

Diagnostic performance of a field-deployable iiPCR platform and viral load dynamics of MPXV Clade IIb in a cohort with high prevalence of HIV

Li-Teh Liu, Chao-Ju Chen, Ping-Chang Lin, Shang-Yi Lin, Po-Chih Chen, Chun-Hong Chen, Ching-Yi Tsai, Yi-Ching Lin & Jih-Jin Tsai

To cite this article: Li-Teh Liu, Chao-Ju Chen, Ping-Chang Lin, Shang-Yi Lin, Po-Chih Chen, Chun-Hong Chen, Ching-Yi Tsai, Yi-Ching Lin & Jih-Jin Tsai (2026) Diagnostic performance of a field-deployable iiPCR platform and viral load dynamics of MPXV Clade IIb in a cohort with high prevalence of HIV, *Annals of Medicine*, 58:1, 2705009, DOI: [10.1080/07853890.2026.2705009](https://doi.org/10.1080/07853890.2026.2705009)

To link to this article: <https://doi.org/10.1080/07853890.2026.2705009>



© 2026 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



[View supplementary material](#)



Published online: 14 Aug 2026.



[Submit your article to this journal](#)



Article views: 189



[View related articles](#)



[View Crossmark data](#)

RESEARCH ARTICLE



Diagnostic performance of a field-deployable iiPCR platform and viral load dynamics of MPXV Clade IIb in a cohort with high prevalence of HIV

Li-Teh Liu^a , Chao-Ju Chen^{b,c,d*}, Ping-Chang Lin^{e*}, Shang-Yi Lin^{b,c,d}, Po-Chih Chen^{b,f}, Chun-Hong Chen^{g,h}, Ching-Yi Tsai^e, Yi-Ching Lin^{b,c} and Jih-Jin Tsai^{c,d,e} 

^aDepartment of Medical Laboratory Science and Biotechnology, College of Medical Technology, Chung Hwa University of Medical Technology, Tainan, Taiwan; ^bDepartment of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; ^cSchool of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; ^dDivision of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; ^eTropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; ^fDepartment of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan; ^gNational Mosquito-Borne Diseases Control Research Center, National Health Research Institutes, Miaoli County, Zhunan, Taiwan; ^hNational Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Zhunan, Taiwan

ABSTRACT

Background: Since 2022, the global outbreak of Mpox caused by the Mpox virus (MPXV) Clade IIb has underscored the need for timely and decentralized molecular diagnostics. Although quantitative real-time PCR (qPCR) remains the diagnostic gold standard, its infrastructure requirements may limit accessibility in resource-constrained settings. Insulated isothermal PCR (iiPCR) represents a potential field-deployable alternative; however, clinical validation data for MPXV remains limited.

Methods: We prospectively enrolled 24 qPCR-confirmed Mpox patients between 2023 and 2024. Multiple specimen types were tested using the POKIT Central MPXV iiPCR system and reference qPCR. Analytical sensitivity was assessed using serial viral dilutions. Viral load dynamics were evaluated using cycle threshold (Ct) values across specimen types and days post-symptom onset. Phylogenetic characterization was performed using a four-gene Sanger sequencing approach.

Results: Both iiPCR and qPCR demonstrated identical analytical limits of detection at 10^{-1} PFU/mL and complete qualitative concordance in cultured samples. Lesion-derived specimens showed the highest detection rates (100%) and consistently lower Ct values, whereas non-lesion specimens exhibited lower and more variable positivity. No clear differences in viral load-based diagnostic performance were observed between people living with HIV receiving antiretroviral therapy (ART) and people without HIV. Phylogenetic analysis confirmed exclusive circulation of MPXV Clade IIb.

Conclusions: Under the conditions evaluated, the POKIT Central iiPCR system achieved diagnostic performance comparable to qPCR, particularly for lesion-derived specimens. In this ART-treated HIV-prevalent cohort with preserved immune function, HIV co-infection was not associated with differences in diagnostic performance, supporting the potential utility of iiPCR for decentralized Mpox testing.

KEY MESSAGES

- A field-deployable iiPCR platform showed diagnostic performance comparable to qPCR for MPXV Clade IIb under the conditions evaluated, particularly in lesion-derived specimens.
- In an ART-treated, HIV-prevalent cohort with preserved immunity, HIV co-infection was not associated with detectable differences in viral load-based diagnostic performance, although this comparison was limited by the small sample size.

ARTICLE HISTORY

Received 9 March 2026

Accepted 13 July 2026

KEYWORDS

Mpox; insulated isothermal PCR; point-of-care testing; HIV; viral load; MPXV clade IIb

CONTACT Jih-Jin Tsai  jjjts@cc.kmu.edu.tw  Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100, Tzyou 1st Rd, Kaohsiung, 807377, Taiwan.

*These authors contributed equally to this work.

 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/07853890.2026.2705009>.

© 2026 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

1. Introduction

Mpox is caused by the double-stranded DNA Mpox virus (MPXV), a member of the genus *Orthopoxvirus*, and was historically endemic in Central and West Africa, where two major clades were recognized and associated with differing clinical severity [1,2]. Since 2022, the emergence of Clade IIb has been associated with a substantial change in the global epidemiology of Mpox, characterized by sustained human-to-human transmission and widespread dissemination across multiple non-endemic regions [3,4].

Compared with classical Mpox described in African outbreaks, Clade IIb infections frequently present with localized anogenital or oral lesions, fewer disseminated skin lesions, and sometimes minimal or absent prodromal symptoms [3,5,6]. These atypical clinical features increase reliance on laboratory confirmation to distinguish Mpox from other sexually transmitted infections or vesiculopustular dermatoses. The World Health Organization (WHO) recommends quantitative real-time PCR (qPCR), performed in appropriately equipped laboratories, as the diagnostic gold standard for Mpox, with lesion-derived material being preferred because of higher viral loads than those detected in non-lesion specimens [7].

