

Effect of long-term storage on the morphology and infectivity of purified Chikungunya virus

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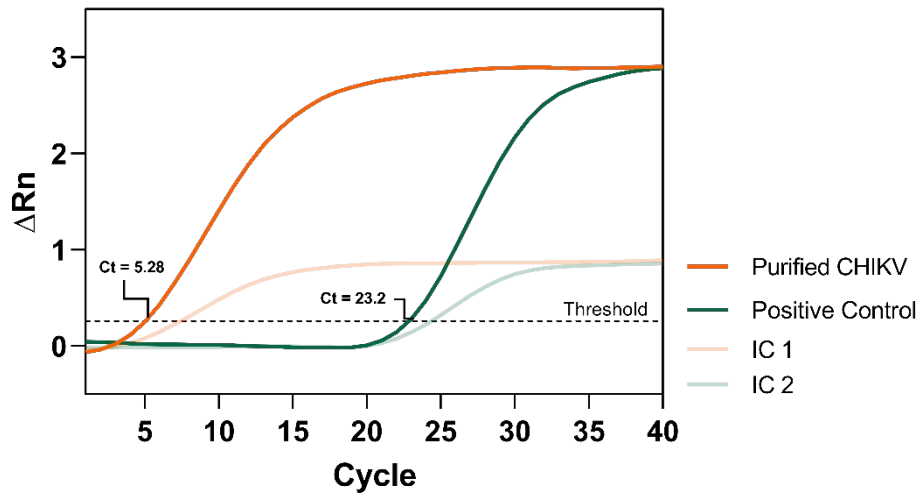


Fig. S1 Amplification curve of purified CHIKV. RT-qPCR was conducted using the Biomol ZDC (IBMP®) kit. The opaque lines represent the amplification of the purified CHIKV (orange) and the positive control (green). The translucent lines represent the internal controls (IC) of the reaction for the purified CHIKV (translucent orange) and the positive control (translucent green). Ct: Cycle threshold.

