How to cite: Serikbayov, O.N., Orazymbetova, N.K., Seisenbayeva, M.S., Umuraliev, B.K., Kaukarbayeva, M.Zh., Koshemetov, Zh.K., Kutumbetov, L.B., Zhuginisov, K.D. (2026). Optimization of culture conditions for vaccinia virus propagation. *Central Asian J. Vet. Science*, 2(014), 45-52. DOI: https://doi.org/10.51452/cajvs.2026.2(014).2226

Research article

Optimization of culture conditions for vaccinia virus propagation

Orazbek N. Serikbayov, Nurgul K. Orazymbetova, Madina S. Seisenbayeva
Bakyt K. Umuraliev, Madina Zh. Kaukarbayeva, Zhumagali K. Koshemetov
Lespek B. Kutumbetov, Kuandyk D. Zhuginisov

Laboratory for Diagnosis of Infectious Diseases, Research Institute of Biological Safety Problems
National Holding «QazBioPharm», Gvardeyskiy, Kazakhstan

Corresponding author: Orazbek N. Serikbayov: o.orazbek@biosafety.kz
Co-authors: (1: NO) n.orazymbetova@biosafety.kz; (2: MS) m.seisenbayeva@biosafety.kz
(3: BU) baha_umuraliev@mail.ru; (4: MK) m.kaukarbayeva@biosafety.kz
(5: ZhK) zh.koshemetov@biosafety.kz; (6: LB) l.kutumbetov@biosafety.kz
(7: KZh) k.Zhugunisov@biosafety.kz
Received: 28 April 2026 **Accepted:** 19 June 2026 **Published:** 30 June 2026

Copyright: Serikbayov, O.N., et al. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

Abstract

Background and Aim. Monkeypox virus has recently re-emerged as one of the most significant zoonotic pathogens and a major global public health concern. Due to its genetic and antigenic similarity to other members of the *Orthopoxvirus* genus, the use of model viruses is essential for the development of vaccines and diagnostic approaches. In this context, Vaccinia virus is widely regarded as a key model system for *Orthopoxvirus* research and is extensively used in the development of vaccines and diagnostic tools. The aim of this study was to determine the optimal conditions for the cultivation of vaccinia virus.

Materials and Methods. Vaccinia virus was cultivated in Vero, HeLa, and LK cell lines. Viral replication activity was evaluated based on cytopathic effect observed in cell cultures and the level of viral biological activity. In addition, the effects of fetal bovine serum concentration, multiplicity of infection, and incubation temperature on viral replication were investigated. For each parameter, the efficiency of viral propagation was comparatively analyzed to determine optimal cultivation conditions.

Results. The results demonstrated that the highest level of viral replication was observed in HeLa cells. The optimal infection dose for efficient viral propagation was determined to be 0.00369 $TCID_{50}$/cell. Furthermore, 2% fetal bovine serum was sufficient to ensure stable and efficient viral growth. The most favorable incubation temperature was 37 °C, which supported the maintenance of viral biological activity.

Conclusion. The optimized cultivation conditions identified in this study enable efficient biomass production of vaccinia virus. These findings provide an important scientific basis for further fundamental and applied research on Orthopoxviruses, including the development of vaccines and diagnostic systems.

Keywords: cultivation parameters; biological activity; *Orthopoxvirus*; vaccinia virus; temperature.

Introduction

Monkeypox is a zoonotic infectious disease caused by the monkeypox virus, a member of the genus *Orthopoxvirus.* The virus demonstrates a high degree of genetic homology with other clinically significant Orthopoxviruses, including variola virus, vaccinia virus, and cowpox virus [1]. Members of the *Orthopoxvirus* genus are characterized by their high pathogenic potential for both humans and animals, represent a continuing concern for public health and veterinary medicine.

Historically, variola virus, the causative agent of smallpox, was responsible for widespread epidemics over several centuries, leading to significant morbidity and mortality worldwide. Following the global vaccination campaign, smallpox was officially declared eradicated in 1980 [2]. Nevertheless, the continued existence of related Orthopoxviruses in natural animal reservoirs sustains the risk of zoonotic transmission, and sporadic epizootic and zoonotic cases are still reported in endemic regions [3].

