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Title: Development and validation of a new and rapid molecular diagnostic tool based on RT-LAMP for Hepatitis C virus detection at Point-of-Care

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Content:

Supplementary Table S1. Characteristics of the samples from people with HCV included in this study, indicating: Sample matrix; HCV genotype and/or subtype; viral load (IU/mL) reported by the hospital (1) or determined by NZYTech RT-qPCR after thaw/freezing at the laboratory (2); LAMP result from serum lysate, where “POS” means positive result, and “UD” undetected..... 3

Supplementary Table S2. GeneBank Access number from HCV reference sequences..... 7

Supplementary Table S3. Oligonucleotide sequences of the different LAMP-oligonucleotide primers designed. 8

Supplementary Table S4. Combination of LAMP-primers tested for detection of GT1, GT2 and GT4. 10

Supplementary Table S5. LAMP-oligonucleotides encompassing HCG124 primer set. 10

Supplementary Fig. S1. Individual representation of real time fluorescence RT-LAMP amplification plots displayed in Fig. 2, using HCDN primer with extracted RNA samples (left panels) or lysed serum samples (right panels). NTC: non-template control; samples PL13, PL14, PL15, PL17, PL19 (GT4) and PL20 (GT2)..... 11

Supplementary Fig. S2. Individual representation of real time fluorescence RT-LAMP amplification plots displayed in Fig. 3 comparing: (a) primer combinations for detection of both GT1, GT2 and GT4 from lysed serum samples, using Mix A, B, C, D, E, F, G; (b) primer sets Mix C and E, and new 9-primer set HCG124, for the detection of GT2 serum samples. 12

Supplementary Fig. S3. Representative real time fluorescence RT-LAMP amplification plot using HCG124 primer set for the detection of the *in vitro* transcripts of GT5 and GT6 5'UTR-Core region..... 13

Supplementary Fig. S4. Determination of the limit of detection of the HCV RT-LAMP assay from purified HCV RNA of GT1, GT2, GT3, and GT4, serially diluted up to less than 1 copy per reaction (copies/rxn)..... 14

Supplementary Fig. S5. Result of the conventional RT-PCR amplification of purified RNA from PLB7a serum sample, visualized by agarose gel electrophoresis. CP: HCV-JFH1 RNA used as positive control. NTC: non template control..... 15

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Sample	Matrix	Genotype	Viral load (IU/mL) ⁽¹⁾	Viral load (IU/mL) ⁽²⁾	LAMP result
PL01	Serum	4	1,597,749.00		POS
PL02	Serum	4	944,970.00		POS
PL03	Serum	4	571,942.00		POS
PL04	Serum	1b	10,958,983.00		POS
PL05	Serum	3	5,082,601.00		POS
PL06	Serum	3	16,940,739.00		POS
PL07	Serum	3	160,519.00		POS
PL08	Serum	1a	250,000.00		POS
PL09	Serum	3	1,750,000.00		POS
PL10	Serum	4	407,000.00		POS
PL11	Serum	3a	-		POS
PL12	Serum	4	337,000.00		POS
PL13	Serum	4	2,230,000.00		POS
PL14	Serum	4	1,230,000.00		POS
PL15	Serum	4	561,000.00		POS
PL16	Serum	4	940,000.00		POS
PL17	Serum	4	10,000,000.00		POS
PL18	Serum	3	6,710,000.00		POS
PL19	Serum	4	2,549,682.00		POS
PL20	Serum	2	22,568,943.00		POS
PLA1a	Serum	1b	389.00		POS
PLA2a	Serum	1b	1,400,000.00		POS
PLA3a	Serum	1b	4,170.00	174.22	UD
PLA4a	Serum	1b	155,000.00		POS
PLA5a	Serum	1b	3,310,000.00		POS
PLA6a	Serum	1b	7,050,000.00		POS
PLA7a	Serum	1b	2,910,000.00		POS
PLA8a	Serum	4	1,880,000.00		POS
PLA9a	Serum	1b	2,010,000.00		POS
PLB1a	Serum	1b	635,000.00		POS
PLB2a	Serum	1a	1,200,000.00		POS
PLB3a	Serum	3a	388,000.00		POS
PLB4a	Serum	1a	2,280,000.00		POS
PLB5a	Serum	1a	87,500.00	427.00	UD
PLB6a	Serum	2	1,660,000.00		POS
PLB7a	Serum	1b	815,000.00	12,306.93	UD
PLB8a	Serum	4d	760,000.00		POS
PLB9a	Serum	1b	2,640,000.00		POS
PLC1a	Serum	1b	2,230,000.00		POS

