



Short Communication

Heat inactivation of monkeypox virus

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ABSTRACT

Different kinds of media spiked with monkeypox virus (MPXV) were subjected to heat inactivation at different temperatures for various periods of time. The results showed that MPXV was inactivated in less than 5 min at 70 °C and less than 15 min at 60 °C, with no difference between viruses from the West African and Central African clades. The present findings could help laboratory workers to manipulate MPXV in optimal biosafety conditions and improve their protocols.

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1. Introduction

Since April 2022, thousands of monkeypox cases have been reported in non-endemic countries. This unprecedented outbreak is caused by a monkeypox virus (MPXV) belonging to the *Poxviridae* family and *Orthopoxvirus* genus. The majority of confirmed cases ($n = 31,800$ on 09 August 2022) are in the WHO European Region.¹ Men who have sex with men and have reported recent sex with new and multiple partners are predominantly infected. Since the discovery of MPXV in 1958, growing numbers of monkeypox cases have been reported in 10 African countries and 4 countries outside of Africa over the past five decades. Thus, the observation of a huge number of cases outside of Africa is highly unusual and reinforces the necessity to better characterize the virus and its transmission and pathogenesis. Manipulation of clinical samples and research on the virus are necessary to help fight and stop the current outbreak. Viral inactivation procedures are therefore necessary to ensure safe experimental laboratory conditions. Heat treatment is a widely used inactivation method for viruses. Personal protective equipment, laboratory materials, hospital equipment, transportation media, and biological samples are often heated to inactivate viruses. Heat is thought to denature the secondary structures of various molecules that constitute viruses. Data on orthopoxviruses are available for vaccinia virus and variola virus, but not for MPXV. The necessary times to achieve a minimum $4\log_{10}$ reduction in citrate-phosphate buffer were 15 min at 60 °C and 90 min at 50 °C.² Due to their close structures, vaccinia virus and MPXV may have similar susceptibilities toward

thermal inactivation, as is the case for biocidal agents.³ To address this question, we submitted two different strains of MPXV to temperatures commonly used in the laboratory for viral inactivation for various periods of time and examined the residual infectivity by a plaque assay method.

2. Materials and methods

2.1. Cell lines and viruses

African green monkey cells (Vero E6) were grown in Dulbecco's modified Eagle's medium (DMEM 1X; Gibco) supplemented with 5% fetal calf serum (FCS) and antibiotics (0.1 U penicillin, 0.1 mg mL⁻¹ streptomycin; Gibco) at 37 °C in a humidified 5% CO₂ incubator.

A strain of MPXV isolated in the Congo Basin (named LK for Lokole region) was also grown on Vero E6 cells for three passages with a titer of 3.49×10^7 plaque-forming units (PFU) mL⁻¹. A human strain of MPXV isolated from a French patient in June 2022 (strain MPXV/2022/FR/CMIP; named CMIP2022) was grown on Vero E6 cells for two passages. The virus was titrated by a plaque assay method and the titer was 3.4×10^7 PFU mL⁻¹.

2.2. Heat inactivation

MPXV strains were diluted 1:2 in two different kinds of media: Viral Transport Media (VTM; 330C; Copan) and FCS (F2442; Sigma-Aldrich). A 200-μL aliquot of each sample was subjected in triplicate to various temperatures for different periods of time, ranging from 30 s to 90 min. The samples were inactivated in a calibrated

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and verified dry water bath, cooled on ice, and tested for infectivity using a plaque assay procedure adapted from Matrosovich et al.⁴. The temperatures tested were 56 °C, 60 °C, 70 °C, and 95 °C to mimic the commonly used temperatures for serosurveys, high-threat pathogen inactivation, MPXV inactivation prior to diagnosis, and rapid virus inactivation compatible with molecular biology techniques, respectively.

All experiments were conducted under strict BSL3 conditions.

2.3. Plaque assay

Vero E6 cells were grown in 6-well plates until they reached appropriate confluence. The cells were washed once with Dulbecco's phosphate-buffered saline and infected with a 10-fold dilution of the heat-inactivated samples. After 1.5 h of adsorption at 37 °C, 3 mL of DMEM containing 1.2% Avicel (RC581; FMC BioPolymers) was added to the cells. After 5 days of incubation at 37 °C, the formed plaques were revealed by staining with crystal violet and paraformaldehyde.

2.4. Quantitative PCR

Viral DNA was extracted from the samples with the longest exposure time using a NucleoSpin® 96 Virus Core Kit in accordance with the manufacturer's instructions. The extracted DNA was amplified using a SsoAdvanced Universal Probes Supermix Kit (Bio-Rad) with the following generic primers and probe targeting the G2R gene⁵: forward primer, 5'-GGAAAATGTAAGACAACGAA TACAG-3'; reverse primer, 5'-GCTATCACATAATCTGGAAGCGTA-3'; hydrolysis probe, 5'-AAGCCGTAATCTATGTTGTCTATCGTGTCC-3'. The cycle quantification (Cq) obtained for each condition was compared with the initial Cq obtained for the virus suspension before heat treatment.

