Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Rapid Differential Diagnosis of The B.1.617.2 (delta) variant of Covid-19 with automated Cas12a-Microfludic System

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Experimental Section

Materials and reagents

LbCas12a Nuclease was purchased from Shanghai Tolo Biotechnology. The centrifugal microfluidic chip and detection equipment were obtained from Shanghai Suxin Biotechnology. sgRNA was purchased from Guangzhou biolifesci Biotechnology. All HPLC-purified sequences were purchased from Sangon Biotech (Shanghai) Co., Ltd. (Table S1). The RT-PCR clinical samples were provided by Qingdao International Travel Healthcare Center.

Table S1 Sequences of the oligonucleotides used in the experiments.

Name	Sequence
Forward Primer	GACAAATCGCTCCAGGGCAAAC
Reverse Primer	GTTGCAATATGGCAGTTTTTGTACACA
Plasmid of the wild type of SARS-CoV-2	GACAAATCGCTCCAGGGCAAACTGGAAAGATTG CTGATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAATTCTAACAATCTTGAT TCTAAGGTTGGTGGTAATTATAATTACCTGTATAG ATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAG AGAGATATTTCAACTGAAATCTATCAGGCCGGTA GCACACCTTGTAATGGTGTTGAAGGTTTTAATTG TTACTTTCCTTTACAATCATATGGTTTCCAACCCA CTAATGGTGTTGGTTACCAACCATACAGAGTAGT AGTACTTTCTTTTGAACTTCTACATGCACCAGCA ACTGTTTGTGGACCTAAAAAGTCTACTAATTTGG TTAAAAACAAATGTGTCAATTTCAACTTCAATGG TTTAACAGGCACAGGTGTTCTTACTGAGTCTAAC AAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAG

	ACATTGCTGACACTACTGATGCTGTCCGTGATCC
	ACAGACACTTGAGATTCTTGACATTACACCATGT
	TCTTTTGGTGGTGTCAGTGTTATAACACCAGGAA
	CAAATACTTCTAACCAGGTTGCTGTTCTTTATCAG
	GATGTTAACTGCACAGAAGTCCCTGTTGCTATTC
	ATGCAGATCAACTTACTCCTACTTGGCGTGTTTAT
	TCTACAGGTTCTAATGTTTTTCAAACACGTGCAG
	GCTGTTTAATAGGGGCTGAACATGTCAACAACTC
	ATATGAGTGTGACATACCCATTGGTGCAGGTATAT
	GCGCTAGTTATCAGACTCAGACTAATTCTCCTCG
	GCGGGCACGTAGTGTAGCTAGTCAATCCATCATT
	GCCTACACTATGTCACTTGGTGCAGAAAATTCAG
	TTGCTTACTCTAATAACTCTATTGCCATACCCACA
	AATTTTACTATTAGTGTTACCACAGAAATTCTACC
	AGTGTCTATGACCAAGACATCAGTAGATTGTACA
	ATGTACATTTGTGGTGATTCAACTGAATGCAGCA
	ATCTTTTGTTGCAATATGGCAGTTTTTGTACACA
Plasmid of the delta variant of SARS-CoV-2	GACAAATCGCTCCAGGGCAAACTGGAAAGATTG
	CTGATTATAATTATAAATTACCAGATGATTTTACAG
	GCTGCGTTATAGCTTGGAATTCTAACAATCTTGAT
	TCTAAGGTTGGTGGTAATTATAATTACCGGTATAG
	ATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAG
	AGAGATATTTCAACTGAAATCTATCAGGCCGGTA
	GCAAACCTTGTAATGGTGTTGAAGGTTTTAATTG
	TTACTTTCCTTTACAATCATATGGTTTCCAACCCA
	CTAATGGTGTTGGTTACCAACCATACAGAGTAGT
	AGTACTTTCTTTTGAACTTCTACATGCACCAGCA
	ACTGTTTGTGGACCTAAAAAGTCTACTAATTTGG

