

Supporting Information

Lyophilised colourimetric LAMP for visual readout with dual colour indicators

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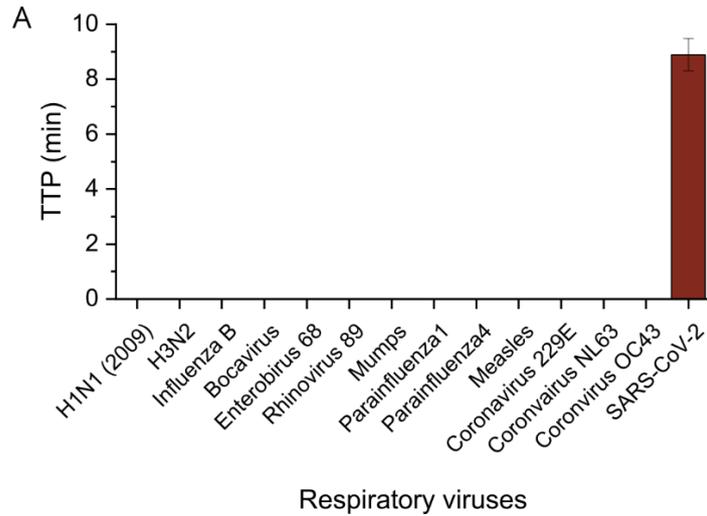


Figure S1. Specificity of the LAMP assay used in this study for the specific detection of SARS-CoV-2 using the Twist Respiratory Virus Research Panel (Cat no. 103730, Twist Bioscience).

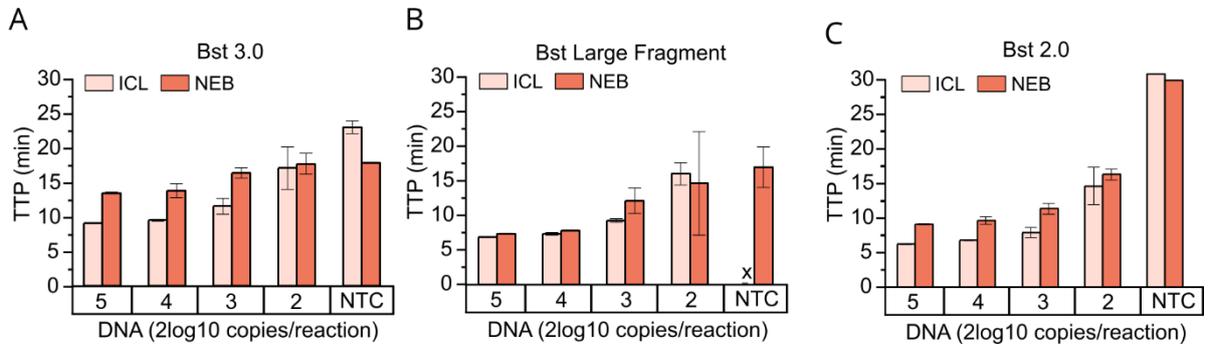


Figure S2. Optimisation of LAMP assessing the performance of Bst DNA polymerases. (A) Comparison between the NEB protocol and the proposed glycerol-free protocol using Bst 3.0 DNA polymerase. (B) Comparison between the NEB protocol and the proposed glycerol-free protocol using Bst Large Fragment. (C) Comparison between the NEB protocol and the proposed glycerol-free protocol using Bst 2.0 DNA polymerase

