

SUPPORTING INFORMATION

Development of a rapid and sensitive single-tube RAA-CRISPR/Cas12a assay for monkeypox virus detection

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Content:

Fig. S1 Screening of RAA reaction primers and conditions.

Fig. S2 Detection of simulated MPXV clinical samples by RAA-CRISPR/Cas12a fluorescence method.

Fig. S3 LFA detection of simulated MPXV clinical samples.

Fig. S4 PCR detection of simulated MPXV clinical samples.

Fig. S5 Sensitivity of PCR detection.

Table S1 Primers and fluorescent probes were used to report the molecular sequences.

Table S2 Comparison of the proposed MPXV single-tube RAA-CRISPR/Cas12a assay with representative CRISPR-based nucleic acid detection methods.

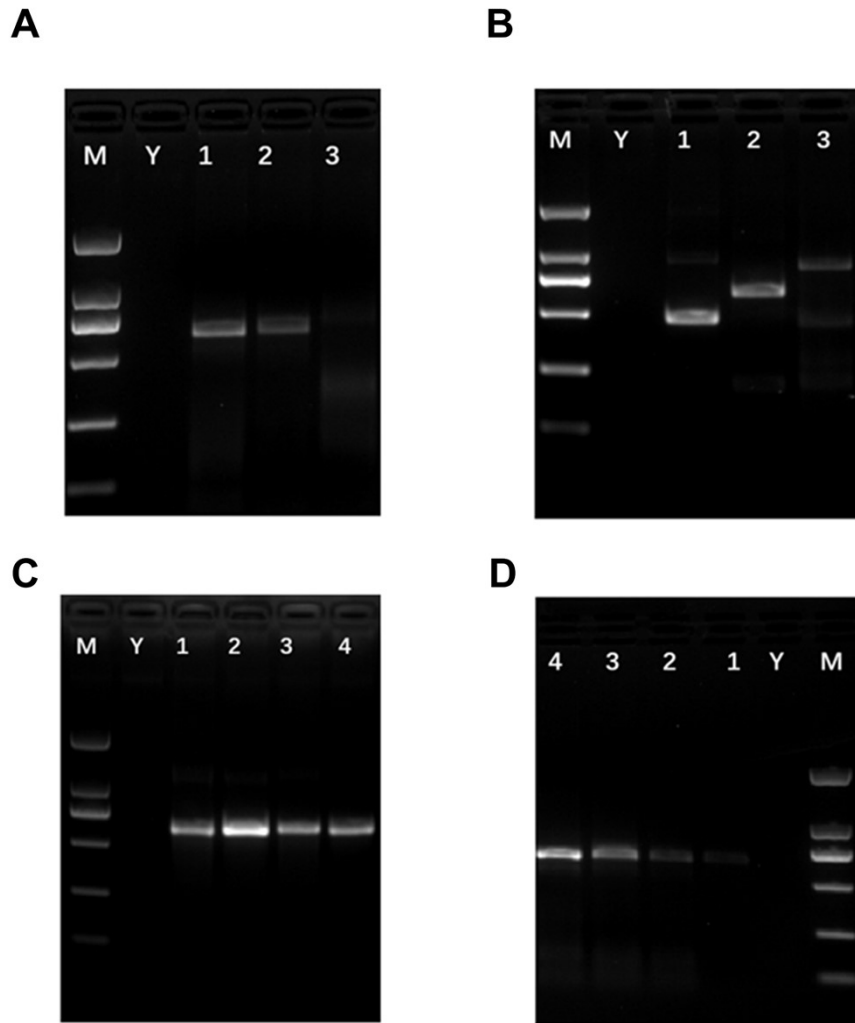
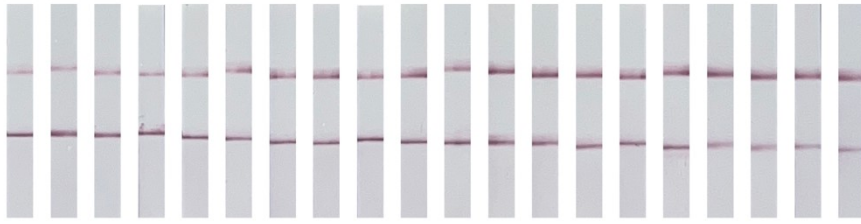