Recent outbreak investigations further highlight the importance of specimen type for molecular testing. Studies quantifying MPXV DNA across different clinical materials have shown that viral loads are highest in skin and mucosal lesion swabs, intermediate in mucosal or nasopharyngeal specimens, and generally lower in blood and urine [5,8]. These findings suggest that diagnostic performance may vary considerably across specimen types, highlighting the need for the evaluation of new assays using diverse sample sets. People living with HIV (PLWH) represent a substantial proportion of Clade IIb cases worldwide [3,4]. Severe immunosuppression has been associated with extensive necrotic lesions, disseminated disease, and increased mortality in some cohorts [4]. However, whether HIV infection, particularly in individuals receiving effective antiretroviral therapy (ART), modifies MPXV viral dynamics or influences diagnostic sensitivity remains unclear. A multicenter study reported that PLWH with well-controlled HIV often exhibited disease courses similar to those of HIV-negative individuals [9], but systematic assessments of viral loads and assay performance according to HIV status remain limited.

Insulated isothermal PCR (iiPCR) platforms were developed to expand molecular testing capacity by providing automated, sample-to-answer nucleic acid detection outside conventional laboratory settings. Previous evaluations have shown that iiPCR assays can achieve analytical performance comparable to qPCR for multiple viral pathogens [10–12]. While whole-genome sequencing provides high-resolution information, it remains a resource-intensive process. Targeted sequencing of selected conserved MPXV genes has therefore been proposed as a pragmatic approach for clade or lineage assignment and coarse-scale phylogenetic placement, particularly in settings where whole-genome sequencing is not readily available [13].

To address these gaps, we prospectively evaluated the analytical and clinical performance of the POCKIT Central MPXV iiPCR assay across multiple specimen types, characterized viral load dynamics in an HIV-prevalent cohort, and applied a four-gene sequencing strategy for clade-level phylogenetic classification during Taiwan's 2023–2024 Clade IIb outbreak.

2. Materials and methods

2.1. Ethics and patient enrollment

This prospective, single-center study was conducted in accordance with the Declaration of Helsinki and reviewed and approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20230132). Patients presenting with clinical features suggestive of Mpox at Kaohsiung Medical University Hospital (KMUH) between May 2023 and August 2024 were screened. Among the suspected cases who provided written informed consent, a total of 24 individuals were subsequently confirmed to have MPXV infection by qPCR and were included in the study. In total, 82 clinical specimens were collected from 24 patients, comprising lesion-derived specimens (pus, vesicular fluid, skin and oral swabs), nasopharyngeal swabs, urine, and plasma.

2.2. Specimen collection, laboratory procedures, and clinical data retrieval

Clinical specimens were collected from each enrolled participant for virological analyses, including lesion-derived specimens, nasopharyngeal swabs, urine, and plasma at the time of enrollment. The analytical sensitivity experiments were performed using a clinical MPXV isolate (KMUH-Mpox-1) that was isolated from a patient enrolled in the present study. Viral titers were quantified in plaque-forming units per milliliter (PFU/mL) prior to serial dilution. For the analytical concordance evaluation (Table 2), the 20 MPXV-positive specimens were culture supernatants obtained by propagating virus from clinical specimens (skin lesions and nasopharyngeal swabs) of confirmed Mpox patients on Vero E6 cells. The 10 MPXV-negative specimens were original clinical specimens (urine and plasma) obtained from suspected cases that were subsequently confirmed qPCR-negative.

Virus propagation was performed in a biosafety level 3 (BSL-3) laboratory. Vero E6 cells were seeded at 1×10^5 cells per well in 24-well plates and incubated overnight to allow attachment. One hundred microliters of a qPCR-positive clinical specimen in UTM (swab, vesicular fluid, or pus) was diluted 2-fold in pretreated Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2× antibiotic-antimycotic (Thermo Fisher Scientific, United States) and added to the cells, which were then incubated at 37°C in 5% CO₂ for 1 h to allow viral attachment. DMEM containing 2% fetal bovine serum (600 µL) was then added to each well, and the cultures were examined daily under a phase-contrast microscope to monitor the cytopathic effect (CPE). Once CPE was observed, the presence of MPXV in the culture supernatant was confirmed by qPCR. The clinically isolated virus was harvested by centrifugation at 3,000 rpm and 4°C, stored at −20°C, and successful cultivation was verified by qPCR.

Demographic and clinical information, including age, sex, days post-symptom onset (PSO), vaccination status, chronic disease history (Charlson Comorbidity Index Score, CCIS), and HIV-related parameters (co-infection status, ART use, CD4 count, CD4/CD8 ratio, and HIV viral load), were retrieved from the electronic medical record system. PCR cycle threshold (Ct) values were documented for all available specimen types. qPCR and iiPCR were performed independently by separate operators (qPCR by P.C. Lin and iiPCR by C.Y. Tsai), each of whom had access only to de-identified specimen codes and was blinded to the results of the other assay and to all clinical information. The ART received by the PLWH in this cohort is HIV-directed treatment and is distinct from MPXV-specific antivirals such as tecovirimat; the Ct values therefore reflect viral load that was not confounded by MPXV-directed antiviral treatment.

2.3. Nucleic acid extraction and MPXV qPCR

Total nucleic acids were extracted from 200 µL of specimen–universal transport medium (UTM) mixtures using the QIAamp MinElute Virus Spin Kit (QIAGEN, Germany). Real-time qPCR was performed on the Mx3000P qPCR system (Agilent, United States). MPXV genomic DNA was detected using a real-time PCR assay targeting the tumor necrosis factor (TNF) receptor gene (G2R), as described by Li et al. [14]. The forward primer 5'-GGA AAA TGT AAA GAC AAC GAA AAC G-3', reverse primer 5'-GCT ATC ACA TAA TCT GGA AGC GTA GTA-3', and probe 5'-FAM-AAG CCG TAA TCT ATG TTG TCT ATC GTG TCC-BHQ1-3' were used on an Mx3000P qPCR system (Agilent, United States), and Ct values were recorded for all positive reactions.