PLC2a	Serum	1a	2,280,000.00		POS
PLC3a	Serum	3a	1,090,000.00		POS
PLC4a	Serum	1a	574,000.00		POS
PLC5a	Serum	3a	3,000,000.00		POS
PLC6a	Serum	2	1,130,000.00		POS
PLC7a	Serum	2	8,870,000.00		POS
PLC8a	Serum	1b	1,400,000.00		POS
PLC9a	Serum	1b	86,200.00		POS
PLD1a	Serum	1a	5,880,000.00		POS
PLD2a	Serum	3a	6,100,000.00		POS
PLD3a	Serum	1b	9,450,000.00		POS
PLD4a	Serum	4	6,100,000.00		POS
PLD5a	Serum	1b	6,980,000.00		POS
PLD6a	Serum	1a	9,770,000.00		POS
PLD7a	Serum	1a	12,800,000.00		POS
PLD8a	Serum	1a	2,460,000.00		POS
PLD9a	Serum	1b	7,510,000.00		POS
PLE1a	Serum	1b	4,050,000.00		POS
PLE2a	Serum	1a	357,000.00		POS
PLE3a	Serum	1b	4,220,000.00		POS
PLE4a	Serum	1b	266,000.00		POS
PLA1b	Serum	1a	2,120,000.00		POS
PLA3b	Serum	3	7,880,000.00		POS
PLA4b	Serum	1a	10,700,000.00		POS
PLA5b	Serum	1b	1,030,000.00		POS
PLA6b	Serum	3a	493,000.00		POS
PLA7b	Serum	4	3,230,000.00		POS
PLA8b	Serum	1a	629,000.00		POS
PLA9b	Serum	1b	773,000.00		POS
PLB1b	Serum	1b	2,350,000.00		POS
PLB2b	Serum	1b	236,000.00		POS
PLB3b	Serum	4	1,510,000.00		POS
PLB4b	Serum	4a/4c/4d	1,020,000.00		POS
PLB5b	Serum	4a/4c/4d	437,000.00		POS
PLB6b	Serum	1b	150,000.00	578.00	UD
PLB7b	Serum	1a	6,910,000.00		POS
PLB8b	Serum	1b	383,000.00		POS
PLB9b	Serum	1a	8,510,000.00		POS
PLC1b	Serum	1b	1,480,000.00		POS
PLC2b	Serum	1b	1,020,000.00		POS
PLC3b	Serum	1b	46,400.00		POS
PLC4b	Serum	3a	1,720,000.00		POS
PLC5b	Serum	3a	3,030,000.00		POS
PLC6b	Serum	1b	38,800.00	149.00	UD

PLC7b	Serum	1a	2,570,000.00		POS
PLC8b	Serum	1b	576,000.00		POS
PLC9b	Serum	3a	663,000.00		POS
PLD2b	Serum	1b	72,300.00		POS
PLD3b	Serum	1b	48,700.00		POS
PLD4b	Serum	1b	291,000.00		POS
PLD5b	Serum	3a	104,000.00		POS
PLD6b	Serum	1a	36,800.00	435.00	UD
PLD7b	Serum	1b	1,790,000.00		POS
PLD8b	Serum	1b	1,060,000.00		POS
PLD9b	Serum	1b	2,160,000.00		POS
PLE1b	Serum	1a	223,000.00		POS
PLE2b	Serum	1b	1,210,000.00		POS
PLE3b	Serum	3a	346,000.00		POS
PLE4b	Serum	1a	2,580,000.00		POS
PLE5b	Serum	1a	110,000.00	1,015.00	UD
PLE6b	Serum	1b	443,000.00		POS
PLE8b	Serum	1b	1,350,000.00		POS
PLE9b	Serum	1b	723,000.00		POS
PLF1b	Serum	2	295,000.00		POS
PLF2b	Serum	1a	127,000.00		POS
PLF3b	Serum	1b	469,000.00		POS
PLF4b	Serum	3a	538,000.00		POS
PLF5b	Serum	1b	17,200,000.00		POS
PLF6b	Serum	3a	463,000.00		POS
PLF7b	Serum	3a	3,850,000.00		POS
PLF8b	Serum	1b	323,000.00		POS
PLF9b	Serum	1a	226,000.00		POS
PLG1b	Serum	1b	31,100.00		POS
PLG2b	Serum	1b	421,000.00		POS
PLG3b	Serum	2	4,270,000.00		POS
PLG4b	Serum	1b	497,000.00		POS
PLG5b	Serum	1b	3,050,000.00		POS
S01	Fresh blood	1a	1,730,000.00		
S02	Fresh blood	1b	860,000.00		
S03	Fresh blood	NA	9,410,000.00		
S04	Fresh blood	1b	198,000.00		
S05	Fresh blood	NA	533.00		
S06	Fresh blood	NA	4,500,000.00		
S07	Fresh blood	1a	1,010,000.00		
S09	Fresh blood	NA	2,150,000.00		
S10	Fresh blood	4	384,000.00		
S11	Fresh blood	1a	975,000.00		
S12	Fresh blood	1a	243,000.00		