TTAAAAACAAATGTGTCAATTTCAACTTCAATGG

TTTAACAGGCACAGGTGTTCTTACTGAGTCTAAC

AAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAG

ACATTGCTGACACTACTGATGCTGTCCGTGATCC

ACAGACACTTGAGATTCTTGACATTACACCATGT

TCTTTTGGTGGTGTCAGTGTTATAACACCAGGAA

CAAATACTTCTAACCAGGTTGCTGTTCTTTATCAG

GATGTTAACTGCACAGAAGTCCCTGTTGCTATTC

ATGCAGATCAACTTACTCCTACTTGGCGTGTTTAT

TCTACAGGTTCTAATGTTTTTCAAACACGTGCAG

GCTGTTTAATAGGGGCTGAACATGTCAACAACTC

ATATGAGTGTGACATACCCATTGGTGCAGGTATAT

GCGCTAGTTATCAGACTCAGACTAATTCTCGTCG

GCGGGCACGTAGTGTAGCTAGTCAATCCATCATT

GCCTACACTATGTCACTTGGTGCAGAAAATTCAG

TTGCTTACTCTAATAACTCTATTGCCATACCCACA

AATTTTACTATTAGTGTTACCACAGAAATTCTACC

AGTGTCTATGACCAAGACATCAGTAGATTGTACA

ATGTACATTTGTGGTGATTCAACTGAATGCAGCA

ATCTTTTGTTGCAATATGGCAGTTTTTGTACACA

Green: the primer sequence

Red: the recognition cites of crRNAs

crRNA-452-1	UAAUUUCUACUAAGUGUAGAUCCGGUAUAGAU UGUUUAGGA
crRNA-452-2	UAAUUUCUACUAAGUGUAGAUCCGUUAUAGAU UGUUUAGGA
crRNA-452-3	UAAUUUCUACUAAGUGUAGAUCCGGUAUAGAU UGUUUAGG
crRNA-452-4	UAAUUUCUACUAAGUGUAGAUCCGGUAUAGAU UGUUUAGGAA

crRNA-478-1	UAAUUUCUACUAAGUGUAGAUGCUACCGGCCU GAUAGAUU
crRNA-478-2	UAAUUUCUACUAAGUGUAGAUGCUACCGGCCU GAUAGAUUUC
crRNA-478-3	UAAUUUCUACUAAGUGUAGAUGCUACCGGCCU GAUAGAUUUCA
crRNA-478-4	UAAUUUCUACUAAGUGUAGAUGCUACCGGCCU GAUAGAUUU
crRNA-681-1	UAAUUUCUACUAAGUGUAGAUUCGUCGGCGGG CACGUAGUG
crRNA-681-2	UAAUUUCUACUAAGUGUAGAUUCGACGGCGGG CACGUAGUG
crRNA-681-3	UAAUUUCUACUAAGUGUAGAUUCGUCGGCGGG CACGUAGUGT
crRNA-681-4	UAAUUUCUACUAAGUGUAGAUUCGUCGGCGGG CACGUAGUGTA

Procedure of PCR for plasmids.

A 50 μL PCR reaction mixture contained 25 μL Phanta Max Master Mix (2x), 2 μL forward primer (10 μM), 2 μL reverse primer (10 μM), 2 μL plasmid sample (10^8 copies/μL), 19 μL DNase/RNasefree water. The thermal cycling protocol included an initial activation of Taq polymerase at 95 ℃ for 3 min, followed by 35 cycles of 95 ℃ for 30 s, 58 ℃ for 30 s, and 72 ℃ for 90 s, and a final extension step at 72 ℃ for 2 min.

PCR samples detection with Cas12a-Microfluidic system.

The CRISPR-Cas12a reaction reagents were preloaded in the outer chamber of the chip, which contained 0.5 μL LbCas12a nuclease (5μM), 0.5 μL crRNA (5 μM), 0.5 μL ssDNA reporter (5'-FAM-TTTTTTT-BHQ1-

3’, 5 μM), 0.5 μL tolo buffer (10x), 0.5 μL 0.5% (w/w) trehalose. After vacuum drying for 15 min at 37 ℃, the side of outer chamber was sealed. Then, a 30 μL PCR reaction mixture was added to the inner chamber and

the chip was sealed (Fig. S1). After that, the chip was put into the detection equipment, followed by low-speed rotation once at 2500 rpm for 5s and high-speed rotation once at 4600 rpm for 20 s. The PCR samples were allocated equally to outer chambers, where the CRISPR-Cas12a reaction occurred at 37 ℃. The incubation time was set for 10 min. Real time fluorescence signals can be read directly from the screen of the detection equipment.