2.4. Insulated isothermal polymerase chain reaction for MPXV detection

MPXV detection by iiPCR was performed using the POKKIT MPXV Premix Reagent Set on the POKKIT Central fully automated sample-to-answer system (GeneReach Biotech, Taiwan). The system automates nucleic acid extraction, reaction setup, amplification, and fluorescence detection within a closed device. Each POKKIT Central run processed up to eight samples with a total turnaround time of approximately 85 min from sample loading to result readout, following the manufacturer's instructions. The POKKIT Central uses insulated isothermal PCR (iiPCR), in which a single constant heat source generates a stable temperature gradient that drives passive thermal convection (Rayleigh-Bénard convection) [15] within the reaction vial, thereby achieving nucleic acid amplification without the programmable thermal cycling required by qPCR or cartridge-based platforms such as GeneXpert. Nucleic acid extraction was performed from a 200 µL sample input, returns a result in approximately 85 min. The instrument is a compact,

portable benchtop unit (approximately 48×31×40 cm; 21 kg) that operates on standard international mains voltage (100–120/200–240 V AC, 50/60 Hz); in this study, ‘field-deployable’ refers to this combination of a closed, automated sample-to-answer workflow and minimal infrastructure requirements, which together allow the assay to be performed outside a centralized molecular laboratory.

2.5. PCR amplification and Sanger sequencing

Briefly, DNA from each sample was used to amplify four target genes from the MPXV genome, including OPG105, OPG109, OPG118, and OPG151 (Table S1). To enable rapid sequencing, PCR was performed using TopPLUS PCR Master Mix (Topgen Biotech., Taiwan) and gene-specific primer pairs (Table S2) at a working concentration of 200 nM on an Applied Biosystems 9700 Thermal Cycler (Applied Biosystems, United States). PCR products were purified using VAHTS DNA Clean Beads (Vazyme Biotech., China) and analyzed using the MultiNA MCE 202 with the DNA 2500 Kit (Shimadzu, Japan) to confirm the expected amplicon size and quantity. Purified products were then subjected to Sanger sequencing using the BigDye Terminator v3.1 (Applied Biosystems., United States) on an ABI 3730 Genetic Analyzer (Applied Biosystems., United States). Finally, sequencing chromatograms were analyzed using Sequencing Analysis Software v5.2 (Applied Biosystems., United States).

2.6. Phylogenetic analysis and lineage identification

To determine the genetic lineage of the identified MPXV strains, the Sanger sequences of four specific core genes (OPG105, OPG109, OPG118, and OPG151) were analyzed. These genes were selected based on previous studies demonstrating their suitability as phylogenetic markers for Poxviridae classification [13]. A comparative phylogenetic analysis was performed using a dataset comprising the six isolates from this study and 17 representative global reference strains retrieved from GISAID and GenBank. Sequences for the four gene targets were aligned using MAFFT v7 with default parameters [16] and subsequently concatenated to generate a final alignment dataset. A maximum-likelihood phylogeny was inferred using IQ-TREE v3.0.1 [17]. The final phylogenetic tree was constructed with 1,000 ultrafast bootstrap replicates to estimate node reliability.

2.7. Statistical analysis

Continuous variables were summarized as medians with ranges. Comparisons between two independent groups were performed using the Mann–Whitney U test. Categorical variables were analyzed using Fisher’s exact test, as specified in the corresponding table footnotes. Between-group comparisons of Ct values in HIV-positive and HIV-negative patients were performed using the Mann–Whitney U test. Analytical sensitivity was assessed using serial 10-fold dilutions tested in triplicate, and the limit of detection (LOD) was defined as the lowest concentration consistently detected. Detection rates were calculated as the proportion of positive samples relative to the total number of samples tested for each specimen type. Exact (Clopper–Pearson) binomial 95% confidence intervals were calculated for the specimen-type detection rates and for overall concordance. All diagnostic performance analyses of alternative specimen types were conducted using qPCR-confirmed cases as the reference standard. Overall agreement between iiPCR and qPCR results was evaluated by calculating the concordance percentage, with qPCR serving as the reference method. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using MedCalc version 23.4.4 (MedCalc Software Ltd, Ostend, Belgium; 2025).

3. Results

3.1. Comparative evaluation of POKKIT central iiPCR and qPCR: sensitivity and specificity

To assess the analytical sensitivity of the POKKIT Central MPXV iiPCR system in comparison with the standard MPXV qPCR, 10-fold serial dilutions of a clinical MPXV isolate (KMUH-Mpox-1) ranging from 10^3

Table 1. Comparative limit of detection of the iiPCR and the qPCR assays for MPXV.^a

Viral load (PFU/ml)	iiPCR results (Pos/Total)	qPCR results Mean Ct (Pos/Total)
10 ³	3/3	23.81 (3/3)
10 ²	3/3	27.68 (3/3)
10 ¹	3/3	31.28 (3/3)
10 ⁰	3/3	34.38 (3/3)
10 ⁻¹	3/3	37.93 (3/3)
10 ⁻²	1/3	41.47 (1/3)
10 ⁻³	1/3	No Ct (0/3)
10 ⁻⁴	0/3	No Ct (0/3)
10 ⁻⁵	0/3	No Ct (0/3)
10 ⁻⁶	0/3	No Ct (0/3)
10 ⁻⁷	0/3	No Ct (0/3)

Abbreviations: PFU, Plaque-Forming Units; Ct, Cycle threshold.

^aSerial 10-fold dilutions of the MPXV were tested in triplicate ($n=3$) for each concentration. The same dilution series was tested in parallel by iiPCR and qPCR. Results for qPCR are presented as mean Ct values of the positive replicates. No Ct: Target not detected (below the limit of detection).

Table 2. Concordance between the iiPCR and the qPCR assays for detection of MPXV in cultured virus and clinical specimens.

Sample ^a	No. Tested	iiPCR results (Pos/Total)	qPCR results (Pos/Total)	qPCR Ct range (Mean $\pm$ SD) ^b	Agreement ^c
MPXV positive	20	20/20	20/20	19.01 – 27.54 (23.21 $\pm$ 2.82)	100%
MPXV negative	10	0/10	0/10	No Ct	100%
Total	30	20/30	20/30	–	100%

Abbreviations: Ct, Cycle threshold; iiPCR, insulated isothermal polymerase chain reaction; qPCR, quantitative real-time polymerase chain reaction.

^aThe 20 MPXV-positive specimens were culture supernatants obtained by propagating virus from clinical specimens (skin lesions and nasopharyngeal swabs) of confirmed Mpox patients. The 10 MPXV-negative specimens were original clinical specimens (urine and plasma) from suspected cases that were subsequently confirmed qPCR-negative.

^bNo Ct: Target not detected (negative result).