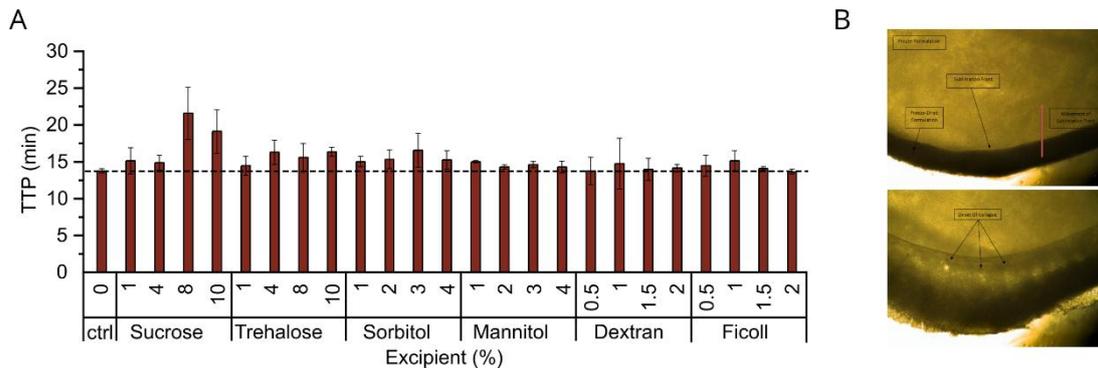


Figure S3. Evaluation of excipients for the development of lyophilised LAMP. (A) TTP values obtained after the incorporation of several excipients in the optimised LAMP protocol using SARS-CoV-2 RNA at 2×10^3 copies per reaction. (B) Light transmission freeze-drying microscopy of an RT-LAMP reaction containing 4% dextran and 2% ficoll depicting pockets of collapse within the sublimation frontier when the drying temperature was raised to -38.5 °C under 5.4 Pa.

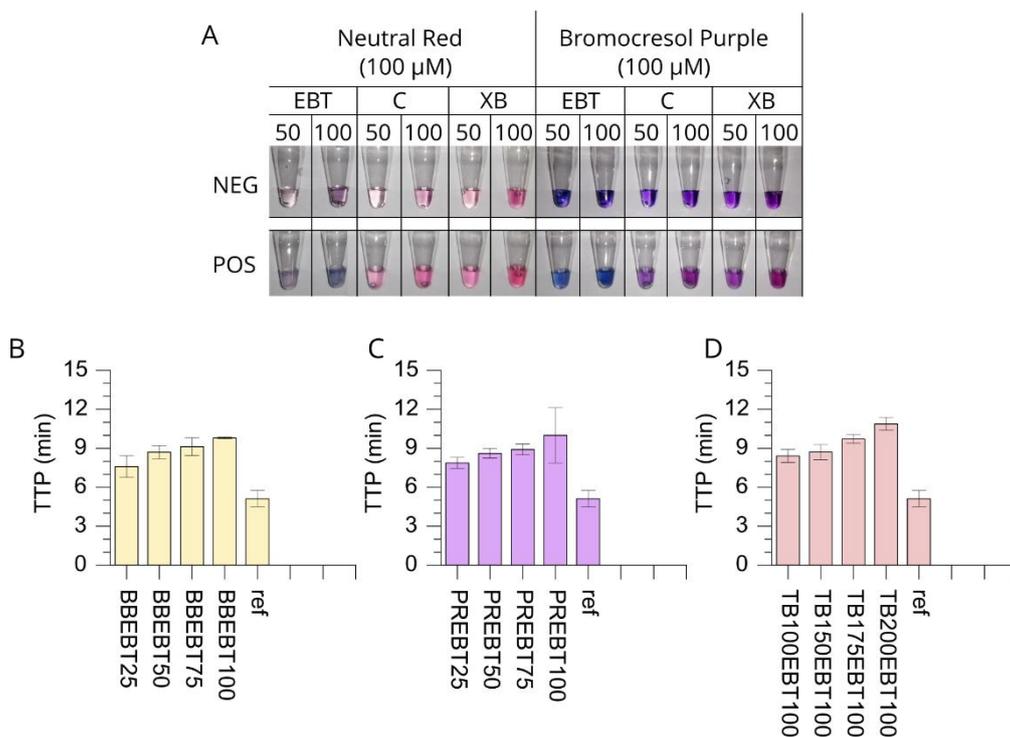


Figure S4. Evaluation of colorimetric dyes combining pH and metal indicators. (A) Cross-titration of neutral red and bromocresol purple with metal indicators eriochrome black T (EBT), calmagite (C), and xylydyl blue (XB), respectively. (B) Real-time data showing TTP values against indicator concentration, including as reference (ref) a reaction without dye. Bromothymol blue (BB) at 100 μ M combined with eriochrome black T (EBT) at 25, 50, 75, and 100 μ M. (C) Real-time data showing TTP values against indicator concentration, including as reference (ref) a reaction without dye. Phenol red (BB) at 100 μ M combined with eriochrome black T (EBT) at 25, 50, 75, and 100 μ M. (D) Real-time data showing TTP values against indicator concentration, including as reference (ref) a reaction without dye. Eriochrome black T (EBT) at 100 μ M combined with thymol Blue (TB) at 100, 150, 175, and 200 μ M.