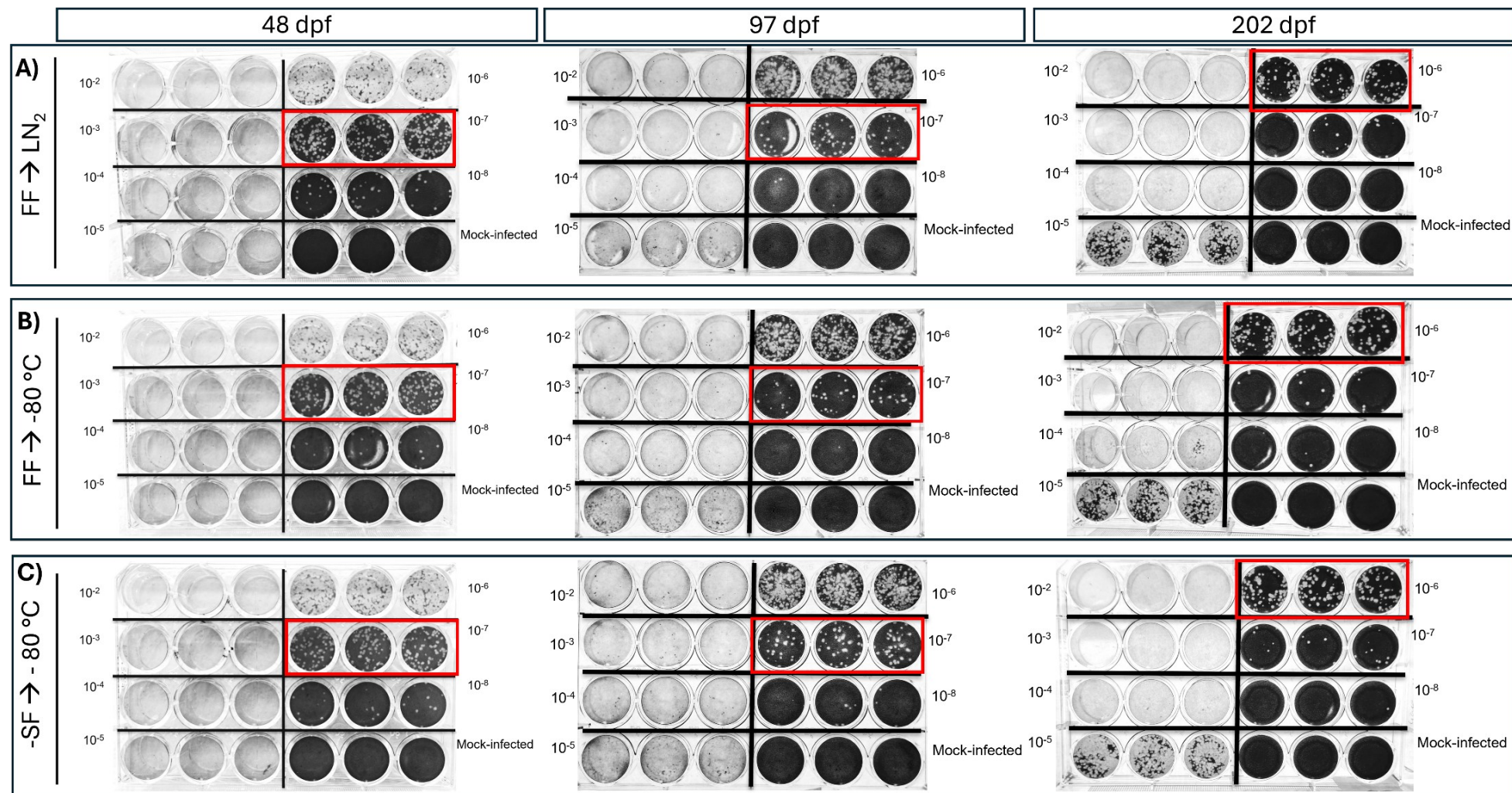


Fig. S2 Plaque assay performed to access the infectivity of the purified CHIKV under different freezing/storage conditions. A) Fast freezing and storage at LN_2 ; B) Fast freezing and storage at $-80^\circ C$; C) Slow freezing at Mr. Frosty® Cryo $1^\circ C$ freezing container followed by storage at $-80^\circ C$. The red squares indicate the dilutions used to calculate the CHIKV titer (PFU/mL). It is possible to observe a decrease in viral titers along the time, being more pronounced at 202 days post-freezing (dpf). Mock-infected wells were performed by adding culture media only.