3. Results and discussion

VTM and FCS were spiked with two different strains of MPXV, one belonging to the West African clade (CMIP2022) and the other to the Central African clade (LK), both with titers of approximately 1×10^7 PFU mL⁻¹. All samples were incubated in triplicate at different temperatures for various periods of time in a calibrated dry water bath. The results are summarized in Tables 1 and 2. The data showed that both strains of MPXV were inactivated under all conditions, except for 30 s at 95 °C and 30 min at 56 °C. Incubation for

Table 2

Viral titers obtained after heat inactivation of viruses diluted in VTM. ND: not detected (below the limit of virus detection: 25 PFU.mL⁻¹).

Strain	Temperature (°C)	Exposure Time (min)	Infectivity (PFU.mL ⁻¹)
LK	56	30	60.8
	60	15	ND
		30	ND
		60	ND
		90	ND
	70	5	ND
	95	0.5	9.6×10^6
		3	ND
CMIP2022	56	30	>2500
	60	15	ND
		30	ND
		60	ND
		90	ND
	70	5	ND
	95	0.5	1.1×10^7
		3	ND

30 s at 95 °C was probably not long enough for the inside of the tube to reach the indicated temperature, as already observed for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).⁶ Because more time to reach the target temperature could be required for volumes greater than 200 µL, we recommend that samples should be treated for 30 min at 60 °C to inactivate the virus, although heat treatment for 15 min at 60 °C for 500 µL of non-diluted viral suspensions with titers of 5.25×10^7 PFU mL⁻¹ (CMIP2022) and 3.49×10^7 PFU mL⁻¹ (LK) led to complete viral inactivation (data not shown). The recommended times and temperature of 70 °C in some extraction kits were also efficient for complete virus inactivation. Our data are in agreement with previous results obtained for vaccinia virus at 60 °C.⁷ However, MPXV was still infectious after 30 min at 56 °C, suggesting that thermal inactivation of serum products must be performed for at least 1 h at this temperature to reach full inactivation of high-titer sera.

Quantitative PCR (qPCR) was performed to explore the impact of heat treatment on viral genome detection. As summarized in Table 3, heat treatment had a very weak impact on viral DNA detection by qPCR, with variations in Cq not exceeding 2.09 at 60 °C or 70 °C and 3.46 at 95 °C, suggesting that the majority of viral DNA remained intact in the virus particles.

Overall, MPXV was relatively sensitive to heat inactivation under laboratory conditions. The present results can help workers

Table 1

Viral titers obtained after heat inactivation of viruses diluted in FCS. ND: not detected (below the limit of virus detection: 25 PFU.mL⁻¹).

Strain	Temperature (°C)	Exposure Time (min)	Infectivity (PFU.mL ⁻¹)
LK	56	30	ND
	60	15	ND
		30	ND
		60	ND
		90	ND
	70	5	ND
	95	0.5	9.4×10^6
		3	ND
CMIP2022	56	30	ND
	60	15	ND
		30	ND
		60	ND
		90	ND
	70	5	ND
	95	0.5	1.3×10^7
		3	ND

Table 3

Quantification of viral DNA in heat-inactivated samples. Mean (\pm SD) cycle quantification (Cq) values were obtained for each sample under the longest inactivation condition performed in triplicate. The initial Cq values were 16.76 ± 0.11 and 20.19 ± 0.18 for the LK and CMIP2022 strains respectively.

	Strain	Temperature (°C)	Exposure time (min)	Cq (mean \pm SD)
VTM	LK	60	90	16.65 ± 1.28
		70	5	18.85 ± 0.13
	CMIP2022	95	3	18.22 ± 0.64
		60	90	18.45 ± 0.31
FCS	LK	70	5	21.39 ± 0.49
		95	3	21.63 ± 0.11
	CMIP2022	60	90	16.96 ± 0.19
		70	5	17.53 ± 0.34
	CMIP2022	95	3	20.22 ± 0.28
		60	90	18.20 ± 0.04
	CMIP2022	70	5	18.33 ± 0.22
		95	3	21.30 ± 0.06

to improve their protocols and can provide a basis for knowledge and comprehension of MPXV survival mechanisms outside the host. The data described in this paper were obtained for viruses suspended in VTM and FCS and subjected to high temperatures, and are useful for laboratory protocols. Further experimental data are necessary to evaluate the persistence of MPXV on various matrices at different temperatures and to determine the potential role of contaminated surfaces in transmission of the virus.

CRediT authorship contribution statement

Christophe Batéjat: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Quentin Grassin:** Investigation. **Maxence Feher:** Investigation. **Damien Hoinard:** Investigation. **Jessica Vanhomwegen:** Writing – review & editing. **Jean-Claude Manuguerra:** Resources, Writing – review & editing. **India Leclercq:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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