Fig. S1 The picture of the microfluidic chip in this work.

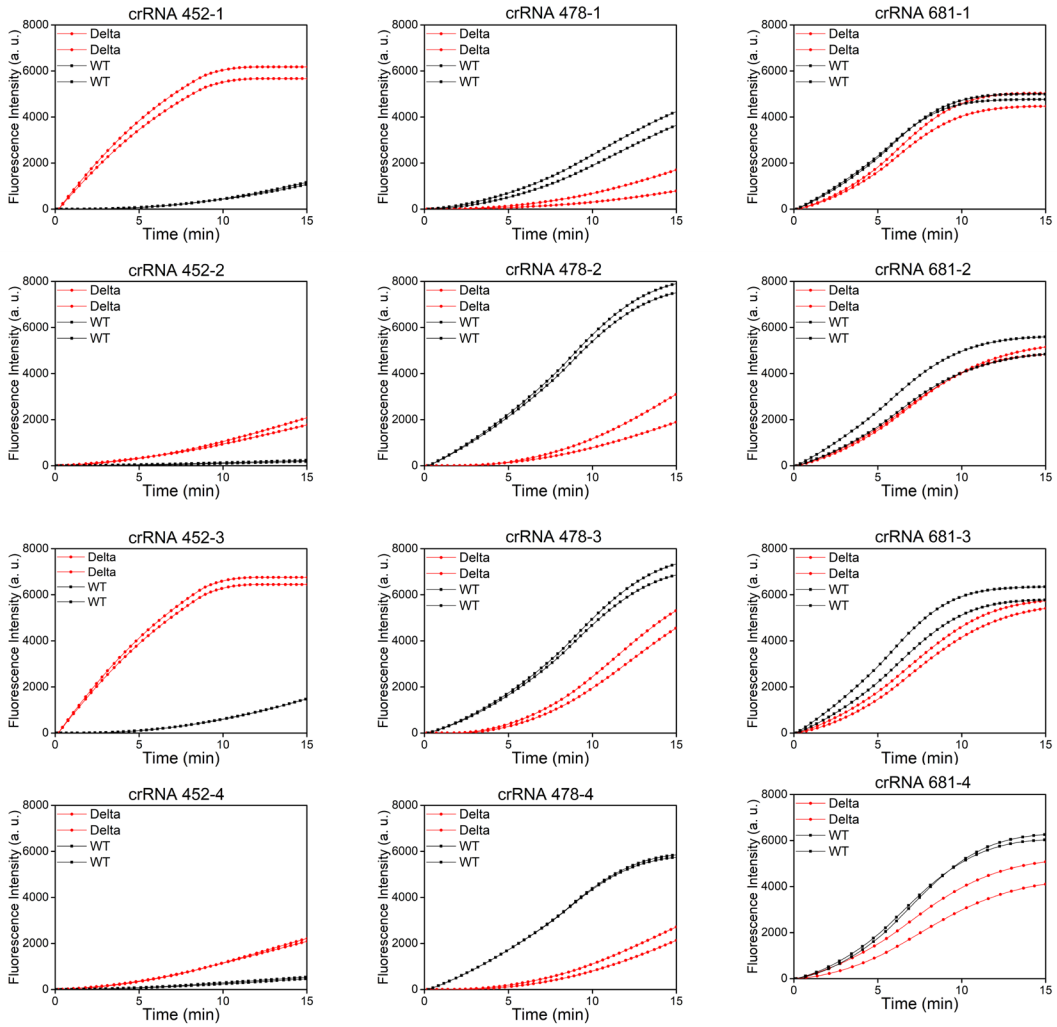

Fig. S2 The screening results of crRNAs.