S13	Fresh blood	1a	159.00		
S14	Fresh blood	1a	3,270,000.00		

Supplementary Table S2. GeneBank Access number from HCV reference sequences

Genotype	GenBank Access Number
1a	AF009606.1
1b	D90208.1
2a (JFH1)	AB047639.1
2b (J8cc)	JQ745652.1
2b	AB559564.1
3a	D17763.1
3b	D49374.1
4a	Y11604.1
4b	FJ462435.1
4c	FJ462436.1
5a	KC844046
6	NC_009827.1

Supplementary Table S3. Oligonucleotide sequences of the different LAMP-oligonucleotide primers designed.

Primer set name	Primer name	Sequence (5'-3')
HCGen	F3_GEN	TGGATCAACCCGCTCAATG
	FIP_GEN_D	CACAAGGCCTTTCGCRACCCACCTGGMGATTTGGGCGTG
	LF_GEN	ACTACTCGGCTAGCAGTCTT
	LB_GEN	TGAGCACGAATCCTAAACCTC
	BIP_GEN_D	CGGGAGGTCTCGTAGACCGTCGGTTGGTGTWCKTTTGGT
	B3_GEN	GGGAACTTGACGTCTCTGTG
HCDN ¹	DN2_F3	TCGAACAGCCTCCAGGACCC
	DN2_FIP	CGGGCATTGAGCGGGTTTATCCTTTTGGTGAGTACACCGGAATTGC
	DN2_LF	AAAGGACCCGGTCTCTCT
	DN2_LB	GTCGCGAAAGGCCTTGTG
	DN2_BIP	CGCGAGACTGCTAGCCGAGTTTTTAGCACCTATCAGGCAGTAC
	DN2_B3	CACGGTCTACGAGACCTCC
GT2-specific primers	DN2_F3-G2	TCGTACAGCCTCCAGGCCCC
	DN2_FIP-G2	CGGGCATAGAGTGGGTTTATCCTTTTGGTGAGTACACCGGAATTGC
	DN2_LF-G2	AAAGGACCCAGTCTTCC
HCDN-G3	DN2_F3-G3	TCGTGCAGCCTCCAGGACCC
	DN2_FIP-G3	TGGGTATTGAGCGGGTTGCTCCTTTTGGTGAGTACACCGGAATCGC
	DN2_LF-G3	AAAGGACCCGGTCACCCC
	DN2_LB	GTCGCGAAAGGCCTTGTG
	DN2_BIP-G3	CGCGAGATCACTAGCCGAGTTTTTAGCACCTATCAGGCAGTAC
	DN2_B3	CACGGTCTACGAGACCTCC
HCGen-G3	F3_GEN-G3	TGGAACAACCCGCTCAATR
	FIP_GEN-G3	CACAAGGCCTTTCGCGACCCACCCRGAAATTTGGGCGTG
	LF_GEN-G3	ACTACTCGGCTAGTGATCTC
	LB_GEN G3	TGAGCACACTTCTAAACCTC
	BIP_GEN-G3	CGGGAGGTCTCGTAGACCGTCGACGGAGKGTGTTTCTTTTKGT
	B3_GEN-G3	GGGAACTTRACGTCTCTKTG
Pangenotypic primers	DN2_F3-Deg	TCGWRCAGCCTCCAGGACCC
	DN2_FIP-Deg	TGGGYATTGAGCGGGTTGMTCCCTTTTGGTGAGTACACCGGAATYGC
	DN2_LF-Deg	AAAGGACCCGGTCAYYCY
	DN2_BIP-Deg	CGCGAGAYYACTAGCCGAGTTTTTAGCACCTATCAGGCAGTAC
	DN2_FIP-InoG2	CGGGCAITGAGCGGGTTAATCCTTTTGGTGAGTACACCGGAATTGC
	DN2_FIP-InoG2T	CGGGCAITGAGIGGGTTAATCCTTTTGGTGAGTACACCGGAATTGC