Initially, the zoonotic nature of monkeypox was identified in the Democratic Republic of the Congo, whereas evidence of human-to-human transmission was subsequently reported in Abia State in southeastern Nigeria [4, 5]. By the end of the 20th century, the epidemiological profile of monkeypox had expanded, and the first documented fatal cases were recorded [6]. In the early 21st century, the geographic spread of the virus extended to a transcontinental level, with confirmed cases reported in the United States and the United Kingdom [7, 8]. In 2022, outbreaks of the disease were reported across multiple countries within the European Union [9, 10], prompting the World Health Organization (WHO) to declare monkeypox a Public Health Emergency of International Concern. This decision was prompted by the spread of the infection beyond previously endemic regions and the need for a coordinated global response [11].

Vaccinia virus historically played a crucial role in the eradication of variola virus, which induces a robust immune response and enabled the interruption of smallpox transmission [12]. Based on this virus, first-generation vaccines such as *Dryvax* and *Lister* were developed in the mid-20th century, making a decisive contribution to global vaccination campaigns and the eventual eradication of variola virus [13]. A later improved vaccine, ACAM2000, demonstrates high immunogenicity; however, its use is limited by safety concerns, as it is a replication-competent virus [14]. Next-generation vaccines, including JYNNEOS and MVA-BN, based on the modified vaccinia Ankara strain, are characterized by strong immunogenicity and improved safety profiles, allowing their use even in immunocompromised individuals. Notably, the JYNNEOS vaccine has demonstrated high efficacy in the prevention of monkeypox [15].

In addition, vaccinia virus antigens are widely used in the diagnosis of monkeypox. Several studies have demonstrated that vaccinia virus antigens exhibit high sensitivity in detecting the humoral immune response induced by monkeypox virus infection [16–18]. Moreover, neutralizing antibodies generated against vaccinia virus have been shown to effectively neutralize monkeypox virus in neutralization assays [19].

Cell culture systems and cultivation conditions for vaccinia virus propagation have been previously described in the scientific literature [20–22]. Therefore, the significance of the present study lies in the investigation of the cultural properties of the vaccinia virus strain in order to obtain a highly biologically active viral suspension for further application in the development of diagnostic methods.

Thus, the presented data indicate that vaccinia virus represents an important biological agent in the context of biosafety related to *Orthopoxvirus* infections. Accordingly, the maintenance, cultivation, and investigation of this virus are of significant importance for scientific research.

The aim of this study was to select an optimal system and parameters for the cultivation of vaccinia virus.

Materials and Methods

Refreshing of the museum strain

In the first stage of the study, vaccinia virus was passaged in lamb kidney (LK) cell culture. Cells were grown in 25 cm^2 culture flasks using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, antibiotics (penicillin, 100 IU/mL; streptomycin, 100 µg/mL), and 1% L-glutamine. Prior to infection, the cell monolayer was washed with phosphate-buffered saline (PBS), after which a virus-containing suspension was added, and adsorption was carried out at 37 °C for 1 h. Subsequently, maintenance medium (DMEM containing 2% serum) was added, and the cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

A total of three consecutive passages of the virus were performed as cytopathic effects (CPE) developed, with daily microscopic monitoring of their progression.

Selection of cell cultures and optimization of vaccinia virus cultivation conditions

In order to select the most suitable cell line providing efficient viral replication, both continuous and primary trypsinized cell cultures maintained at the Laboratory of Cell Biotechnology (RIBSP) were

used. The study employed African green monkey kidney (Vero), human cervical carcinoma (HeLa), and lamb kidney (LK) cell lines.

The susceptibility of cell cultures to the virus was assessed by infecting monolayers of Vero, HeLa, and LK cells, followed by incubation at 37 °C in a 5% CO_2 atmosphere for 5 days, with daily microscopic observation of the development of CPE. To optimize infection conditions, cells were infected at doses ranging from 0.000369 to 3.69 $TCID_{50}$/cell, and the effects of temperature (33 ± 0.5 °C, 36.5 ± 0.5 °C, and 39 ± 0.5 °C) and serum concentration (2.5%, 10%) on the development of viral infection.

