

Table S2. N6 assay false-positive product sequencing

Primers to establish library	Forward	Reverse	index in red	underscore incates the F2 of N6	
Sequencing results	AATGATACGGGGACCACGAGATCTACAC <u>TGCACGGTA</u> ACACTCTTCCCTACAGAGCTCTCCGATCT <u>GTCAAGGCTCTCTCGTTCCT</u>	CAAGCAGAAAGCGCATACGAGATT <u>GCAACGTA</u> GTGACTGGAGTTCAGACGTGTGCTCTCCGAT <u>GCTCAAGCTGGTTC</u> AATCT			underscore incates the B2 of N6
	Top 10 reads				
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGATC			2120402	35.36%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			66114	1.10%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			1432573	23.89%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			917862	15.31%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			217254	3.62%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			70644	1.18%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			55081	0.92%
	GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			31607	0.53%
GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			48189	0.80%	
GTCAATCTCTCTCGTTCCTCTGCTAGATTGAACCAAGCTTGAGGCAAAAGCCATTCAAGCAGGGCTGCTGGAGTTGAATGTCAAGCCTCTCTCGTTCCTCTGCTAGATTAAACCACTTGAGCGCACCGCCAGTCAAGCAGGGAT			27643	0.46%	
			Total	5996593	
				83.17%	

Table S3. The loop stem (F1c and B1c), LAMP amplification efficiency, and specificity

primer sets	F1c	Length	Tm (°C)	B1c	Length	Tm(°C)	TT (min)	FP
N gene								LAMP
N7	tgccaatgtgatctt	15	47.9	aatcgtgctacaac	14	45.2	19.7±0.8	1
N8	tccctctcgcta	13	46.7	gtcgcaacagttc	13	45.9	43.9±5.3	3
N9	catcaccgccatt	13	47.2	ttgctttgctgct	13	47.2	24.4±1.7	2
N10	gccttgctctoga	13	49	caatagcagtcocaga	15	47.4	28.6±3.1	1
N11	ttgttagcaggattg	15	45.7	caaggaacaacattg	15	45.2	28.4±6.6	1
N12	gcacgattgcag	12	45.1	acaacattgccaaa	14	45.2	32.9±7.1	1
N13	cctttggcaatgtt	15	47.6	agtcaagcctcttc	14	46.4	26.0±3.5	3
N14	cctctgcgtagaa	14	46.7	tcatcagtagtcg	14	46.7	27.5±3.0	3
N15	cctctgcgtagaa	14	46.7	acgtagtcgcaac	13	47.1	26.6±2.0	2
N16	cctctgcgtagaa	14	46.7	agtcaagcctcttc	14	46.4	25.7±2.3	2
N17	aacgagaagaggc	13	45.4	tcgcaacagttca	13	45.9	30.5±3.3	1
N18	gcctggagtgaat	14	47.3	ctgctctgctttg	14	47	35.2±1.7	2
N19	gcctggagtgaat	14	47.3	ctgctctgctttg	14	47	31.2±3.5	2
N20	caggagaagtctcc	14	47	tgatgctgctctg	14	48	27.4±3.1	2
N21	ctagcaggagaagt	14	45.1	ctgctctgctttg	14	47	42.6±4.9	2
N22	catcaccgccat	12	45.2	ttgctttgctgct	13	47.2	41.7±4.4	2
N23	tcaccgccattg	12	45.7	gcaaaaatgtctggt	14	45.6	22.6±2.4	2
N24*	catcaccgccatt	13	47.2	gcaaaaatgtctggt	14	45.6	21.6±2.6	1
N25*	tcaatctgcaagca	15	47.5	atgtctgtaagaggc	15	48.1	18.0±3.9	1
N26	attatgggtaaacctt	17	46	ggcaaggaagacc	13	47.1	34.9±6.6	1
N27*	gagagcggtagac	13	47.6	tcaggacaagggc	13	49	18.4±1.8	1
N28	gcaglattattgggta	16	45.4	ttcaccgctctca	13	47.7	27.5±6.7	1
RT-LAMP								
N2(-5)	aggctccctcagttgc	16	57.7	attggcaccggcaatc	16	57.1	15.3±0.3	1
N3(-4)	attattgggtaaacctt	17	46	caaggaagacctt	12	38.8	23.1±3.2	1
N4(-4)	ccaatgtgatctt	13	38.8	taacaatgctgct	12	38.7	21.4±4.9	1
N6(-4)	ctggagtgaat	12	35.9	ctagaatggctg	12	35.9	21.9±3.4	1
N7	tgccaatgtgatctt	15	47.9	aatcgtgctacaac	14	45.2	14.3±0.6	1
N24*							20.4±1.8	3
N25*							20.2±5.5	1
N27*							11.7±0.1	1
M gene								
M1	tccttagcaggctc	14	45.9	actgttgctacatca	15	47.1	22.6±2.9	1
M2	gtccttgatgtcac	14	45.1	caaattgggagcttc	15	47.6	22.0±0.9	2
M3	cagaatagtgccatg	15	46.6	agaagtgactgct	15	47.1	11.1±0.8	1
M4	gcaggctctgat	13	45.4	ggagcttcgacg	14	46.8	31.1±2.3	1
M5	aaacagctgaaagaa	16	45.7	ttctcaacgtgctc	13	47.2	16.5±0.4	1
M6	acatggaacgcg	12	46.6	ttcttctcaacgtgctc	15	49.6	15.2±0.4	1
S gene								
S1	gcaaagggtggca	12	46.4	tgattgctcaatacac	16	47.1	24.8±2.1	1
S2	aggccgttaaaactt	14	46.6	gttttgccacctt	14	46.3	15.5±1.4	3
S3	gaggctctagcag	14	46	gtttaacggccttac	15	47.4	26.1±4.9	3
S4	gcaaagggtggcaaaa	14	49.9	tgattgctcaatacac	16	47.1	23.1±2.9	1
S5	gtgtctgtggatca	14	46.5	acaccaggaacaaa	14	46.2	21.5±1.8	4
S6	tcaagtgctgtgg	14	46.8	caccaggaacaaata	15	45.1	17.5±0.8	4
S7	tgaggctctagca	14	46.5	tttaacggccttact	15	47.1	19.2±2.9	1