^cAgreement was calculated as overall percent concordance between iiPCR and qPCR qualitative results, with qPCR serving as the reference method.

to 10⁻⁷ PFU/mL were analyzed (Table 1). Both platforms demonstrated a consistent LOD at 10⁻¹ PFU/mL, with 100% detection of replicates (3/3) at this concentration, which was defined as the lowest concentration at which all triplicate reactions were consistently detected. For qPCR, mean Ct values increased inversely with viral concentration, ranging from 23.81 at 10³ PFU/mL to 37.93 at 10⁻¹ PFU/mL.

To further evaluate diagnostic accuracy, 30 specimens were analyzed. The 20 MPXV-positive specimens were culture supernatants obtained by propagating virus from clinical specimens (skin lesions and nasopharyngeal swabs) of confirmed Mpox patients, whereas the 10 MPXV-negative specimens were original clinical specimens (urine and plasma) from suspected cases that were subsequently confirmed qPCR-negative (Table 2). The iiPCR showed 100% concordance (30/30; 95% CI 88.4–100%, Clopper-Pearson) with the standard qPCR assay. All 20 positive culture supernatants were identified as positive by both platforms. In addition, all 10 negative clinical specimens tested negative on both systems ('No Ct'), supporting the analytical specificity of the test under the conditions tested.

3.2. Clinical characteristics of the study population and viral lineage identification

The study cohort consisted of 24 confirmed Mpox patients (Table 3), all of whom were male, with a median age of 33.5 years (range, 21–45). Vaccination history varied within the cohort: 16 patients (66.7%) were unvaccinated, 6 (25.0%) had received one dose, and 2 (8.3%) had received two doses of Mpox vaccine at the time of diagnosis. All vaccinated participants received the JYNNEOS (MVA-BN) Mpox vaccine (Bavarian Nordic), the only Mpox vaccine available and administered in Taiwan during the study period (2023–2024). HIV co-infection was common, with 18 of 24 patients (75.0%) being PLWH; all PLWH were receiving antiretroviral therapy at the time of diagnosis. Among PLWH, the median CD4 count was 709 cells/ μ L (range, 236–1253), the median CD4/CD8 ratio was 0.81 (range, 0.19–1.65), and 14 of 18 (77.8%) had HIV viral loads below 20 copies/mL. The median time from symptom onset to specimen collection was 4 days (range, 0–14), indicating that most patients were sampled during the acute phase of infection. Lesion-derived specimens demonstrated lower Ct values, consistent with higher viral loads

Table 3. Aggregated demographic, clinical, and virological characteristics of the 24 mpox cases in the study cohort.^a

Variable	Overall cohort (n = 24)
Sex	24 male (100%)
Age, years	33.5 (21–45)
Days post-symptom onset at sampling, days	4.0 (0–14)
Mpox vaccination status (JYNNEOS, MVA-BN)	
Unvaccinated	16 (66.7%)
One dose	6 (25.0%)
Two doses	2 (8.3%)
HIV co-infection ^b	18 (75.0%)
Receiving antiretroviral therapy among PLWH	18/18 (100%)
HIV viral load < 20 copies/mL among PLWH	14/18 (77.8%)
CD4 count, cells/ μ L, among PLWH	709 (236–1253)
CD4/CD8 ratio among PLWH	0.81 (0.19–1.65)
MPXV qPCR Ct values ^c	
Lesion-derived specimens	23.3 (18.0–32.0)
Nasopharyngeal swab	33.3 (25.4–39.2)
Urine, positive/tested	5/8 (62.5%)
Plasma, positive/tested	2/6 (33.3%)

^aData are presented as n (%) for categorical variables and median (range) for continuous variables.

^bHIV-related variables are presented for the 18 PLWH only. All PLWH were receiving antiretroviral therapy at the time of Mpox diagnosis.

^cCt values indicate cycle threshold values from MPXV qPCR. Lower Ct values correspond to higher viral loads. Lesion-derived specimens include pus, vesicular fluid, skin swabs, and oral swabs. Urine and plasma are summarized as positive/tested counts rather than individual Ct values because detection was sporadic and these specimen types are described in detail in Table 4.

Table 4. Diagnostic detection rates of MPXV across different clinical specimen types, with identical results obtained by qPCR and iPCR.

Specimen type	Positive	Total tested	Detection rate (%) ^b	95% CI ^c
Skin/oral swabs ^a	5	5	100.0%	47.8%–100%
Pus	21	21	100.0%	83.9%–100%
Vesicular fluid	19	19	100.0%	82.4%–100%
Nasopharyngeal swab	19	23	82.6%	61.2%–95.0%
Plasma	2	6	33.3%	4.3%–77.7%
Urine	5	8	62.5%	24.5%–91.5%

^aThis category includes skin and oral swabs (e.g. one oral swab from Patient 24).

^bDetection rate is calculated as the number of positive samples divided by the total number of samples tested for each specimen type and reflects identical qualitative results obtained by both qPCR and iPCR across all specimen types. The detection rate reflects positivity by either validated platform.

^c95% confidence interval (CI) calculated using the exact Clopper-Pearson binomial method.

(median Ct, 23.3; range, 18.0–32.0), whereas nasopharyngeal swabs showed higher Ct values (median Ct, 33.3; range, 25.4–39.2). In contrast, MPXV DNA detection in urine and plasma was less frequent, with positivity observed in 5 of 8 urine samples (62.5%) and 2 of 6 plasma samples (33.3%).

To determine the genetic lineage of the MPXV strains circulating in this cohort, Sanger sequencing of four specific genes (OPG105, OPG109, OPG118, and OPG151) was performed for six representative isolates (KMUH-Mpox-1 to KMUH-Mpox-6). All nucleotide sequences generated in this study have been deposited in GenBank under the accession numbers listed in Table S3. A maximum-likelihood phylogenetic tree was constructed based on the concatenated alignment of these four gene sequences together with 17 global reference strains (Figure 1). All six isolates from this study clustered within Clade IIb, grouping closely with strains associated with the 2022–2023 global outbreak (e.g. sequences from the United States and Italy) and segregating from Clade I and Clade IIa lineages. These findings indicate that the cases in this cohort were caused by Clade IIb MPXV, with no evidence of Clade I or IIa lineages among the sequenced isolates.