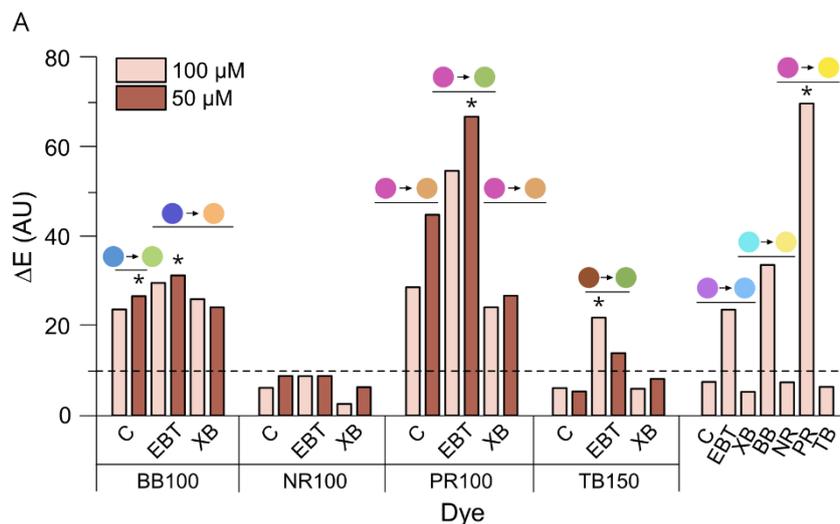


Figure S5. Adjusted ΔE values showing the perceived difference between positive and negative colorimetric reactions. Selected dye combinations are marked with asterisks (*). The top combinations were: PR100, PR100/EBT50, BB100/EBT50, BB100/C50, and TB150/EBT100 for each pH dye. No combination was selected for the neutral red (NR100) group. Two combinations were selected for the bromothymol blue (BB) group given the different color hues of such combinations. Selected combinations were lyophilised.

Reaction	pH indicator (BB100)	Metal indicator (EBT50)	Combination (BB100EBT50)	If only pH works (initial pH right) (initial metal low)	If only metal works (initial pH right) (initial metal right)	If only pH works (initial pH right) (initial metal right)	If only metal works (initial pH low) (initial metal right)
MM4 (BB100 EBT50)	- + 	- + 	- + 	- + 	- + 	- + 	- + 

Figure S6. Dual colour indicators robustness in extreme cases. As example, various starting pH and metal conditions were used to illustrate the reaction colour palette obtained with MM4 (BB100EBT50) which includes a pH and a metal indicator.

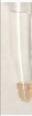
		Lyo-cLAMP									
		MM5	MM5-A	MM4	MM4-A	MM1	MM1-A	MM1-A2	MM5-A2	MM5-A3	MM4-A2
63 °C	t = 0 min										
63 °C	t = 35 min										
TTP (min)		N/A	9.62	N/A	9.71	N/A	8.65	8.32	9.16	9.33	9.40

Figure S7. Evaluation of 12-month-old lyo-cLAMP strips. The colour after rehydration was out of range (MM5, MM4 and MM1) but could be restored by raising the pH with NaOH as shown in the adjusted tubes (MM1-A, MM4-A, and MM5-A). Additionally, only the adjusted ones amplified as shown in TTP section, assessed in a real-time thermocycler. MM5-A, MM4-A and MM1-A included 0.25 mM NaOH; MM1-A2, MM5-A2 and MM4-A2 included 0.18 mM and MM5-A3 included 0.20 mM.