TT represented 3-10 independent measures presented with mean± SD. * same primer sets were used both for LAMP and RT-LAMP assays. FP was determined as described in "Method". From 3-4 replicates, the highest FP score was listed as the final FP for the primer.

Table S4. The M Gene primer sets

Primer sets	Amplification Len	Sequence (5'-3')	Sequence (5'-3')		Sequence (5'-3')		Sequence (5'-3')			
#	B2-F2(Len)	F3	Len	Tm	F2	Len	Tm	FL	Len	Tm
M1	123	actattctgaccagaccgcttc	22	62	tctcgtggacatcttcgtattgc	22	60.7	gatgtcacagcgtcctagatgg	22	62
M2	157	cattcttctcaacgtgccaactc	22	61	cggagctgtgatcctctcgtg	20	62.6	gtgtccagcaatcgaagatgt	22	60
M3	129	gctatcgcaatggcttgctctg	22	62	ccatgtggctcattcaatccaga	22	60	ggcacgttgagaagaatgttagt	23	61
M4	166	cattcttctcaacgtgccaactc	22	61	tgtgatccttcgtggacatcctt	22	61.1	tcctagatggtgtccagcaatc	23	61
M5	160	ggccagtaaccttagcttgTTTTg	24	61	cgcaatggcttgctctgttagg	21	61.6	tgaagttagctgagccacatcaa	22	61
M6	141	gttttTgcttgctgctgttta	22	60	aggcttgatgtggctcagcta	21	63.3	gcaaacagctctgaaagaagcaatg	24	61

Primer sets	Sequence (5'-3')	Sequence (5'-3')		Sequence (5'-3')			
#	F1c	Len	Tm	B1c	BL	Len	Tm
M1	tctttaggcaggtc	14	46	actgttgctacatca	15	47.1	cttattacaaattgggagcttcgc
M2	gtccttgatgtcac	14	45	caaattgggagcttc	15	47.6	gcgtgtagcagggtgactcag
M3	cagaatagtgccatg	15	47	agaaagtgaactcgt	15	47.1	gatccttcgtggacatcttcgt
M4	gcaggctcctgat	13	45	ggagcttcgcag	12	46.8	ggtgactcaggttttctgctgeat
M5	aaacagctctgaaagaa	16	46	ttctcaacgtgcc	13	47.2	tattctgaccagaccgcttcta
M6	acatggaacgcg	12	47	ttcttctcaacgtgc	15	49.6	tattctgaccagaccgcttcta

Primer sets	Sequence (5'-3')	Sequence (5'-3')		Sequence (5'-3')		
#	B2	Len	Tm	B3	Len	Tm
M1	aaacctgagtcacctgctacac	22	62	attgtcactgctactggaatgg	22	60
M2	tgtagcagctgtatgcagcaaa	22	62	gctactggaatggtctgtgttt	22	60.2
M3	cgtcctagatggtgtccagcaa	22	64	gaaagcgttcgtgatgtagcaa	22	61.4
M4	ttatagttgccaatcctgtagcg	23	60	gctactggaatggtctgtgttt	22	60.2
M5	cagctccgattacagattcact	22	62	gtccagcaatcgaagatgtcc	22	60.7
M6	agctccgattacagattcactt	22	61	gtccagcaatcgaagatgtcc	22	60.7

