Supplementary Information

Development of a Novel Loop-Mediated Isothermal Amplification Method for the Rapid Detection of Monkeypox Virus Infections

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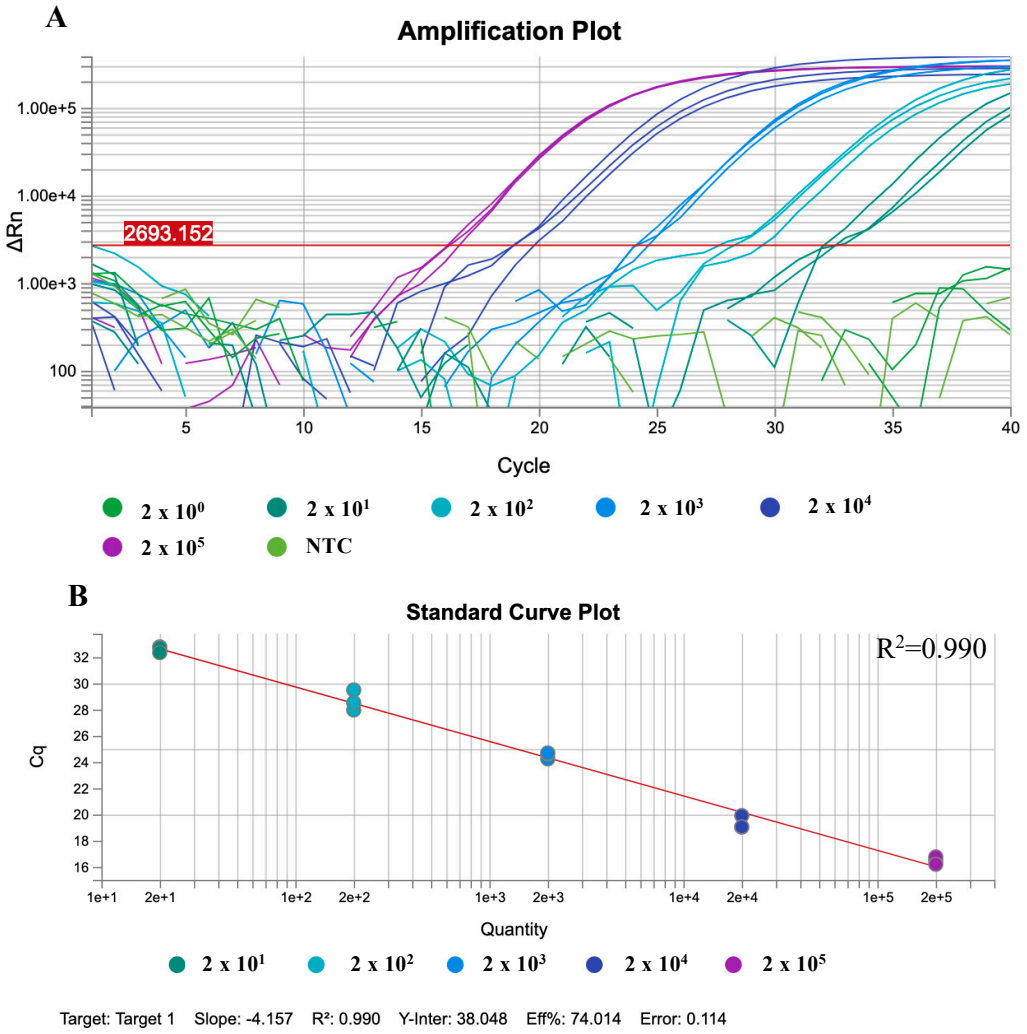

Figure S1. Sensitivity of the qPCR assay for mpox detection using DNA standard plasmid. The plasmid concentrations ranged from 2×10^5 to 2×10^0 copies; non-template control (NTC). (A) amplification curve of the qPCR assay. (B) Standard curve graph generated from serially diluted copies of DNA standard plasmid templates with correlation coefficient value ($R^2 = 0.99$).