Determination of the biological activity of the viral strain under study

The infectious activity of the virus was determined by cell culture titration. The infectious titer was calculated using the Reed and Muench method and expressed in lg $TCID_{50}/cm^3$.

Statistical analysis of data

Statistical analysis of the data was performed using GraphPad Prism software. Differences between groups were evaluated and considered statistically significant at $p < 0.05$.

Results

Vaccinia virus was passaged sequentially in LK cell culture to assess the dynamics of CPE development and viral adaptation to the cellular system. During cultivation, daily microscopic observations were performed to monitor the condition of the cell monolayer and the degree of morphological changes.

During the first passage, CPE was weakly expressed and minimal within the initial 1–2 days. By day 4, CPE development reached approximately 30–40%, indicating the onset of active virus –cell interaction. By day 6, the level of cell monolayer destruction increased to 60–70%, reflecting pronounced viral replication in the system. The dynamics of viral growth during the first passage are presented in Figure 1, illustrating the temporal development of the cytopathic process.

During the first passage, CPE was weakly expressed and minimal within the initial 1–2 days. By day 4, CPE development reached approximately 30–40%, indicating the onset of active virus –cell interaction. By day 6, the level of cell monolayer destruction increased to 60–70%, reflecting pronounced viral replication in the system. The dynamics of viral growth during the first passage are presented in Figure 1, illustrating the temporal development of the cytopathic process.

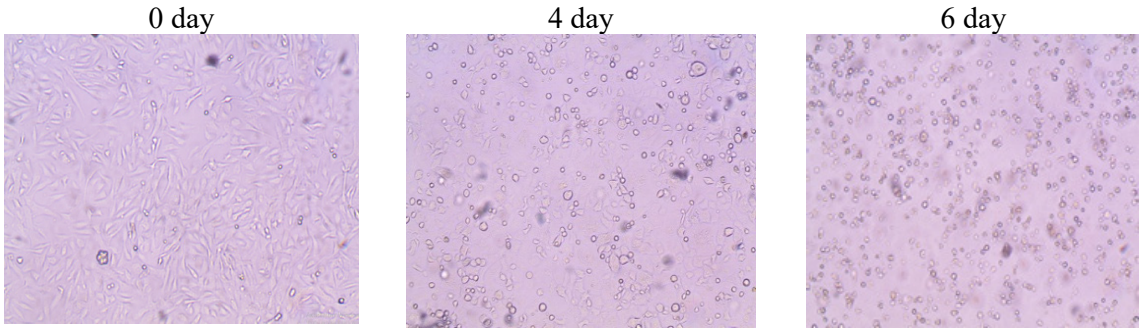

Figure 1. Cytopathic effect of vaccinia virus in LK cell culture during the first passage (×2000)

During the study, the smallpox vaccine virus was cultured in monolayers of Vero, HeLa, LK cell lines, with successive passages and assessment of the dynamics of CPE.

In the first passage, the development of CPE in the studied cell cultures was gradual. In the Vero culture, the first signs of cytopathic changes were observed on day 3, whereas in HeLa cells, cytopathic effects were detected as early as day 2, and in the LK culture – on day 3. By day 6 of cultivation, the degree of monolayer damage reached 70–80% in all cell types, indicating active viral replication.

In the second passage, an acceleration of CPE development was observed in all investigated cell lines; however, the dynamics of its manifestation varied depending on the cell type. In Vero cells, CPE was detected on day 2, with pronounced monolayer destruction (80–90%) observed by day 4 of cultivation. In HeLa cells, initial signs of CPE also appeared on day 2, while the maximum level of cell damage (80–90%) was recorded by day 3. In LK cells, CPE development occurred slightly more slowly: initial changes were observed on day 2, whereas pronounced CPE reaching 80–90% was achieved by day 5 of cultivation.