DN2_LF_1	AAAGGACCCGGTCGTCC
DN2_LF-In02	AAAGGACCCGGTCITCC
DN2_LF-In03	AAAGGACCCIGTCITCC

¹Nyan DC, Swinson KL. A method for rapid detection and genotype identification of hepatitis C virus 1-6 by one-step reverse transcription loop-mediated isothermal amplification. *Int J Infect Dis.* 2016;43:30-36.

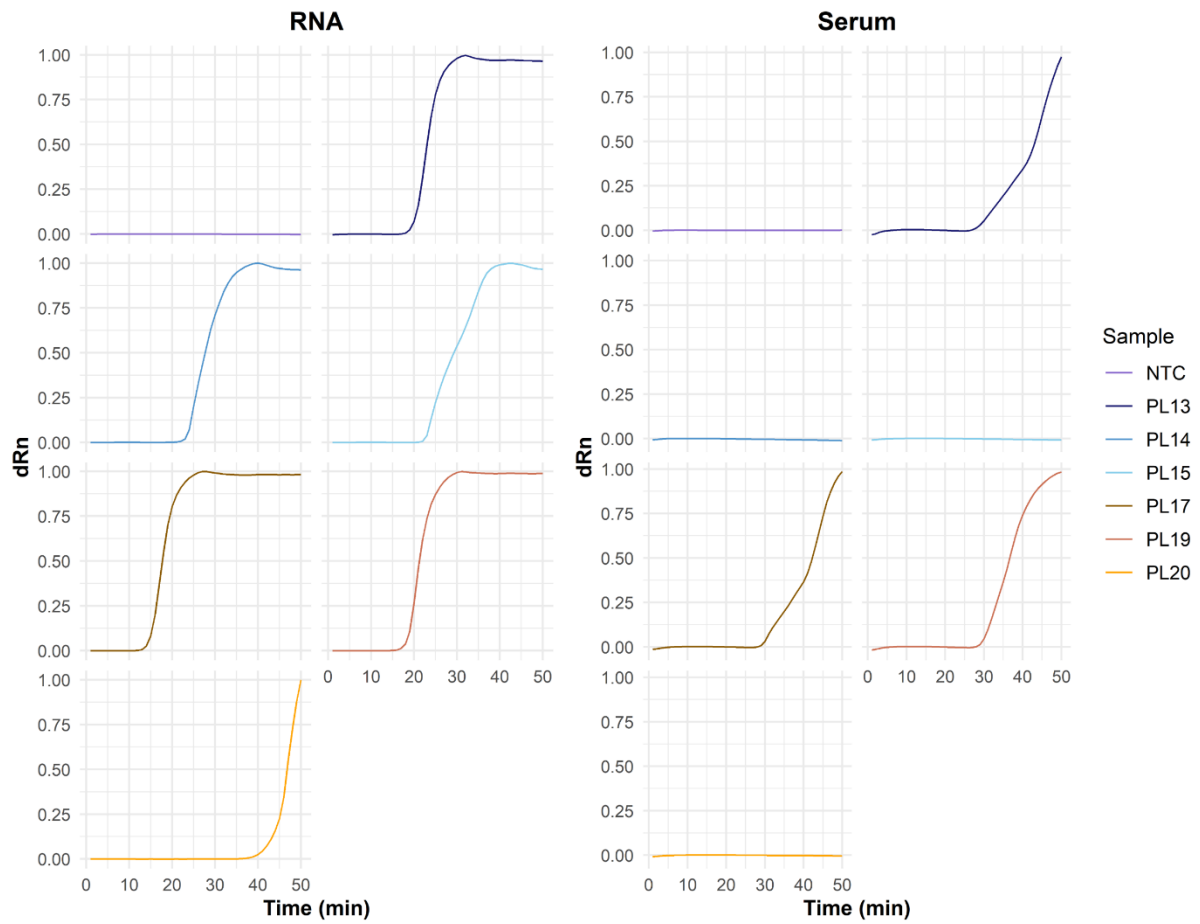
Supplementary Table S4. Combination of LAMP-primers tested for detection of GT1, GT2 and GT4.