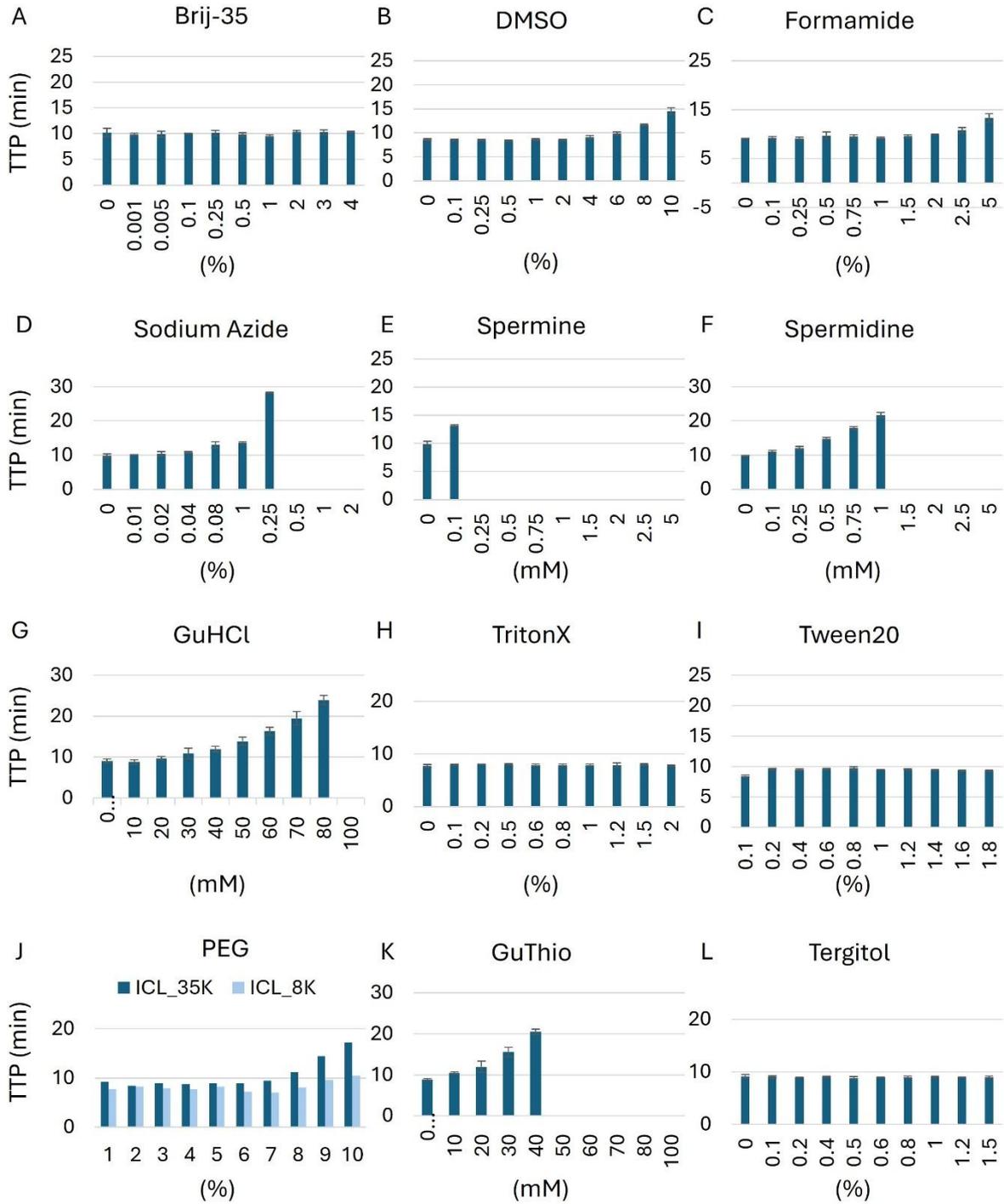


Figure S8. Effect of additives in LAMP. (A) Addition of Brij-35. (B) Addition of DMSO. (C) Addition of formamide. (D) Addition of Sodium Azide. (E) Addition of Spermine. (F) Addition of Spermidine. (G) Addition of guanidine hydrochloride (GuHCl). (H) Addition of Triton X-100. (I) Addition of Tween20. (J) Addition of polyethylene glycol (PEG) with molecular weight of 35KDa and 8KDa. (K) Addition of guanidine thiocyanate (GuThio). (L) Addition of Tergitol.

