogens. The error bars represent the standard deviation for three parallel experiments.

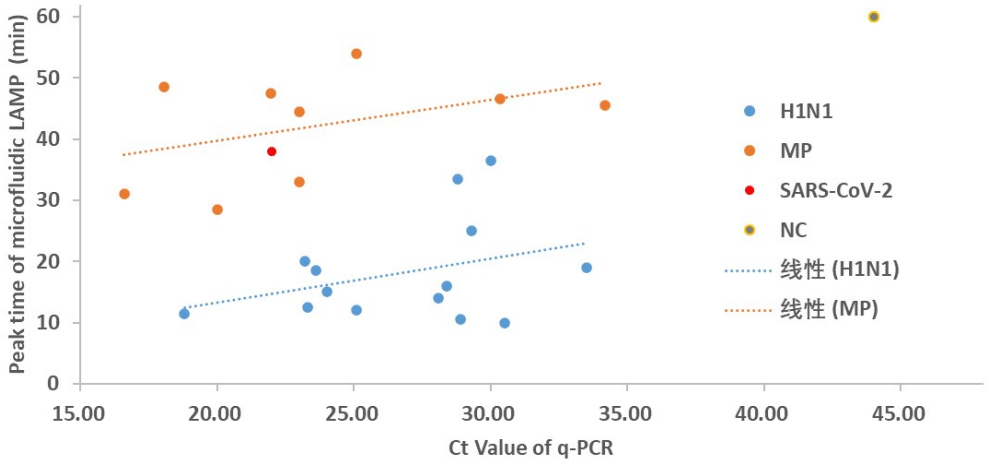

Fig. S6 The relation between PCR Ct value and the peak time of microfluidic LAMP assay for the clinical sample tests.

Table S1. The LAMP target sequences used in this work.

Pathogen	Target sequence(5'-3')
RSVA-LAMP-target	GACGATGATCCTGCATCGCTTACAATATGGGTGCCCATTTTCAATCATCCATGCCAGCA GATTTACTTATAAAAGAACTAGCTAATGTCAATATACTAGTGAAACAAATATCCACACC CAAGGGACCCTCATTAAGAGTCATGATAAACTCAAGAAGTGCAGTGCTAGCACAAATG CCCAGCAAATTCACCATATGCGCCAATGTGTCCTTGGATGAAAGAAGCAAGCTGGCAT ATGATGTAACCACACCCTGTGAAATTAAGGCATGCAGTCTAACATGCCTAAAATCAAA AAATATGTTGACTACAGTTAAAGATCTCACTATGAAAACACTCAACCCAACACATGAC ATCATTGCTTTATGTGAATTTGAAAATATAGTAACATCAAAAAAAGTCATAATACCAAC ATACCTAAGATCCATCAGTGTCAGAAATAAAGATCTGA
SARS-CoV-2-LAMP-	AAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAA GACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATG ACCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAAAT

target	GAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGCCAGAAGCTGGA CTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAA TACACCAAAAGATCACATTGGCACCCGCAATCCTGCTAACAATGCTGCAATCGTGCTAC AACTTCCTCAAGGAACAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGG CAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTC CCCCAAGACAAGTTCATGGCCCAATCATGACTCGAACAAAGGTGTAACGGCAGCATGT CCTCATGCTGGAGCAAAAAGCTTCTACAAAAATTTAATATGGCTAGTTAAAAAAGGAA ATTCATACCCAAAGCTCAGCAAATCCTACATTAATGATAAAGGGAAAGAAGTCCTCGT
H1N1-LAMP-target	GCTATGGGGCATTCACCATCCATCTACTAGTGCTGACCAACAAAGTCTCTATCAGAATG CAGATGCATATGTTTTTGTTTCAAGCCGGAAATAGCGGGGACATCAAGATACAGCAAG AAGTTCAAGCCGGAAATAGCAATAAGACCCAAAGTGAGGGATCAAGAAGGGAGAATG AACTATTACTGGACACTAGTAGAGCCGGGAGACAAAATAACATTCGAAGCAACTGGAA ATCTAGTGGTACCGAGATATGCATTCGCAATGGAAAGAAATGCTGGATCTGGTATTATC ATTTCAGATACACCAGTC
MP-LAMP-target	AAAATGGCTTGTTCTGAAATACCAAGAGTGGTGGTTCACAACACGATTTGTATGTATGT CCTTTGAAAAATCCACCTAGTGATTTGGAAGAATTACAAATAATTGTTGATGAATGTAC TACCCATGCGCAGTTTGTTACTATGCGTGACGTGTTCTTTCTCCATAATAAACAGCTAGC ACCTTCTTTGTTGATGTTCAGCTAGGCTGGTATTGAAGGGGTTATTACTATACCCCACAA TTAAGTGGTTGATCTTATCAGATGAAAACACCAGATGGACAGATATTCTATGATCTAAA AACTTCGAAAATCTTCTTTGTCCAGGACAACCAAAACGTGTTCTTTCTCCATAATAAAC TCAACAAACAAACTGGTTACAGCTGGGATTGAGTAGAATGGCTAAAACATGACATGAA TGAGGACAAAGACGAAAACTTTAAATGGTACTTTTCGCGTGATGACCTTACCATTCCTT CCGTTGA

Table S2. The LAMP primer sequences used in this work.