Mix A	Mix B	Mix C	Mix D	Mix E	Mix F	Mix G
DN2_F3	DN2_F3	DN2_F3	DN2_F3	DN2_F3	DN2_F3	DN2_F3
DN2_FIP- InoG2	DN2_FIP	DN2_FIP	DN2_FIP- InoG2	DN2_FIP	DN2_FIP- InoG2	DN2_FIP- InoG2T
DN2_LF-1	DN2_LF-1	DN2_LF-Ino2	DN2_LF-Ino2	DN2_LF-Ino3	DN2_LF-Ino3	DN2_LF-Ino2
DN2_LB	DN2_LB	DN2_LB	DN2_LB	DN2_LB	DN2_LB	DN2_LB
DN2_BIP	DN2_BIP	DN2_BIP	DN2_BIP	DN2_BIP	DN2_BIP	DN2_BIP
DN2_B3	DN2_B3	DN2_B3	DN2_B3	DN2_B3	DN2_B3	DN2_B3

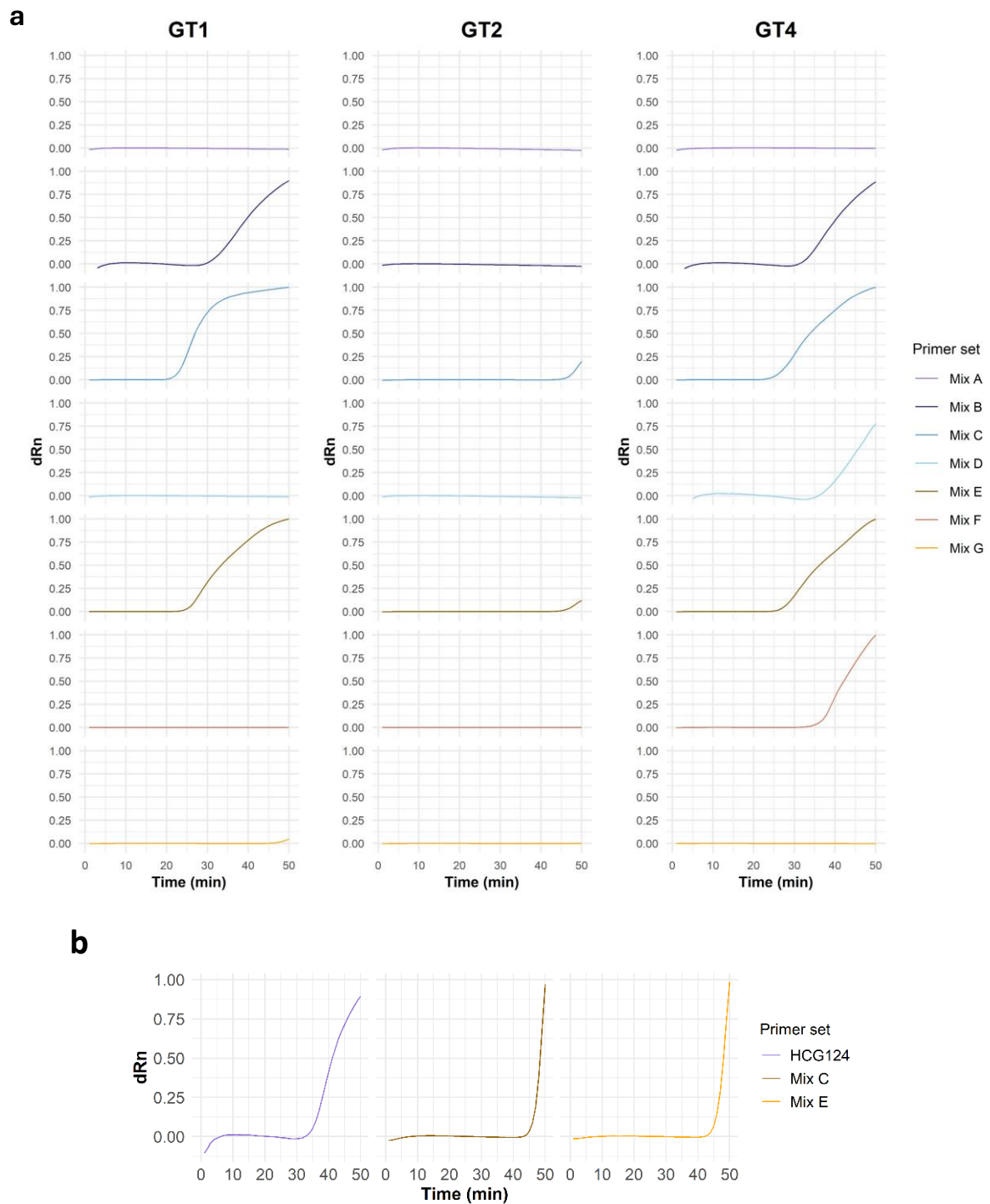
Supplementary Table S5. LAMP-oligonucleotides encompassing HCG124 primer set.