3.3. Diagnostic performance and viral load dynamics across clinical specimen types

Diagnostic performance was evaluated across various clinical specimen types collected from the 24 confirmed cases (Table 4). The detection results were identical between qPCR and iPCR across all specimen types. No discrepant, borderline, or equivocal results were observed between the two platforms in either the culture-fluid panel (Table 2) or the clinical specimens (Table 4); consequently, no repeat testing or adjudication was required. Lesion-derived specimens exhibited the highest diagnostic sensitivity; pus (21/21), vesicular fluid (19/19), and skin/oral swabs (5/5) all showed a 100% detection rate. Among

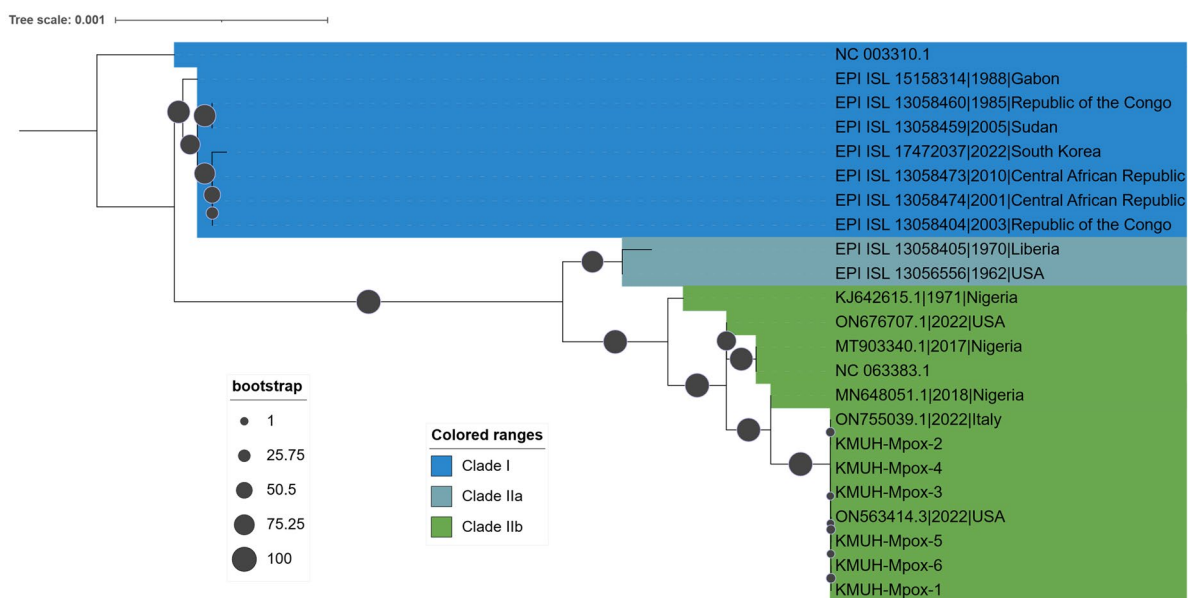


Figure 1. Phylogenetic analysis of MPXV isolates based on concatenated sequences of four selected genes. The phylogenetic tree was inferred using the Maximum Likelihood method based on the concatenated alignment of four specific gene fragments: OPG105, OPG109, OPG118, and OPG151. The analysis included sequences from six isolates obtained in this study (labeled KMUH-Mpox-1 to KMUH-Mpox-6) and 17 global reference strains retrieved from databases. Shaded regions denote the major viral lineages: Clade I (blue), Clade IIa (gray-blue), and Clade IIb (green). All six isolates from this study clustered tightly within Clade IIb, demonstrating high genetic similarity to strains associated with the 2022–2023 global outbreak. Bootstrap support values (1,000 replicates) are represented by the size of the nodal circles, with larger circles indicating higher statistical confidence. The scale bar represents 0.001 nucleotide substitutions per site. Reference sequences were obtained from the GISAID and GenBank databases.

qPCR-confirmed Mpox cases, nasopharyngeal swabs demonstrated high diagnostic utility when tested by iPCR, with a positivity rate of 82.6% (19/23). In contrast, non-lesion specimens showed lower and more variable iPCR detection rates: urine samples yielded a positivity rate of 62.5% (5/8), while plasma samples had the lowest detection rate at 33.3% (2/6). These results indicate that, although nasopharyngeal swabs provide additional diagnostic yield, lesion-derived specimens constitute the primary source for consistent detection of MPXV in this cohort. Detection rates are reported with exact (Clopper-Pearson) 95% confidence intervals in Table 4; the intervals were wide for the non-lesion specimens, reflecting the small numbers tested.

To evaluate the temporal patterns of viral shedding, we analyzed the distribution of the lowest qPCR Ct values across four specimen types relative to the number of days post-symptom onset (PSO) (Figure 2). Detailed longitudinal Ct values for each patient are provided in Table S4. These temporal patterns are descriptive and should be interpreted with caution, as they are constrained by the modest sample size and the non-standardized, clinically driven sampling schedule (see Limitations). In contrast, nasopharyngeal swabs exhibited higher and more variable Ct values, primarily ranging from 25 to 39. Detection in urine and plasma was sporadic; when detectable, these specimens typically yielded higher Ct values (>30), consistent with lower levels of viral DNA. An exception was observed in one sample (Ct 12.0), which was attributed to likely contamination from an active penile lesion during collection (Table 3). Collectively, these findings show that lesion specimens provided the most consistent detection across the observed course of infection in this dataset.