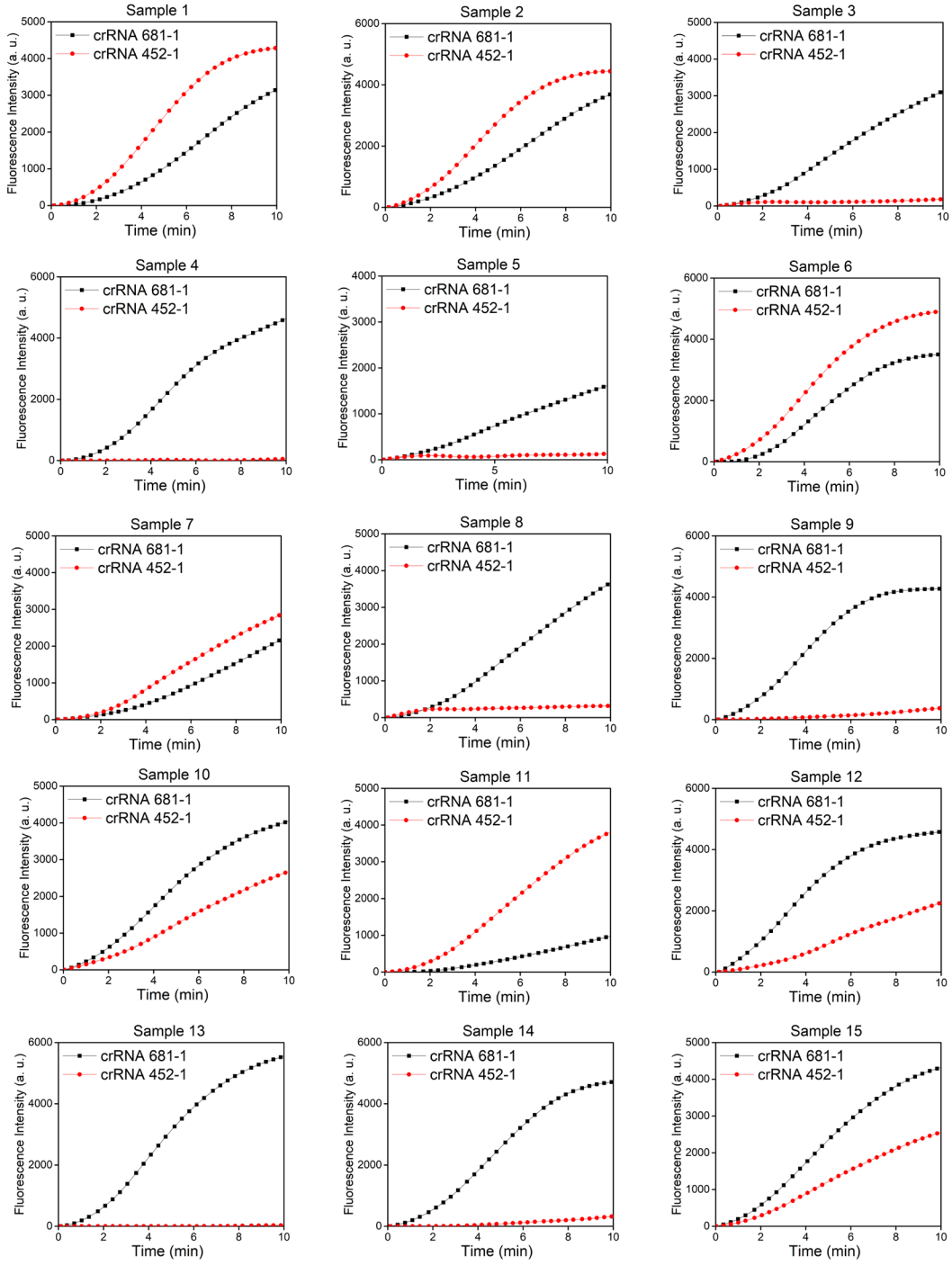

Fig. S3 The real time fluorescence signals of clinical samples by Cas12a-Microfluidic system (sample 1 to sample 15).