Mix HCG124
DN2_F3
DN2_F3-G2
DN2_FIP
DN2_FIP-G2
DN2_LF-1
DN2_LF-G2
DN2_LB
DN2_BIP
DN2_B3

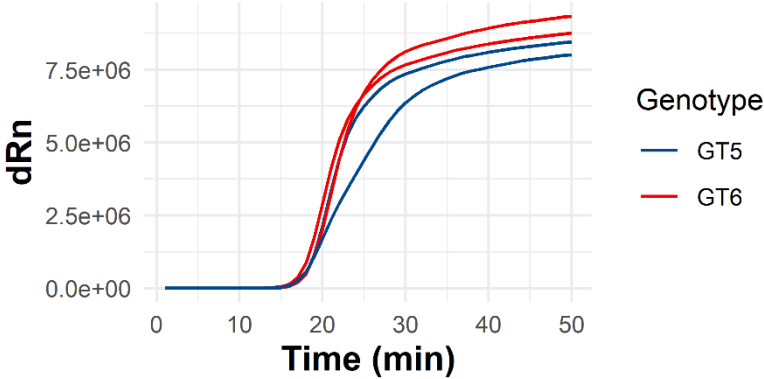
Supplementary Fig. S1. Individual representation of real time fluorescence RT-LAMP amplification plots displayed in Fig. 2, using HCDN primer with extracted RNA samples (left panels) or lysed serum samples (right panels). NTC: non-template control; samples PL13, PL14, PL15, PL17, PL19 (GT4) and PL20 (GT2).