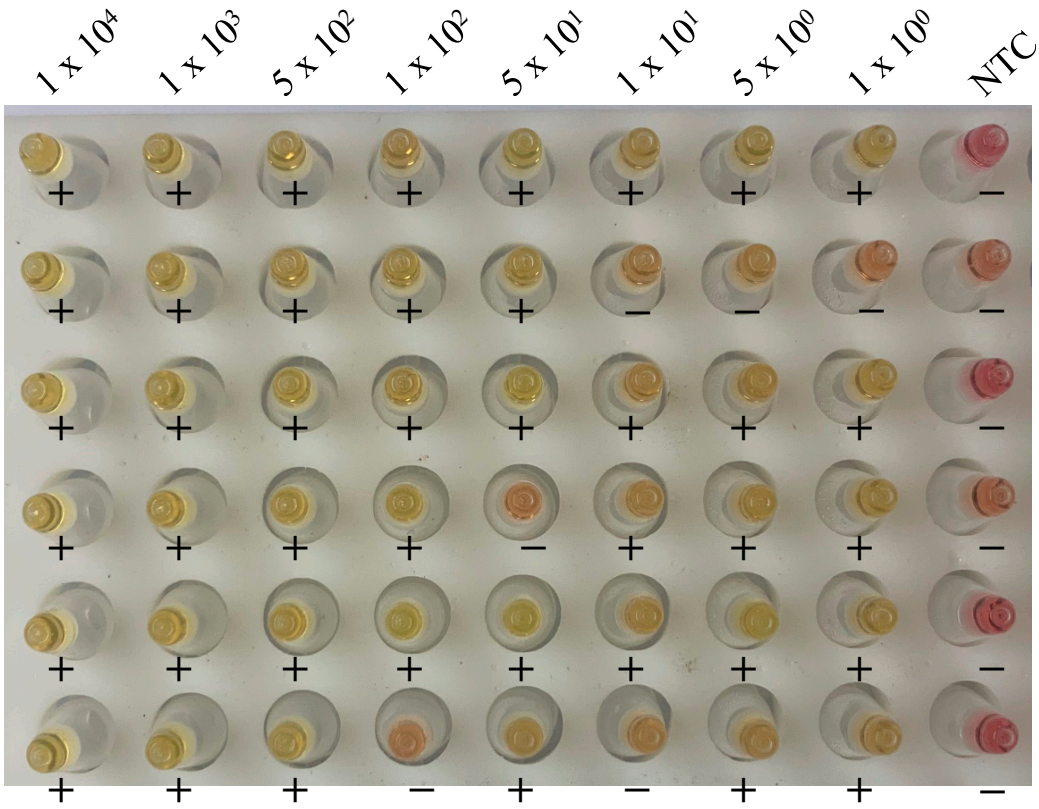

Figure S2. Limit of detection of the mpox visual LAMP assay. The limit of detection was assessed using diluted DNA standard plasmid from 1×10^4 to 1×10^0 copies in 8 replicates. NTC: non-template control.

Table S1. Primer sequences used for the detection of mpox via LAMP assay and qPCR.

Primers	Sequences (5'-3')	Length (nt)
F3	GCGAATAAGACAGTGCGATT	20
B3	TCATACAGAACATCTACAGGAT	22
FIP	GACCAAAGATCGAGGTCGTCGATGGAGTCGGTAGATTTCATG	42
BIP	TGGATTAGGTGTTGACTGTTATGTTCACAAATTGGTTCAAGGAGAA	46
LF	GAAACTGCTCATCGACAGC	19
LB	CTAGAACCAGTTGTTGACAGGA	22
Forward	GGAAAATGTAAAGACAACGAATACAG	27
Reverse	GCTATCACATAATCTGGAAGCGTA	24
Probe	FAM-AAGCCGTAATCTA<BHQ-1dT>GTTGTCTATCGTGTCC-Spacer C6	30

Table S2. Detailed information of mpox biological samples used in this study.

Sample ID	Sample Type	Real-time qPCR (Ct)		LAMP (Tp)
1	Crusts	20.69	+	6.84
2	Pus	33.42	+	10.00
3	Crusts	26.33	+	9.93
4	Crusts	25.59	+	9.17
5	Crusts	31.04	+	8.51
6	Crusts	23.25	+	7.41
7	Pus	23.63	+	7.00
8	Pus	25.67	+	9.23
9	Pus	30.93	+	9.11
10	Pus	22.09	+	6.88
11	Pus	24.66	+	8.12
12	Pus	19.50	+	6.27
13	Pus	23.24	+	6.97
14	Pus	23.98	+	7.57
15	Serum	31.42	+	9.56