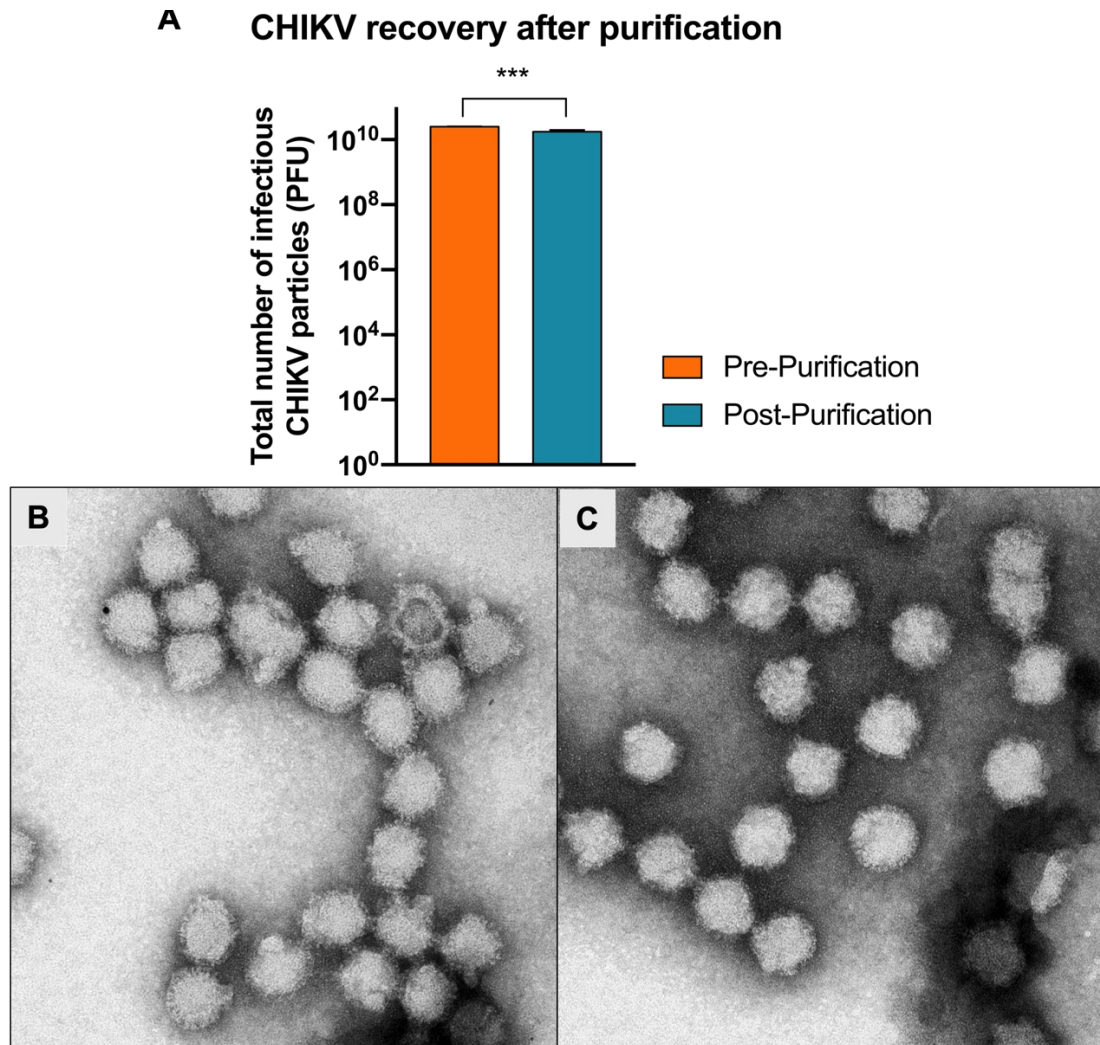


Fig. S3 CHIKV purification efficiency and morphological evaluation of purified particles. A) Total viral yield quantification before and after purification, expressed in plaque-forming units (PFU). The pre-purification baseline (starting volume of 800 mL) yielded 2.5×10^{10} PFU, whereas the post-purification sample yielded 1.76×10^{10} PFU (in a final volume of 3.2 mL), demonstrating a 70.4% recovery rate and a 250-fold concentration factor. The result of an unpaired T-test is shown (p-value = 0.0006). B-C) Morphological analysis of freshly purified CHIKV virions visualized via negative-staining transmission electron microscopy (TEM). Scale bars represent 100 nm.

Freezing/ Storage Method	48 days post-freezing					
	Rep1	Rep2	Rep3	AVG	SD	SEM
FF/LN2	4.4E+ 09	3.95E +09	4.95E +09	4.43E +09	5.01E +08	2.89E +08
FF/-80 °C	3.5E+ 09	4.45E +09	2.95E +09	3.63E +09	7.59E +08	4.38E +08
SF/-80 °C	4.25E +09	3.55E +09	3.8E+ 09	3.87E +09	3.55E +08	2.05E +08

Table S1 Infectivity of purified CHIKV under different freezing and storage conditions after 48 days. Viral titers were obtained from three replicates (Rep1, Rep2, and Rep3) by plaque assay. Data are expressed as PFU/mL, accompanied by the mean, standard deviation, and standard error of the mean. Abbreviations: AVG, average; SD, standard deviation; SEM, standard error of the mean; FF/LN2, fast freezing and storage in liquid nitrogen; FF/-80 °C, fast freezing and storage at -80 °C; SF/-80 °C, slow freezing and storage at -80 °C.

Freezing/ Storage Method	97 days post-freezing					
	Rep1	Rep2	Rep3	AVG	SD	SEM
FF/LN2	1.2E+ 09	2.05E +09	X	1.63E +09	6.01E +08	4.25E +08
FF/-80 °C	1.3E+ 09	1.15E +09	7E+08	1.05E +09	3.12E +08	1.80E +08
SF/-80 °C	1.55E +09	1.55E +09	1.45E +09	1.52E +09	5.77E +07	3.33E +07

Table S2 Infectivity of purified CHIKV under different freezing and storage conditions after 97 days. Viral titers were obtained from three replicates (Rep1, Rep2, and Rep3) by plaque assay. Data are expressed as PFU/mL, accompanied by the mean, standard deviation, and standard error of the mean. Abbreviations: AVG, average; SD, standard deviation; SEM, standard error of the mean; FF/LN2, fast freezing and storage in liquid nitrogen; FF/-80 °C, fast freezing and storage at -80 °C; SF/-80 °C, slow freezing and storage at -80 °C.

Freezing/ Storage Method	202 days post-freezing					
	Rep1	Rep2	Rep3	AVG	SD	SEM
FF/LN2	4E+08	5E+08	2.5E+08	3.83E+08	1.26E+08	7.26E+07
FF/-80 °C	4E+08	3.5E+08	3.5E+08	3.67E+08	2.89E+07	1.67E+07
SF/-80 °C	5.5E+08	3E+08	4E+08	4.17E+08	1.26E+08	7.26E+07

Table S3 Infectivity of purified CHIKV under different freezing and storage conditions after 202 days. Viral titers were obtained from three replicates (Rep1, Rep2, and Rep3) by plaque assay. Data are expressed as PFU/mL, accompanied by the mean, standard deviation, and standard error of the mean. Abbreviations: AVG, average; SD, standard deviation; SEM, standard error of the mean; FF/LN2, fast freezing and storage in liquid nitrogen; FF/-80 °C, fast freezing and storage at -80 °C; SF/-80 °C, slow freezing and storage at -80 °C.