Table S1. Primer sequences used in this study.

Name	Sequence (5' to 3')	Reference
LAMPcov_F3	ACC AAT AGC AGT CCA GAT GA	Rodriguez-Manzano <i>et al.</i> , <i>ACS Cent Sci</i> 2021
LAMPcov_B3	CAC GAT TGC AGC ATT GTT AGC	
LAMPcov_LF	GGA CTG AGA TCT TTC ATT TTA CCG T	
LAMPcov_LB	ACT GAG GGA GCC TTG AAT ACA	
LAMPcov_FIP	TCTGGCCCCAGTTCCTAGGTAGTCCAGACAAATTCGTGGTGG	
LAMPcov_BIP	GGACTTCCCTATGGTGCTAACAAACGGGTGCCAATGTGATCT	
nCoV_N1-F	5'-GAC CCC AAA ATC AGC GAA AT-3'	CDC COVID-19 assay, Integrated DNA Technologies (IDT)
nCoV_N1-R	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	
nCoV_N1-P	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	
nCoV_N2-F	5'-TTA CAA ACA TTG GCC GCA AA-3'	
nCoV_N2-R	5'-GCG CGA CAT TCC GAA GAA-3'	
nCoV_N2-P	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	
RNaseP-F	AGA TTT GGA CCT GCG AGC G	
RNaseP-R	GAG CGG CTG TCT CCA CAA GT	
RNaseP-P	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1	

Table S2. Accelerated aging and shelf-life. Accelerated and real-time ageing was performed across two temperatures at 250 copies per reaction of SARS-CoV-2 extracted RNA to determine the long-term stability of LAMP.

Temperature 20°C		Temperature 35°C	
Day	Response	Day	Response
0	3/3	-	-
60	3/3	17	3/3
90	3/3	26	3/3
120	0/3	34	0/3
150	0/3	42	0/3
180	0/3	52	0/3

Table S3. C_t values obtained by qPCR from extracted nucleic from nose and throat swabs collected at North West London Pathology laboratory. CDC COVID-19 assay (N1 and N2) and RNaseP (R) assay from IDT were used.

ID	N1	N1	N1 mean	N1 std	N2	N2	N2 mean	N2 std	R	R	R mean	R std
1	16.43	16.28	16.36	0.10	15.53	15.68	15.61	0.11	29.01	29.81	29.41	0.57
2	17.95	18.05	18.00	0.07	16.46	16.39	16.43	0.05	29.88	29.70	29.79	0.13
3	18.31	18.23	18.27	0.06	17.10	17.08	17.09	0.01	26.14	24.59	25.37	1.10
4	19.81	19.71	19.76	0.07	17.82	17.83	17.83	0.01	29.57	29.75	29.66	0.13
5	20.02	19.96	19.99	0.05	18.92	18.78	18.85	0.10	25.91	24.73	25.32	0.83
6	20.53	20.48	20.51	0.04	21.57	21.40	21.49	0.12	29.00	27.25	28.13	1.24
7	20.61	20.54	20.58	0.05	19.80	19.85	19.83	0.04	33.06	33.11	33.09	0.04

8	21.76	21.69	21.72	0.05	21.35	21.20	21.28	0.11	35.10	32.62	33.86	1.75
9	21.83	21.75	21.79	0.05	21.06	21.09	21.08	0.02	29.21	26.81	28.01	1.70
10	21.88	21.83	21.85	0.04	15.67	15.50	15.59	0.12	29.91	28.61	29.26	0.92
11	21.89	21.94	21.91	0.04	25.68	26.48	26.08	0.57	29.77	29.79	29.78	0.01
12	23.01	22.97	22.99	0.03	22.13	22.66	22.40	0.37	27.78	27.67	27.73	0.08
13	23.14	23.07	23.10	0.05	22.87	22.87	22.87	0.00	28.29	28.29	28.29	0.00
14	23.28	23.32	23.30	0.03	22.34	22.31	22.33	0.02	30.44	30.51	30.48	0.05
15	26.93	26.95	26.94	0.01	26.73	27.05	26.89	0.23	31.32	30.89	31.11	0.30