Primer sets	Sequence (5'-3')	Sequence (5'-3')		PubMed	
#	FIP (F1c+F2)	Len	BIP (B1c+B2)	Len	Accession
M1	tctttaggcaggctcttcgtggacatcttcgtattgc	36	actgttgctacatcaaacctgagtcacctgctacac	37	NC_045512
M2	gtccttgatgtcaccggagctgtgatccttcgtg	34	caaattgggagctctcgtagcagctgtatgcagcaaa	37	NC_045512
M3	cagaatagtgccatgccaatggtcattcaatccaga	37	agaaagtgaactcgtcgtcctagatggtgtccagcaa	37	NC_045512
M4	gcaggctcctgattgtgatccttcgtggacatctt	35	ggagcttcgcagttatagttgccaatcctgtagcg	35	NC_045512
M5	aaacagctctgaaagaacgcaatggcttgctctgtagg	37	ttctcaacgtgccagctccgattacagattcact	35	NC_045512
M6	acatggaacgcgaggcttgatgtggctcagcta	33	ttcttctcaacgtgcagctccgattacagattcactt	37	NC_045512

* Note: Each primer set is composed of eight oligos, in which FIP is made by F1c+F2, and BIP is made by B1c+B2.

Table S5. The S Gene primer sets

Primer sets			Amplification Len		Sequence (5'-3')		Sequence (5'-3')		Sequence (5'-3')	
#	B2-F2 (Len)	F3	Len	Tm	F2	Len	Tm	FL	Len	Tm
S1	124	acacttgcatgctggctt	20	62.4	tattgctgtagagacctcattt	23	59	agtaaggccgttaaaccttttgg	23	59.4
S2	122	agtgacacttgcatgctg	20	60.3	aatatggtagtgcttgggtga	22	59.7	gcacaaatgaggtctctagcag	22	60.7
S3	140	attaccagatccatcaaaacca	22	57.1	agtgacacttgcatgctg	20	60.3	accaaggcaatcaccatattgt	22	60
S4	133	agtgacacttgcatgctg	20	60.3	cttggtagattgctgctagag	22	58.3	gtaaggccgttaaaccttttgg	22	58.1
S5	174	tcaatggtttaacaggcacagg	22	60.8	aagttctgctttccaacaat	22	58.6	cagtagtgcagcaatgtctct	22	59.4
S6	169	tcaatggtttaacaggcacagg	22	60.8	ctgctttccaacaatttggc	21	60.6	tcagtagtgcagcaatgtctc	22	59.4
S7	136	attaccagatccatcaaaacca	22	57.1	cacttgcatgctggctt	19	60.7	accaaggcaatcaccatattgt	22	60

Primer sets			Sequence (5'-3')		Sequence (5'-3')		Sequence (5'-3')			
#	F1c	B1c	Len	Tm	B1c	Len	Tm	BL	Len	Tm
S1	gcaaaggtggca	12	46.4	tgattgctcaatacac	16	47.1	ctgttagcgggtacaatcaactt	22	60	
S2	aggccgttaaacct	14	46.6	gttttgccacctt	14	46.3	cacagatgaaatgattgctcaa	22	57	
S3	gaggtctctagcag	14	46	gtttaacggccttac	15	47.4	cccttgctcacagatgaaatga	22	58.6	
S4	gcaaaggtggcaaa	14	49.9	tgattgctcaatacac	16	47.1	gttagcgggtacaatcaacttct	22	59.8	
S5	gtgtctgtggatca	14	46.5	acaccaggaacaaa	14	46.2	taaccaggtgctgtcttcttat	22	57.3	
S6	tcaagtgctctgtgg	14	46.8	caccaggaacaaaata	15	45.1	aaccaggtgctgtcttcttatc	22	58.5	
S7	tgaggtctctagca	14	46.5	tttaacggccttact	15	47.1	cccttgctcacagatgaaatga	22	58.6	

Primer sets			Sequence (5'-3')		Sequence (5'-3')		Sequence (5'-3')	
#	B2	B3	Len	Tm	B3	Len	Tm	
S1	ctgcaccaaaggtccaacc	19	60.9	acattctgtgtaactccaatacca	24	59.4		
S2	aagtgtatgtaccgctaacag	22	60	ccattaaacctataagccatttgc	24	58.1		
S3	gctaacaagtgcagaagtgtatt	22	58.3	ctgcaccaaaggtccaacc	19	60.9		
S4	ccctgcaccaaaggtccaacc	19	60.9	ccattaaacctataagccatttgc	24	58.1		
S5	acagggacttctgtgcagtt	20	61	ggagtaagttgatctgcatgaa	22	57.8		
S6	aacagggacttctgtgcagtt	20	61	ggagtaagttgatctgcatgaa	22	57.8		
S7	cgctaacaagtgcagaagtgtat	22	60.4	ctgcaccaaaggtccaacc	19	60.9		