3.4. Impact of HIV co-infection on clinical characteristics and viral load

To evaluate whether HIV co-infection was associated with differences in clinical presentation or diagnostic parameters, the 24 patients were stratified into HIV-positive ($n=18$) and HIV-negative ($n=6$) groups (Table 5). For patients with multiple sampling days, only the lowest Ct value was used to represent the highest detectable viral load for between-group comparisons, consistent with the analytical approach

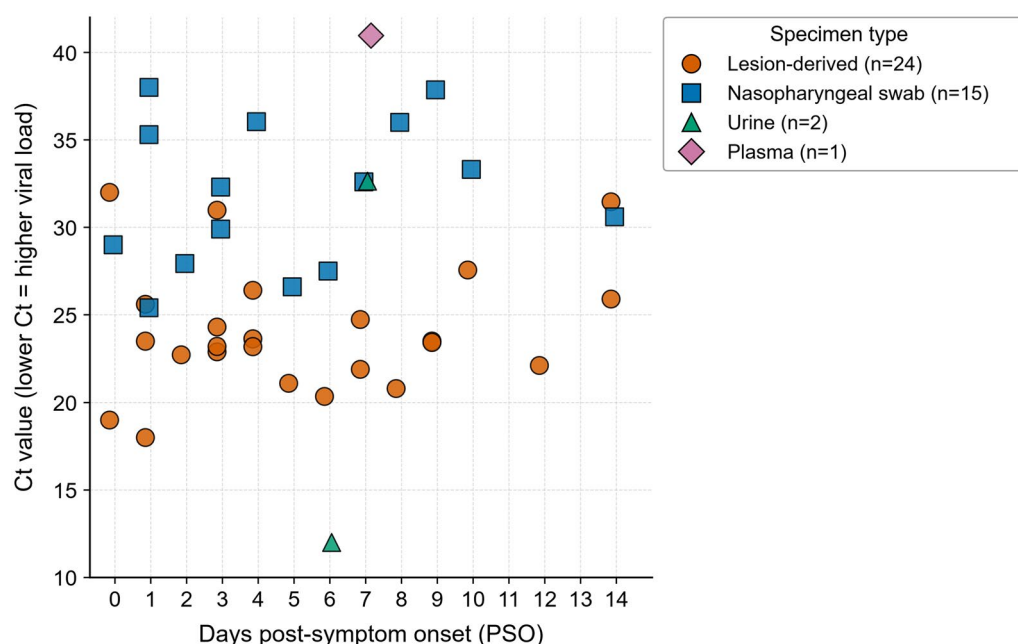


Figure 2. Temporal dynamics of qPCR Ct values across different clinical specimen types according to days post-symptom onset.

The scatter plot illustrates the distribution of qPCR cycle threshold (Ct) values from four distinct specimen types collected from 24 confirmed Mpox cases, plotted against the days post-symptom onset (PSO). Lesion-derived specimens (orange circles), including pus, vesicular fluid, skin, and oral swabs, consistently exhibit the lowest Ct values, indicating high viral loads, and remain detectable throughout the observation period. Nasopharyngeal swabs (blue squares) show intermediate Ct values, while urine (green triangles) and plasma (purple diamonds) samples demonstrate sporadic detection with higher Ct values. One urine specimen with a markedly low Ct (approximately 12) reflects likely contamination from an adjacent active lesion during collection rather than intrinsic urinary shedding, as discussed in the text. Note that the lower Ct values correspond to higher viral loads. For each individual and specimen type, the lowest qPCR Ct value obtained on each day PSO was selected to represent the highest detectable viral load for temporal analysis. Markers are horizontally offset by specimen type to reduce overlap. Full longitudinal Ct data for all available specimens are shown in [Table S4](#). Lesion-derived specimens include pus, vesicular fluid, skin, and oral swabs.

described in the Methods section. Baseline characteristics were comparable between the two groups, with no statistically significant differences observed in age (median, 33.5 years in both groups; $p=0.871$), days post-symptom onset (4.5 vs. 3.0 days; $p=0.177$), or Charlson Comorbidity Index Score (CCIS; $p=0.721$). Co-infections other than HIV were observed only among the PLWH and are detailed in [Table 5](#), comprising syphilis (4/18, 22.2%), hepatitis B virus (3/18, 16.7%), and hepatitis C virus (3/18, 16.7%); no such co-infections were recorded in the HIV-negative group. Notably, all 18 PLWH were receiving ART at the time of diagnosis, and all maintained preserved immune function with CD4 counts above 200 cells/ μ L (median: 709 cells/ μ L; range: 236–1253). In terms of HIV viral suppression, 77.8% (14/18) had undetectable HIV viral loads (<20 copies/mL). Comparison of virological data showed no statistically significant differences in viral loads across key diagnostic specimens according to HIV status, although several specimen-specific comparisons were limited by small sample sizes ([Table 5](#)). The median Ct values for vesicular fluid were similar between HIV-positive and HIV-negative patients (26.8 vs. 26.7; $p=0.574$). Likewise, no significant differences were observed in pustule Ct values (23.6 vs. 23.4; $p=1.00$) or nasopharyngeal swab Ct values (32.6 vs. 30.7; $p=0.753$).

4. Discussion

This study evaluated the performance of the POKIT Central iiPCR system for detecting MPXV across various clinical specimen types. The analytical sensitivity of iiPCR was similar to that of conventional qPCR, with both platforms demonstrating a consistent LOD at 10^{-1} PFU/mL. Taken together, these data suggest that, within this dataset, iiPCR can provide qPCR-comparable performance in a format that is compatible with decentralized or resource-limited clinical settings. Previous studies have likewise shown that iiPCR-based platforms can provide reliable detection for other viral pathogens, supporting their potential applicability in decentralized diagnostic settings [10–12]. Beyond Mpox, the growing interest in

Table 5. Comparison of viral load and key clinical parameters between HIV-positive and HIV-negative mpox patients.^a

Variable	HIV-positive (n = 18)	HIV-negative (n = 6)	p value ^d
Demographics			
Age (years)	33.5 (21–44)	33.5 (29–45)	0.871
Days PSO	4.5 (1–14)	3.0 (0–9)	0.177
CCIS	0 (0–8)	0.5 (0–1)	0.721
Mpox vaccination status			
Unvaccinated	12 (66.7%)	4 (66.7%)	NA
One dose	5 (27.8%)	1 (16.7%)	
Two doses	1 (5.6%)	1 (16.7%)	
Sex-transmitted disease or co-infection			
AIDS	6 (33.3%)	0 (0.0%)	0.277
Syphilis	4 (22.2%)	0 (0.0%)	0.539
Hepatitis B virus	3 (16.7%)	0 (0.0%)	0.546
Hepatitis C virus	3 (16.7%)	0 (0.0%)	1.00
HIV characteristics^b			
HIV VL >20 copies/mL	4 (22.2%)	NA	NA
HIV VL <20 copies/mL	14 (77.8%)	NA	NA
CD4 count (cells/ μ L)	709 (236–1253)	NA	NA
CD4/CD8 ratio	0.81 (0.19–1.65)	NA	NA
Virological data (Ct values)^c			
Skin/oral swabs	19.2 (n = 1)	22.1 (18.5–25.7)	NA
Pus	23.6 (18.0–31.5)	23.4 (19.0–32.0)	1.00
Vesicular fluid	26.8 (18.3–36.9)	26.7 (22.9–31.0)	0.574
Nasopharyngeal swab	32.6 (25.4–38.0)	30.7 (27.5–37.9)	0.753
Urine	32.6 (n = 1)	12.0 (n = 1) ^e	NA
Plasma	41.0 (n = 1)	NA (n = 0)	NA

Abbreviations: PSO, days post-symptom onset; CCIS, Charlson comorbidity index score; VL, viral load; Ct, cycle threshold; NA, not applicable or insufficient data for statistical calculation.