Fig. S1 Screening of RAA reaction primers and conditions. (A) Screening of RAA primers for the N4R gene. M: 2K Marker; Y: no template control; 1: F1+R1; 2: F2+R2; 3: F3+R3. (B) Screening of RAA primers for the L6R gene. M: 2K Marker; Y: no template control; 1: F1+R1; 2: F2+R2; 3: F3+R3. (C) Screening of the optimal primer reaction temperature for RAA of the N4R gene. M: 2K Marker; Y: no template control; 1: 34°C; 2: 37°C; 3: 40°C; 4: 43°C. (D) Screening of the optimal RAA primer reaction time for N4R gene. 4: 25min; 3: 20min; 2: 15min; 1: 10min. Y: no template control; M: 2K Marker.

A



B

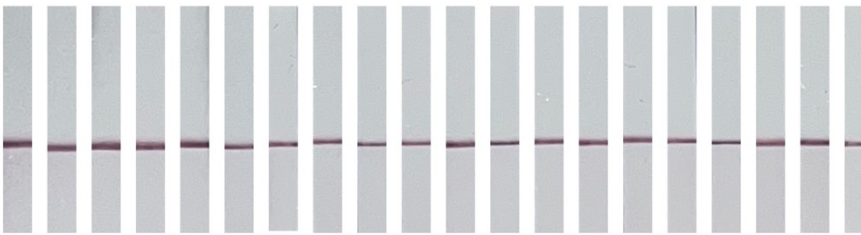
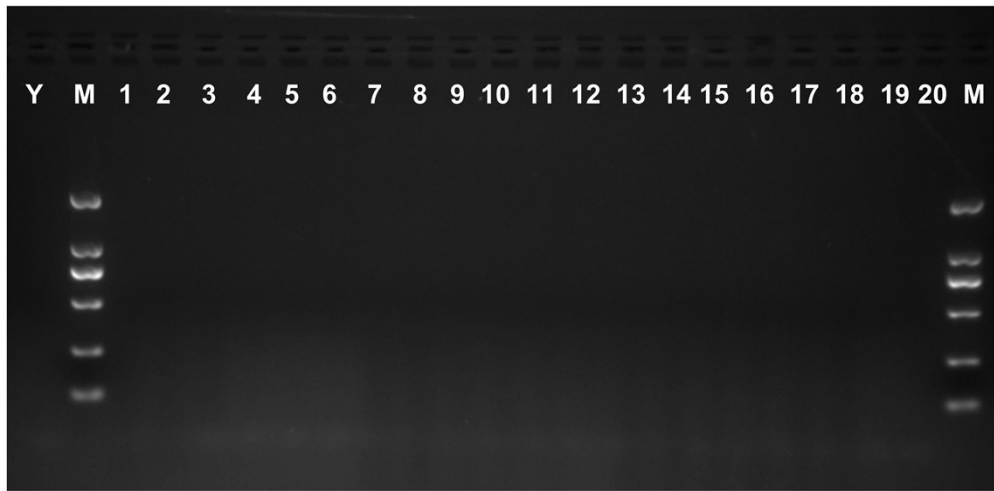


Fig. S3 LFA detection of simulated MPXV clinical samples. (A) Positive samples. (B) Negative samples.