In the third passage, a similar trend of accelerated CPE development was observed in all investigated cell lines. The cytopathic effect during the third passage is shown in Figure 2.

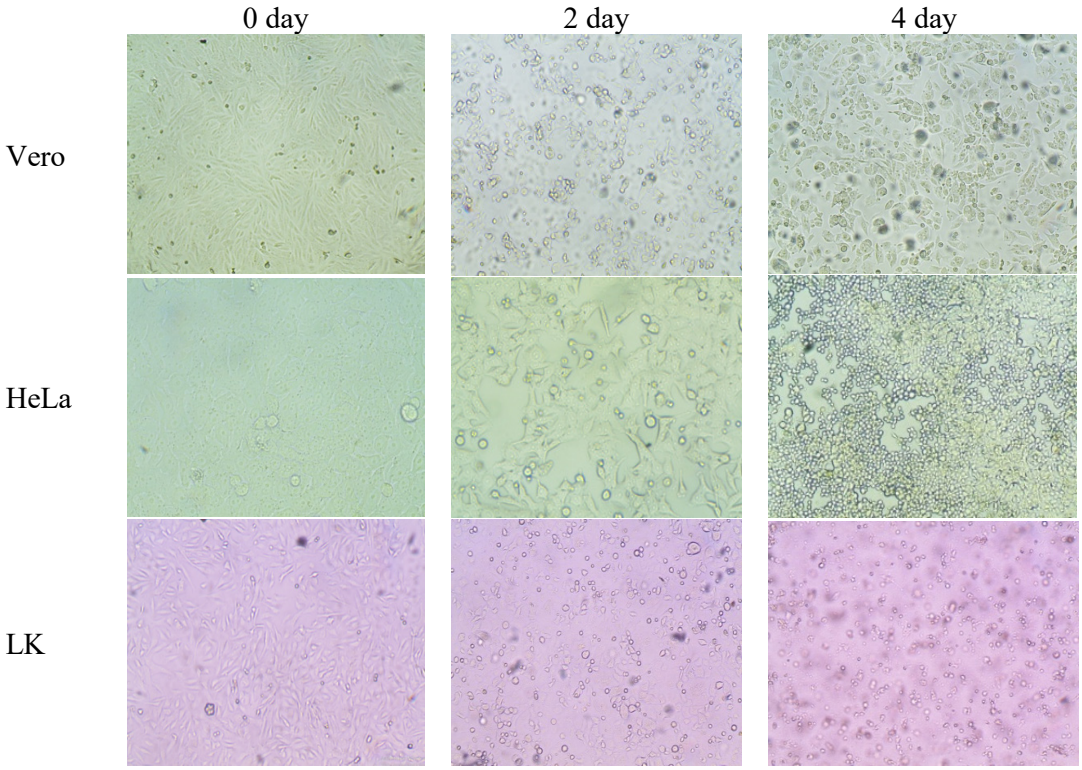

Figure 2. Morphological changes in cell cultures infected with vaccinia virus during the third passage

The obtained results indicate that the most pronounced replication of vaccinia virus was observed in HeLa cell culture, where cytopathic effect developed more rapidly compared to the other investigated cell lines. The infectivity data confirmed these observations: the highest viral titer was recorded in HeLa cells, reaching 8.50 ± 0.083 lg $TCID_{50}/cm^3$, whereas lower values were obtained in Vero and lamb kidney (LK) cells, amounting to 5.75 ± 0.08 and 6.33 ± 0.08 lg $TCID_{50}/cm^3$, respectively. The dynamics of vaccinia virus biological activity are presented in Figure 3.