^aData are presented as no. (%) for categorical variables and median (range) for continuous variables (including Ct values), unless otherwise indicated.

^bData are presented for the 18 PLWH only. All PLWH were receiving antiretroviral therapy at the time of diagnosis.

^cCt value was used to represent the highest detectable viral load (lowest Ct) for comparative analysis. Skin/oral swabs: This category includes skin and oral swabs (e.g. one oral swab from Patient 24). Some Ct values were presented as median only due to limited sample size.

^dContinuous variables were compared using the Mann-Whitney U test, and categorical variables using Fisher's exact test.

^eThe low Ct value in this urine specimen likely resulted from contamination by viral shedding from an active penile lesion during sample collection, rather than intrinsic urinary viral load.

rapid, field-deployable molecular diagnostics reflects a broader shift in outbreak preparedness strategy – one that prioritizes decentralized, sample-to-answer platforms capable of functioning outside conventional laboratory infrastructure. Such approaches have demonstrated utility across a range of emerging pathogens, including dengue and SARS-CoV-2, and are increasingly recognized in global health frameworks as essential tools for early outbreak detection and response [11,12,18].

In practical terms, the iiPCR workflow differs substantially from conventional centralized qPCR. Conventional qPCR typically requires a dedicated molecular laboratory, trained personnel, and a multi-step manual workflow lasting several hours, whereas the POKIT Central system automates extraction, amplification, and detection within a single closed unit, accepts a small input volume (200 μ L), and returns a qualitative result in approximately 85 min, thereby lowering the operator-skill and infrastructure thresholds for decentralized testing. Several practical constraints nonetheless remain: throughput is limited to up to eight samples per run, the readout is qualitative rather than quantitative, per-test consumable costs apply, and confirmatory or quantitative qPCR is still required when a precise viral load is needed or when discordant results arise. Broader, multicenter validation is also needed before routine decentralized deployment.

The analysis of multiple specimen types revealed distinct patterns of MPXV detection that are relevant to sampling strategies. As shown in Figure 2 and Table S4, skin lesion specimens consistently demonstrated lower Ct values, corresponding to higher viral loads, from symptom onset through Day 14. The sustained low Ct values in lesion specimens are consistent with the current understanding of Mpox pathogenesis, in which viral replication predominantly occurs in epidermal tissues, resulting in high viral concentrations in lesion material [2,19]. These findings are consistent with current WHO recommendations that prioritize lesion material for MPXV diagnosis [7]. Ct values across different specimen types are not strictly comparable in a biological sense, because collection method, sampled volume, specimen matrix, and local tissue viral burden differ between lesion and respiratory or systemic specimens; the Ct values therefore reflect relative detectability within each specimen type rather than an absolute, cross-compartment

measure of viral load. In contrast, detection in urine and plasma was intermittent and generally associated with higher Ct values, indicating lower levels of viral DNA in these compartments. Although one urine specimen yielded a low Ct value (12.0), this was likely an artifact due to cross-contamination from a concurrent penile lesion, reinforcing that intrinsic urinary shedding is typically low [8,19]. Nasopharyngeal swabs exhibited a relatively high detection rate (82.6%), although Ct values were generally higher than those observed in lesion specimens. This finding is consistent with previous reports showing lower viral loads in upper respiratory specimens than in skin lesion material [2,19]. Collectively, these observations indicate that while mucosal swabs may provide supplementary diagnostic information, lesion-based sampling remains the most reliable approach for laboratory confirmation of MPXV infection. This specimen-dependent viral load dynamic, characterized by sustained high viral loads in skin lesions and lower or transient detection in body fluids, has also been described in a larger longitudinal cohort of Mpox patients with multiple specimen types sampled over time [20]. Our findings reinforce current recommendations that lesion-based sampling should remain the primary diagnostic approach, with nasopharyngeal swabs serving as complementary specimens when lesions are inaccessible or atypical.

Phylogenetic analysis showed that all isolates sequenced in this study clustered within Clade IIb, consistent with the lineage associated with the 2022–2023 global outbreaks [21,22]. A targeted sequencing strategy focusing on four specific genes, including OPG105, OPG109, OPG118, and OPG151, was applied instead of whole-genome sequencing as a cost-effective alternative. Previous studies have demonstrated that selected conserved gene targets can provide phylogenetic resolution comparable to whole-genome approaches for poxviruses [13]. In the present study, this strategy proved sufficient for clade-level differentiation. These findings are also in agreement with Nextstrain analyses, which indicate limited genetic diversity among currently circulating Clade IIb strains [23]. No Clade I or Clade IIa lineages were identified among the sequenced isolates in this cohort. Given that Clade I viruses have been associated with higher virulence and fatality rates [1], the absence of these lineages during the study period is noteworthy. Nevertheless, the recent emergence of a novel Clade I lineage in Central Africa [24] highlights the need for continued genomic surveillance to enable early detection of potential lineage introductions in Taiwan and other regions [25].