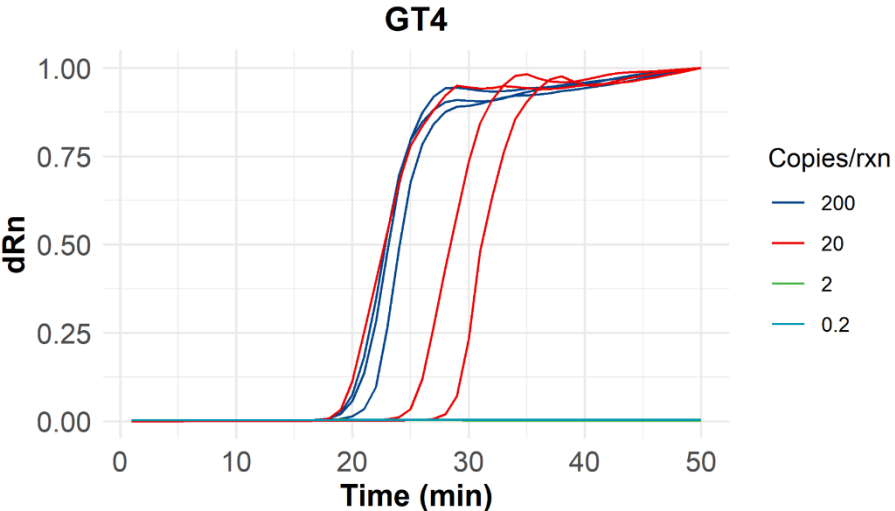
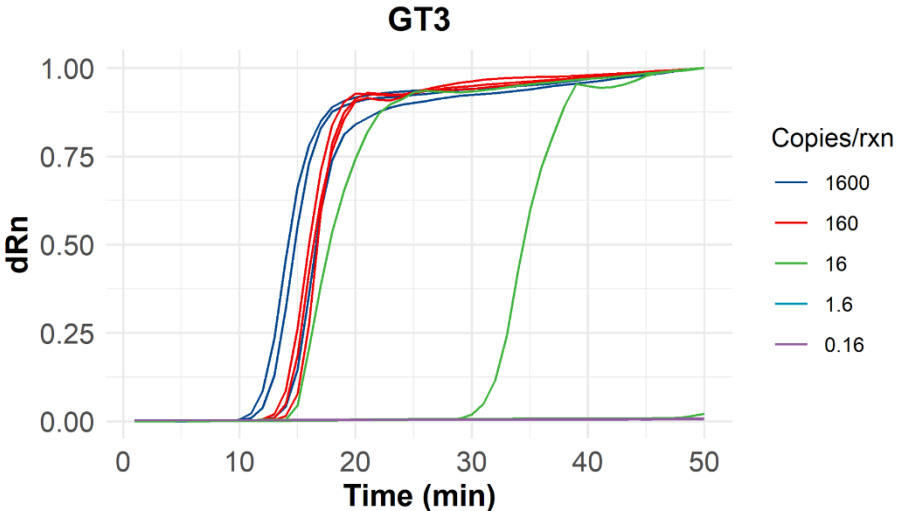
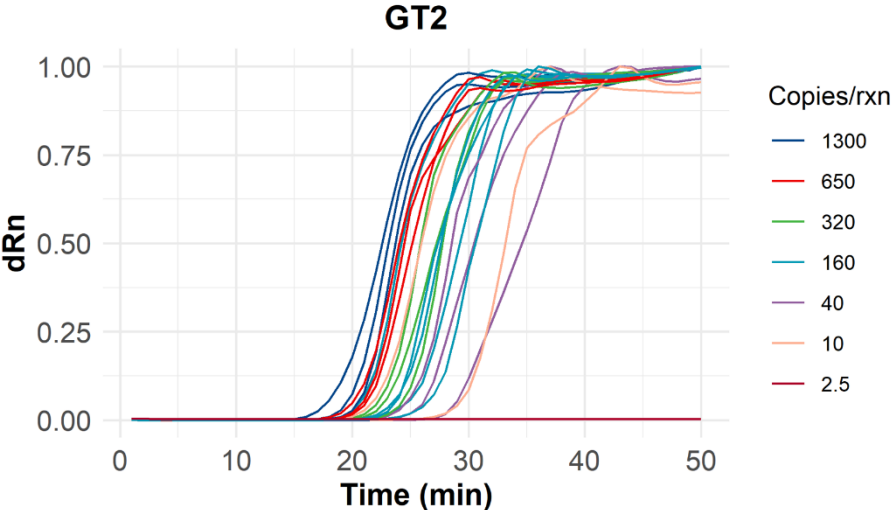
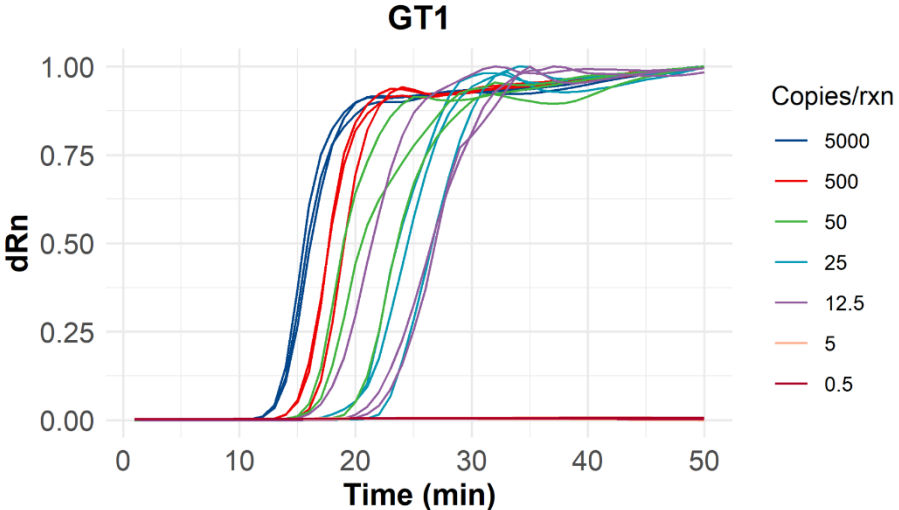
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