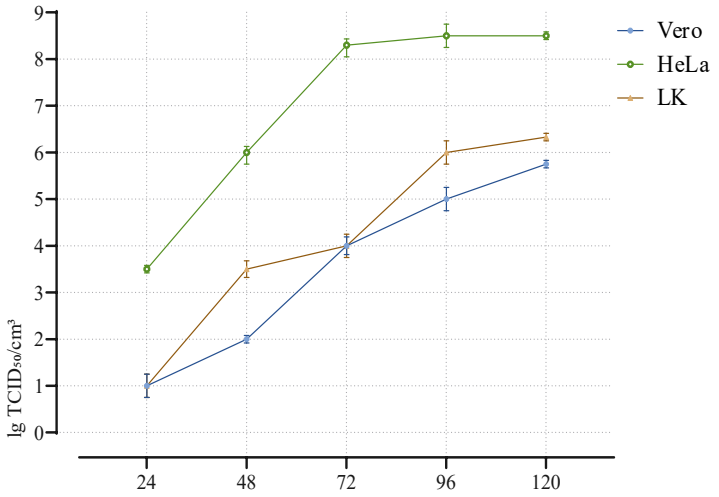

Figure 3. Dynamics of the biological activity of vaccinia virus

The infectious dose of vaccinia virus in HeLa cell culture was determined. It was established that viral biological activity depends on the magnitude of the infectious dose. At high virus concentrations (3.69; 0.369; 0.0369; and 0.00369 $TCID_{50}$/cell), a stable high level of biological activity was observed, with an infectious titer of 8.16 ± 0.083 lg $TCID_{50}/cm^3$. When the dose was reduced to 0.000369 $TCID_{50}$/cell, the infectious activity decreased to 6.6 ± 0.016 lg $TCID_{50}/cm^3$, accompanied by a reduction in the severity of CPE (Figure 4).

Based on the obtained results, an infectious dose of 0.00369 $TCID_{50}$/cell was selected as the optimal condition, under which stable levels of biological activity and pronounced CPE of vaccinia virus were maintained.

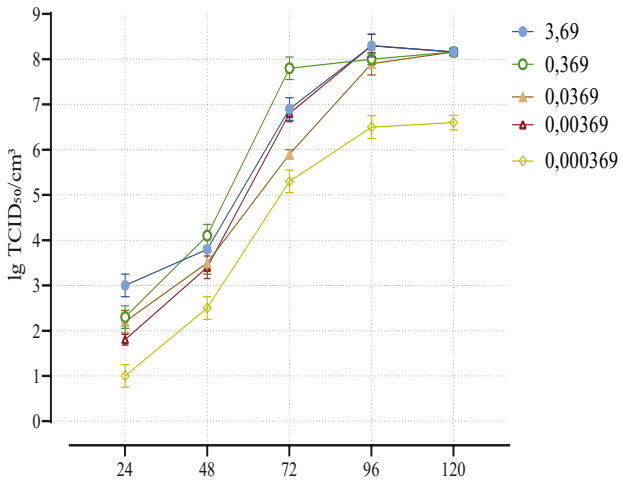

Figure 4. Dynamics of vaccinia virus biological activity at different infectious doses

During the selection of fetal bovine serum concentration in the culture medium, it was found that the biological activity of vaccinia virus showed only minor variation depending on serum content. At 2% and 5% FBS, the biological activity was 7.5 ± 0.083 lg $TCID_{50}/cm^3$, whereas at 10% FBS it reached 8.00 lg $TCID_{50}/cm^3$. No significant differences were observed between the tested conditions (Figure 5).

Thus, a 2% FBS concentration was determined to be optimal, as it ensures a sufficient level of viral biological activity while allowing more economical use of culture medium components.

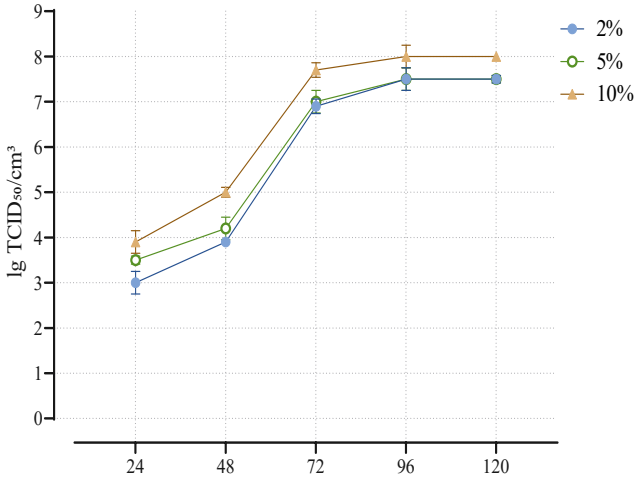

Figure 5. Biological activity of vaccinia virus at different fetal bovine serum concentrations