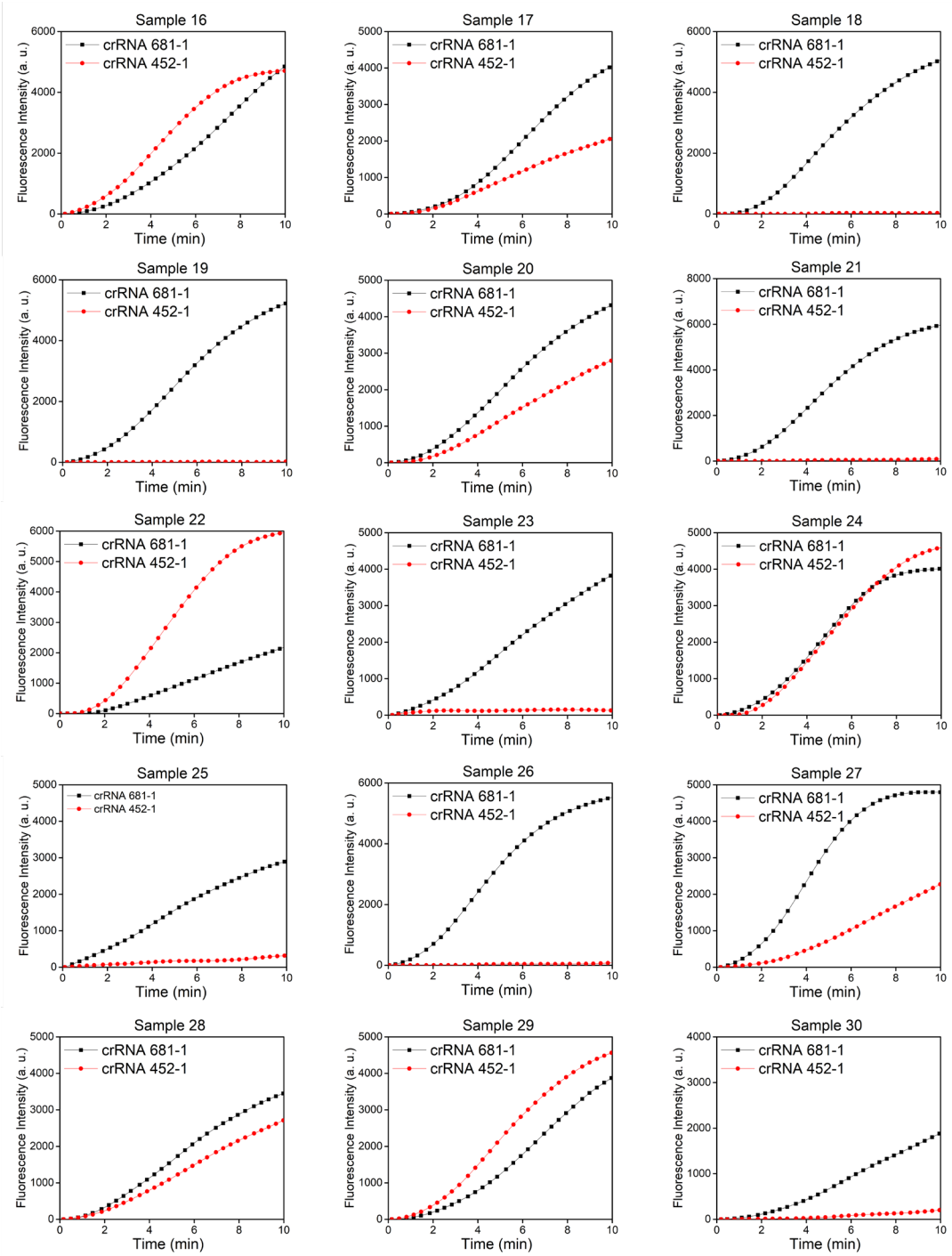

Fig. S4 The real time fluorescence signals of clinical samples (sample 16 to sample 30).