A



B

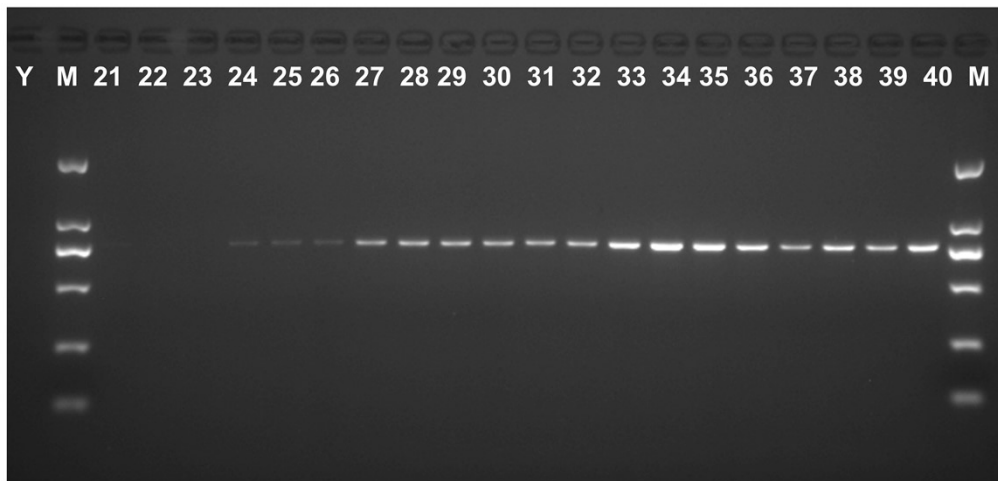


Fig. S4 PCR detection of simulated MPXV clinical samples. (A) Negative samples. (B) Positive samples. M: 2K marker; Y: no template control.; 1~20: negatives; 21~40: positives.

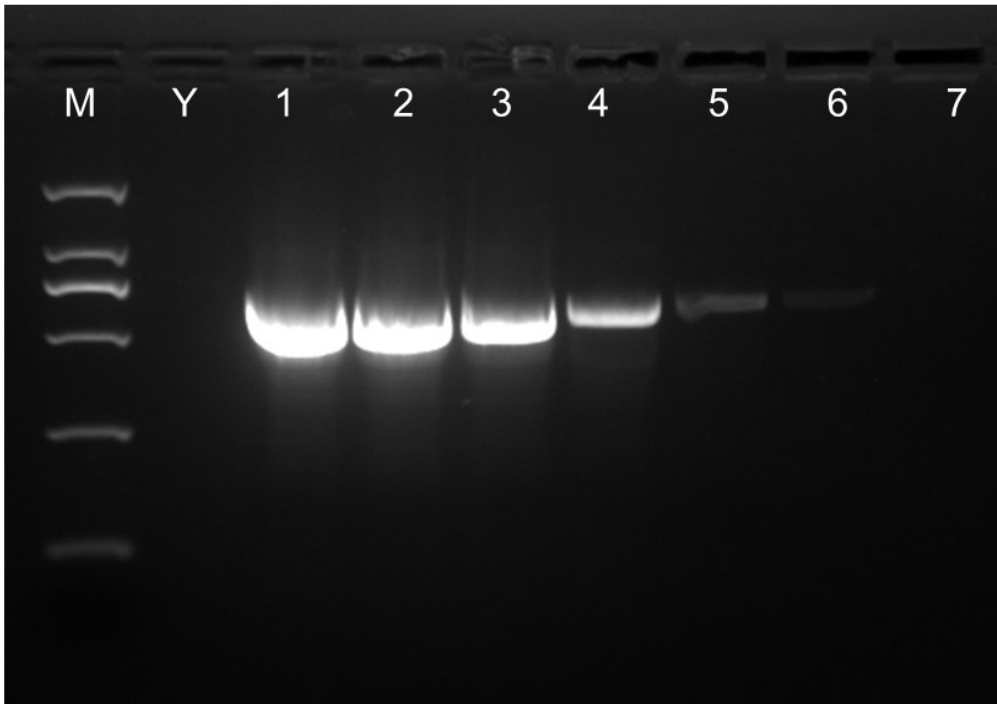


Fig. S5 Sensitivity of PCR detection. M: 2K Marker; Y: no template control; 1-7: 10^8 to 10^2 copies/ μL .

Table S1 Primers and fluorescent probes were used to report the molecular sequences.