In the experiment aimed at determining the optimal cultivation temperature for the Orthopoxvirus strain, three temperature regimes (33 °C, 37 °C, and 39 °C) were evaluated. Cell monolayers were

infected with vaccinia virus at an infectious dose of 0.00369 $TCID_{50}$/cell, followed by incubation under the specified temperature conditions.

It was established that viral biological activity depended on the incubation temperature. At 33 °C, the infectious titer was 3.75 ± 0.25 lg $TCID_{50}/cm^3$, whereas at 39 °C it reached 6.5 ± 0.00 lg $TCID_{50}/cm^3$. The highest viral activity was observed at 37 °C, where the titer reached 7.50 lg $TCID_{50}/cm^3$. These results indicate that 37 °C is the optimal temperature for virus cultivation. The results are presented in Figure 6.

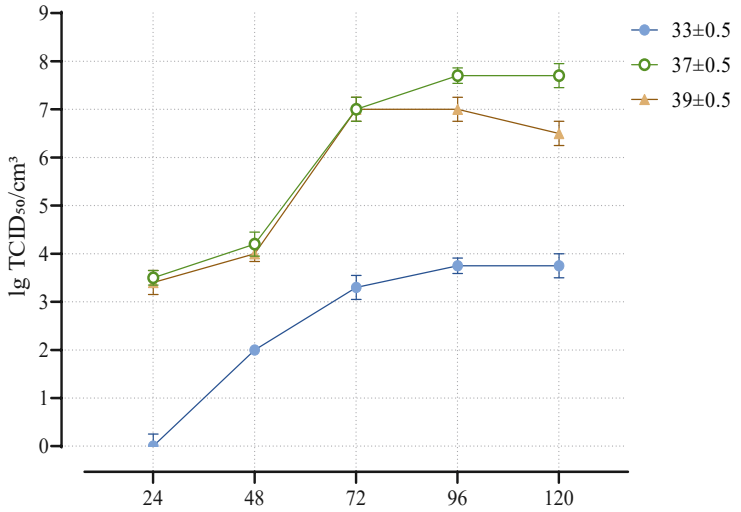

Figure 6. Biological activity of vaccinia virus at different cultivation temperatures

Discussion

Monkeypox, caused by viruses of the genus *Orthopoxvirus*, has recently regained significant scientific attention due to changes in its epidemiological pattern. In this context, vaccinia virus represents an important model for studying the biology of *Orthopoxviruses,* including viral replication mechanisms and theoptimization of cultivation conditions.

The results of the present study demonstrated that vaccinia virus efficiently replicates in various cell cultures; however, the level of replication is strongly dependent on the cell line used. The highest biological activity and the most rapid development of CPE were observed in HeLa cells, indicating their high susceptibility to the virus. These findings are consistent with classical studies showing that continuous cell lines provide more efficient viral replication compared to primary cultures [20].

In Vero and LK cells, virus replication was less pronounced, and CPE developed more slowly. Nevertheless, the ability of vaccinia virus to efficiently replicate in LK cells was previously demonstrated. For instance, *Subramanyam* et al. [21] showed that LK cells can support active viral replication and may be used for virus cultivation, which supports the results obtained in the present study.

In this study, high viral titers were observed at infection doses ranging from 3.69 to 0.00369 $TCID_{50}$/cell. At the lowest dose (0.000369 $TCID_{50}$/cell), a significant reduction in viral titer was detected. Thus, the effective infectious dose was determined to be 0.00369 $TCID_{50}$/cell. Although a high viral titer was also observed at a dose of 3.69 $TCID_{50}$/cell, this condition was considered unsuitable, as high infection doses induce rapid cytotoxic effects in cells, leading to alterations in viral phenotypic properties and the formation of free virions in subsequent passages.