Pathogen	Primer sequence(5'-3')
RSVA-1-F3	TCAAAAATGCCATTACAAATGC
RSVA-1-B3	GGTTTGATTGCAAATCGTGT
RSVA-1-FIP	TGCTCCTTTGTTGTCAGTCACTTTAAAAATCATCCCTTACTCAGGA
RSVA-1-BIP	AGTAGATCTTGGAGCTTACCTAGAATTTAGCTGTGTGCTTCCAATT
RSVA-2-F3	ATACACAGCTGCTGTTCA
RSVA-2-B3	GTGAATTTGCTGGGCATT
RSVA-2-FIP	GATTGAAACATGGGCACCCATATTTAATGTCCTAGAGAAAGACGAT
RSVA-2-BIP	CTAGTGAAACAAATATCCACACCCATTTGCACTGCACTTCTTGAGTT
RSVA-3-F3	ACTAGTGAAACAAATATCCACAC
RSVA-3-B3	GAGATCTTTAACTGTAGTCAACA
RSVA-3-FIP	GGTGAATTTGCTGGGCATTTGTTTTCATTAAGAGTCATGATAAACTCAAG
RSVA-3-BIP	GCCAATGTGTCCTTGGATGAATTTTGTTAGACTGCATGCCTTA
RSVA-4-F3	CTAGCACAAATGCCCAGC
RSVA-4-B3	GCAATGATGTCATGTGTTGG
RSVA-4-FIP	TGGTTACATCATATGCCAGCTTGTTTAATTCACCATATGCGCCAA
RSVA-4-BIP	CACCCTGTGAAATTAAGGCATGCTTTATAGTGAGATCTTTAACTGTAGTC
RSVA-5-F3	CCACTGAATTCAAAAATGCCA
RSVA-5-B3	GGTTTGATTGCAAATCGTGT
RSVA-5-FIP	CCTTTGTTGTCAGTCACTGTGATGTTTTTACAAATGCAAAAATCATCCCT
RSVA-5-BIP	AGTAGATCTTGGAGCTTACCTAGAATTTAGCTGTGTGCTTCCAATT

RSVA-2-LB1	AGGGACCCTCATTAAGAGTCAT
RSVA-2-LB2	AGGGACCCTCATTAAGAGTCATG
RSVA-2-LB3	AGGGACCCTCATTAAGAGTCATGAT
RSVA-2-LB4	GGGACCCTCATTAAGAGTCATGAT
RSVA-2-LB5	GGGACCCTCATTAAGAGTCATGATA
MP-1-F3	GGTGGTTCACAACACGATT
MP-1-B3	CATCTGATAAGATCAACCACTT
MP-1-FIP	GCATGGGTAGTACATTCATCAACAATTTTGTATGTCCTTTGAAAAATCCA
MP-1-BIP	TTGTTACTATGCGTGCAGCTAGTTTGGGTATAGTAATAACCCCTTCA
MP-2-F3	TCAGTTTCGGGTCTCCTC
MP-2-B3	TGCACGCATAGTAACAAAC
MP-2-FIP	TGTGAACCACCACTCTTGGTTTTTGAACTGTTAGGTCAAACTGAA
MP-2-BIP	ATGTCCTTTGAAAAATCCACCTAGTTTTGCATGGGTAGTACATTCATCA
MP-3-F3	TGAAAATGGCTTGTTCTGAA
MP-3-B3	CTTCAATACCAGCCTAGCT
MP-3-FIP	ATCACTAGGTGGATTTTTCAAAGGATTTATACCAAGAGTGGTGGTTCA
MP-3-BIP	GAATGTACTACCCATGCGCAGTTTACATCAACAAAGAAGGTGCTA
MP-4-F3	GCAGTTTGTTACTATGCGTG
MP-4-B3	TTTATTATGGAGAAAGAACACGT
MP-4-FIP	TTGTGGGGTATAGTAATAACCCCTTTTTAGCACCTTCTTTGTTGATGT
MP-4-BIP	TTATCAGATGAAAACACCAGATGGATTTGGACAAAGAAGATTTTCGAAGTT