Primer Name	Sequences (5'–3')
N4R-PCR-F	CGATGAGCAGTTTCACGACG
N4R-PCR-R	CCTACGGGCGATGACAGAT
L6R-PCR-F	AAAGAGTTATCGGGACACGC
L6R-PCR-R	GATTCAATTCCTCCGGTTCA
N4R-RAA-F1	TTGATAGATTTCTCCTTGAACCAATTTGTGAT
N4R-RAA-R1	GTATATCCATTACTCATTACTGCGTTGACATT
N4R-RAA-F2	TATTTGATAGATTTCTCCTTGAACCAATTTGTGAT
N4R-RAA-R2	GTATATCCATTACTCATTACTGCGTTGACATT
N4R-RAA-F3	ATGGATTAGGTGTTGACTGTTATGTTCTAG
N4R-RAA-R3	TATATCCATTACTCATTACTGCGTTGACATT
L6R-RAA-F1	TTACTAAGATTCAATTCCTCCGGTTCAACG
L6R-RAA-R1	CCTATTGGAATTATTTCTGCACAGGTATTG
L6R-RAA-F2	CTTACTAAGATTCAATTCCTCCGGTTCAAC
L6R-RAA-R2	TGATGTGAGAGAGAAATACTTCTTTACGGT
L6R-RAA-F3	CTCCGTTGATTATTAGACCGGCTATTAGTT
L6R-RAA-R3	GAACTCTCTCCATATTTCCCAAGATGTT
N4R-crRNA1	UAAUUUCUACUCUUGUAGAUCAUAUCCCCUAAAAUAGGCAUU
N4R-crRNA2	UAAUUUCUACUCUUGUAGAUAUCGCGCUAUCUAUGCAGGUUAUA
N4R-crRNA3	UAAUUUCUACUCUUGUAGAUGGAUUCAUAGAGAUAAUAUUGAU
N4R-crRNA4	UAAUUUCUACUCUUGUAGAUAUCAUAUAUUAAGAAUUGUAA

N4R-crRNA5	UAAUUUCUACUCUUGUAGAUUGGUAGAUCAUGGGGCUGUAAUA
L6R-crRNA1	UAAUUUCUACUCUUGUAGAUGAAGAGUGAUGUCUGGAUU
L6R-crRNA2	UAAUUUCUACUCUUGUAGAUGAGAUAUCAUCGGAUACCAGA
L6R-crRNA3	UAAUUUCUACUCUUGUAGAUUGGAGUAUAUAUUCUUGACGCAU
L6R-crRNA4	UAAUUUCUACUCUUGUAGAUUAAUUCUAAUUCUAGAAGGAUUA
L6R-crRNA5	UAAUUUCUACUCUUGUAGAUGGUAGACAGUAAAUCUAAUUAU
FQ-reporter	FAM-TTATT-BHQ1
FB-reporter	FAM-TTATT-Biotin

Table S2 Comparison of the proposed MPXV single-tube RAA-CRISPR/Cas12a assay with representative CRISPR-based nucleic acid detection methods.

Study	Pathogen	Sensitivity/Time	Chemistry/format	Key advantage
This study	MPXV	0.5 copies/ μ L, 35 min (Fluorescence); 5 copies/ μ L, 40 min (LFA)	single-tube RAA-Cas12a	High sensitivity; reduced reagent cost; closed-tube format with lower contamination risk; reliable performance in complex matrices
1	MPXV	6.5 copies/ μ L, 40 min (Fluorescence)	one-step LAMP-Cas12b	Clinical validation
2	MPXV	1 copies/ μ L, 30 min (Fluorescence)	single-tube RPA-Cas12a	Rapid detection
3	MPXV	10 copies/ μ L, 40 min (Fluorescence); 10^4 copies/ μ L, 45 min (LFA)	Two-step RAA+CRISPR	No thermal cycling required
4	Feline Parvovirus	4.277 copies/ μ L, 35 min (Fluorescence); 42.77 copies/ μ L, 55 min (LFS)	Two-tube, One-tube, and One-tube-LFS RAA-Cas12a	Clinical validation
5	Pigeon Circovirus	6.08 copies/ μ L, 30 min (Fluorescence)	One-Step	Clinical validation

References

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