The accumulation of virus in cell cultures depends not only on the cell type, temperature, and infectious dose, but also on the composition of the culture medium, including serum concentration. In serum-free conditions, cells experience a deficiency of essential nutrients, leading to cellular damage and accelerated senescence, which in turn negatively affects viral replication. Conversely, excessively high serum concentrations may promote the presence of non-specific inhibitors that suppress viral replication. Therefore, in the present study, a 2% serum concentration was used, which enabled the achievement of a high viral titer.

Viral replication in cell cultures is also temperature-dependent, and this factor is regulated by the viral genome. For most viruses, the optimal growth temperature is 36–37 °C. In the present study, 33 °C was shown to be unfavorable for the replication of the vaccinia virus strain, whereas at 39 °C an increase in viral titer was observed during the first 72 hours, followed by suppression of viral replication between 96 and 120 hours. Our findings demonstrate that a temperature of 37 °C provides optimal conditions for achieving a high viral titer of the vaccinia virus strain.

Conclusion

The present study demonstrated that vaccinia virus efficiently replicates in various cell cultures, with the highest biological activity observed in HeLa cells. The optimal infectious dose was determined to be 0.00369 $TCID_{50}$/cell, ensuring stable viral replication. It was shown that a 2% fetal bovine serum concentration is sufficient to maintain high viral biological activity, while the optimal cultivation temperature is 37 °C.

The established cultivation parameters provide favorable conditions for vaccinia virus propagation and may be of interest for further fundamental and applied research.

Authors' contributions

OS, NO, MS, BU, MK: conceptualized and designed the study, conducted a comprehensive literature search, analyzed the collected data, and prepared the manuscript; ZhK: wrote the original draft; LB, KZh: writing-review & editing.

Acknowledgements

This work was supported by the Scientific and Technical Program BR28712529, “Development of Specific Prevention and Diagnostic Tools for Monkeypox”

The authors express their gratitude to the Head of the Laboratory of Cell Biotechnology of the Research Institute for Biological Safety Problems, Aziz Nakhnov, and to junior researcher Aibol Torebek for providing the cell lines used in this study.

Statement on Competing Interests

The authors declare that they have no conflicts of interest.

References

1 Mitjà, O., Ogoina, D., Titanji, B.K., Galvan, C., Muyembe, J.J., Marks, M., Orkin, C.M. (2023). Monkeypox. *The Lancet*, 401(10370), 60–74. DOI:10.1016/S0140-6736(22)02075-X.

2 World Health Organization. (2023). *Smallpox overview*. https://www.who.int/emergencies/situations/smallpox

3 Bunge, E.M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L.R., et al. (2022). The changing epidemiology of human monkeypox – A potential threat? A systematic review. *PLOS Neglected Tropical Diseases*, 16(2), e0010141. DOI:10.1371/journal.pntd.0010141.

4 Ladnyj, I.D., Ziegler, P., Kima, E. (1972). A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bulletin of the World Health Organization,* 46(5), 593–597.

5 Arita, I., Henderson, D.A. (1976). Monkeypox and whitepox viruses in West and Central Africa. *Bulletin of the World Health Organization,* 53(4), 347–353.

6 Likos, A.M., Sammons, S.A., Olson, V.A., Frace, A.M., Li, Y., Olsen-Rasmussen, M., et al. (2005). A tale of two clades: Monkeypox viruses. *Journal of General Virology*, 86(10), 2661–2672.

7 Centers for Disease Control and Prevention. (2003). U*pdate: Multistate outbreak of monkeypox – Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin. Morbidity and Mortality Weekly Report (MMWR).* https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5226a5.htm

8 Vaughan, A., Aarons, E., Astbury, J., Balasegaram, S., Beadsworth, M., Beck, C.R., et al. (2018). Two cases of monkeypox imported to the United Kingdom. *Eurosurveillance,* 23(38), 1800509. DOI:10.2807/1560-7917.ES.2018.23.38.1800509.