Primer sets			Sequence (5'-3')		Sequence (5'-3')		PubMed	
#	FIP (F1c+F2)	BIP (B1c+B2)	Len	Tm	BIP (B1c+B2)	Len	Tm	Accession
S1	gcaaaggtggcatattgctgctagagacctcattt	35		tgattgctcaatacacactgcaccaaaggtccaacc	35		NC_045512	
S2	aggccgttaaaccttaatatggtgattgccttgggtga	36		gttttgccaccttaagtattgaccgctaacag	36		NC_045512	
S3	gaggtctctagcagagtgcacttgcatgctg	34		gtttaacggccttacgtaacagtgacagaagtgtatt	37		NC_045512	
S4	gcaaaggtggcaaacctgggtgatattgctgctagag	36		tgattgctcaatacacactgcaccaaaggtccaacc	35		NC_045512	
S5	gtgtctgtggatcaaaagttctgcctttccaacaat	36		acaccaggaacaaaacagggacttctgtgcagtt	34		NC_045512	
S6	tcaagtgctctgtggctgcctttccaacaatttggc	35		caccaggaacaaaataaacagggacttctgtgcagtt	35		NC_045512	
S7	tgaggtctctagcaaaccttgcatgctggctt	33		tttaacggccttactgcctaacaagtgcagaagtgtat	37		NC_045512	

* Note: Every primer set is composed of eight oligos, in which FIP is made by F1c+F2, and BIP is made by B1c+B2.

Table S6. Case #16 RT-LAMP product sequencing results

Primers to establish library	Forward	AATGATACGGCGACCCAGAGATCTACACCTAGACTGCACACTCTTCCCTACACGACGCTCTTCGGATCTGGGCGGATCAAAAAGAGC			
Sequencing results	Reverse	CAAGCAGAGACCGCATACGAGATCTAGACTGGGTGACTGGAGTTCAGACGTGTGCTCTCCGATTCGGGTAGGCAATTGGTC	SARS-CoV-2 genomic RNA (MN908947.3), % identities	index in red index in red	underscore incates the F2 of N27 underscore incates the B2 of N27
	Top 10 reads			read count	% of total reads
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	1497377	38.33%
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	1193621	30.55%
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		95%	182513	4.67%
	TGGCAACCCAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		94%	106275	2.72%
	ACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	93284	2.39%
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	68159	1.74%
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	60954	1.56%
	TGGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		94%	48579	1.24%
	CGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	33875	0.87%
	CGGCCCCAAGGTTTACCCAAATAACTGGCTTGGTTCAACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATCCCTCGAGGACAAGGCGTTCCAAATTAACCAAAAACCCATACAAAATAACA		100%	23569	0.60%
	Total			3906852	84.68%

Table S7. ACTB RT-LAMP primer set

ACTB

FIP	GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA
BIP	CTGAACCCCAAGGCCAACCGGCTGGGGTGTGAAGGTC
FL	TGTGGTGCCAGATTTTCTCCA
BL	CGAGAAGATGACCCAGATCATGT
F3	AGTACCCCATCGAGCACG
B3	AGCCTGGATAGCAACGTACA

Reference

Zhang, Y., Ren, G., Buss, J., Barry, A. J., Patton, G. C., and Tanner, N. A. (2020)

Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. *Biotechniques* 69, 178-185

Table S8. Real-time PCR primers

E-Sarbeco*	F	ACAGGTACGTTAATAGTTAATAGCGT
	R	ATATTGCAGCAGTACGCACACA
Beta-actin (ACTB)	F	CTATCCCTGTACGCCTCTGG
	R	GTCACCGGAGTCCATCACC
Human coronavirus 229E	F	CGTGTTGGGACAGACATACTATC
	R	CTGAACACCTGAAGCCAATCTA
Human coronavirus OC43	F	TGGAGCGTATGGCTGATTTG
	R	GCATAGTTTGCAAGGCAGAAAC
Influenza A virus (H1N1)	F	GGGAACGGTGACCCAAATAA
	R	CAAGTGCACCAGCAGAATAAC
Human respiratory syncytial virus	F	GGCAGTAGAGTTGAAGGGATTT
	R	TGCACACTAGCATGTCCTAAC
Human rhinovirus 16	F	GTCAAATGGATCCAGCCTCA
	R	GGGTCTTGAGAGAAGTCCAATC

*Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brunink, S., Schneider, J., Schmidt, M. L., Mulders, D. G., Haagmans, B. L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J. L., Ellis, J., Zambon, M., Peiris, M., Goossens, H., Reusken, C., Koopmans, M. P., and Drosten, C.

(2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25