9 European Centre for Disease Prevention and Control. (2023). *Weekly communicable disease threats report.* https://www.ecdc.europa.eu/sites/default/files/documents/communicable-disease-threats-report-week-32-2023_0.pdf

10 World Health Organization. (2022). *Multi-country monkeypox outbreak: Situation update (2022 DON396).* https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON396

11 World Health Organization. (2022). *Second meeting of the International Health Regulations Emergency Committee regarding the multi-country outbreak of monkeypox*. https://www.who.int/news/item/23-07-2022-second-meeting-of-the-international-health-regulations-%282005%29-%28ihr%29-emergency-committee-regarding-the-multi-country-outbreak-of-monkeypox

12 Kaynarcalidan, O., Moreno Mascaraque, S., Drexler, I. (2021). Vaccinia virus: From crude smallpox vaccines to elaborate viral vector vaccine design. *Biomedicines,* 9(12), 1780. DOI:10.3390/biomedicines9121780.

13 Sánchez-Sampedro, L., Perdiguero, B., Mejías-Pérez, E., García-Arriaza, J., Di Pilato, M., Esteban, M. (2015). The evolution of poxvirus vaccines. *Viruses,* 7(4), 1726–1803. DOI:10.3390/v7041726.

14 Wahid, M., Mandal, R.K., Sikander, M., Khan, M.R., Haque, S., Nagda, N., Ahmad, F., Rodriguez-Morales, A.J. (2025). Safety and efficacy of repurposed smallpox vaccines against mpox: A critical review of ACAM2000, JYNNEOS, and LC16. *Journal of Epidemiology and Global Health,* 15(1), 88. DOI:10.1007/s44197-025-00432-8.

15 Dalton, A.F. (2023). Estimated effectiveness of JYNNEOS vaccine in preventing mpox: A multijurisdictional case-control study – United States. *Morbidity and Mortality Weekly Report (MMWR)*, 72.

16 Hicks, B., Jones, S., Callaby, H., Bailey, D., Gordon, C., Rampling, T., et al. (2024). Evaluation of a multiplexed immunoassay for assessing long-term humoral immunity to orthopoxviruses. *Vaccine,* 42(26), 126453. DOI:10.1016/j.vaccine.2024.126453.

17 Jones, S., Hicks, B., Callaby, H., Bailey, D., Gordon, N.C., Rampling, T., et al. (2025). Assessment of MpoxPlex, a high-throughput and multiplexed immunoassay: A diagnostic accuracy study. *The Lancet Microbe,* 6(4), 100987. DOI:10.1016/j.lanmic.2024.100987.

18 Jones, S., Hicks, B., Callaby, H., Bailey, D., Gordon, C., Rampling, T., et al. (2024). MpoxPlex: A high-throughput and versatile multiplexed immunoassay for assessing and discriminating between IgG responses to mpox infection and vaccination. *medRxiv.* DOI:10.1101/2024.06.03.24308186.

19 Pettke, A., Keszei, M., Christ, W., Mayola Danés, N., Gredmark-Russ, S., Söderholm, S., et al. (2025). Serological differentiation between naturally acquired mpox and MVA-BN-vaccine induced antibody responses using ratios of MPXV and VACV antigen pairs in the MSD immunoassay. *Microbiology Spectrum*, 13, e00182-25. DOI:10.1128/spectrum.00182-25.

20 Nishmi, M., Niecikowski, H. (1963). Interaction of vaccinia virus and cells in primary and continuous culture. *Nature,* 199, 1117–1118. DOI:10.1038/1991117a0.

21 Subramanyam, P., Divakaran, S., Vinodraj, P. (1961). Cultivation of vaccinia virus in sheep kidney cell cultures. *Bulletin of the World Health Organization,* 25(1), 33–40.

22 Kroon, E.G., Abrahão, J.S., Trindade, G.S., Oliveira, G.P., Luiz, M.F., Costa, G.B., Lima, M.T., et al. (2018). Natural vaccinia virus infection: Diagnosis, isolation, and characterization. *Current Protocols in Microbiology*, 42(1), 14A.5.1–14A.5.43. DOI:10.1002/cpmc.13.