This study further examined the potential influence of HIV co-infection on MPXV viral dynamics. A high prevalence of HIV co-infection was observed in the cohort (75.0%), consistent with the epidemiology of the global Clade IIb outbreak [22,26]. This high proportion likely reflects both the local epidemiology of the Taiwanese Clade IIb outbreak, which has been concentrated among men who have sex with men with a high prevalence of HIV co-infection, and a referral pattern specific to our single tertiary center; it should therefore be regarded as a potential source of selection bias, consistent with the single-center limitation noted below. Previous large-scale studies have reported severe clinical outcomes, prolonged viremia, and increased mortality among HIV-positive individuals with advanced immunosuppression, particularly those with CD4 counts below 200 cells/mm³ [4]. In contrast, all PLWH in the present study were receiving ART, and uniquely, none presented with severe immunosuppression (all CD4 counts >200 cells/μL, median 709 cells/μL). Under these conditions, no statistically significant differences in viral loads, as reflected by Ct values, were observed between HIV-positive and HIV-negative patients across key diagnostic specimen types (Table 5). This observation differs from reports involving patients with advanced HIV (CD4 < 100 cells/μL) who exhibit severe outcomes [4]. Within this small, ART-treated cohort with preserved immunity, MPXV viral dynamics in PLWH did not differ significantly from those in people without HIV; however, given the limited and unbalanced sample size, this should not be interpreted as evidence of equivalence. This observation aligns with recent reports from other settings indicating that Mpox clinical and virological outcomes in ART-treated PLWH may not differ substantially from those in HIV-negative patients [27]. From a diagnostic perspective, our data suggest that, within this ART-treated cohort with preserved immune function, routine Mpox testing algorithms and assay cutoffs did not appear to require modification, although this requires confirmation in larger cohorts.

This study has several limitations. First, the overall sample size was modest ($n=24$), particularly for non-lesion specimen types, which reduced the precision of detection rate estimates and limited the generalizability of the diagnostic yield across diverse clinical and demographic settings; the study was not based on a formal a priori sample-size calculation. In addition, although Mpox vaccination history varied within the cohort, the limited sample size and uneven distribution of vaccine doses precluded a meaningful assessment of vaccine-associated differences. Second, the cohort was drawn from a single center

and comprised exclusively male patients, predominantly men who have sex with men; thus, findings may not be applicable to other gender groups, age ranges, or transmission contexts. Third, no participants with untreated or advanced immunosuppression (e.g. severely low CD4 counts) were included; therefore, the present observations regarding viral load dynamics and diagnostic performance may not be applicable to severely immunocompromised individuals. Fourth, specimen collection and sampling frequency were governed by routine clinical practice rather than a standardized longitudinal protocol, which may introduce sampling bias and limit the capacity to define precise dynamics of viral shedding over time in non-lesional body fluids. Fifth, phylogenetic characterization was based on a targeted four-gene sequencing strategy rather than whole-genome sequencing; while sufficient for clade-level classification, this approach lacks the resolution needed to detect minor variants or to infer fine-scale transmission chains. Finally, although a preliminary isothermal amplification platform (iiPCR) was evaluated, and other isothermal methods (e.g. LAMP) have been described [28], further large-scale, multicenter validation across wider geographic and demographic populations is warranted before advocating widespread deployment.

5. Conclusions

This study shows that the POKKIT Central iiPCR system achieved diagnostic performance comparable to that of the standard CDC qPCR assay for MPXV detection under the conditions evaluated, particularly when applied to skin lesion-derived specimens. In this cohort of individuals with well-controlled HIV infection receiving ART and preserved immune function (median CD4 > 700 cells/ μ L), no clear differences were observed in viral load-based diagnostic performance according to HIV status. These findings apply specifically to ART-treated PLWH with preserved immune function and cannot be extrapolated to individuals with advanced or uncontrolled HIV infection. In addition, phylogenetic analysis based on a targeted four-gene sequencing approach indicated that the circulating strains in this cohort belonged exclusively to Clade IIb. Taken together, these findings suggest that the POKKIT Central iiPCR system may serve as a practical molecular diagnostic option for the rapid detection of MPXV in decentralized clinical settings, particularly when lesion-derived specimens are available.

Acknowledgments

The authors extend their sincere appreciation to all the data contributors, including the authors and their originating laboratories responsible for specimen acquisition, as well as the submitting laboratories for generating the genetic sequences and metadata. This research is built upon data shared *via* GISAID and GenBank, and we acknowledge their valuable contributions.

Ethics approval and consent to participate

The current study was reviewed and approved by the Institutional Review Board of KMH (approval no. KMHIRB-E(I)-20230132). We obtained written informed consent before sample collection.

Authors contributions

CRedit: **Li-Teh Liu**: Conceptualization, Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing; **Chao-Ju Chen**: Investigation, Validation; **Ping-Chang Lin**: Data curation, Formal analysis, Investigation, Methodology; **Shang-Yi Lin**: Formal analysis, Resources, Validation; **Po-Chih Chen**: Investigation, Methodology, Validation; **Chun-Hong Chen**: Methodology; **Ching-Yi Tsai**: Data curation, Formal analysis, Methodology; **Yi-Ching Lin**: Investigation, Validation; **Jih-Jin Tsai**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. All authors confirm that they had full access to all the data in the study and take responsibility for its integrity and the accuracy of the analysis. All authors approved the final version for submission.

Disclosure statement

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. In particular, the authors have no financial, proprietary, or

collaborative relationship with GeneReach Biotech, the manufacturer of the POKKIT Central iiPCR system; the company provided no funding, reagents, or sponsorship and had no role in the study design, data collection, analysis, interpretation, or the decision to publish.

Funding

This work was supported by the Kaohsiung Medical University Hospital, Taiwan (S11417, <https://www.kmuh.org.tw>). The funders had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

ORCID

Li-Teh Liu  <http://orcid.org/0000-0002-7993-7263>
Jih-Jin Tsai  <http://orcid.org/0000-0003-2226-8916>

Data availability statement

The data supporting the conclusions of this article are included within the article and its Supplementary Materials. The 24 MPXV sequences generated in this study have been deposited in GenBank under the accession numbers listed in Table S3. These records are publicly available under accession numbers PX782045–PX782068. The reference sequences used in the phylogenetic analysis are publicly available from GISAID and GenBank.

Declaration of generative AI in scientific writing

During the preparation of this manuscript, the authors used ChatGPT 5.2 and Claude Opus 4.8 as language assistance tools to improve the clarity and readability of the text. All content was critically reviewed and revised by the authors, who take full responsibility for the accuracy, integrity, and final content of the publication.

References

- [1] Bunge EM, Hoet B, Chen L, et al. The changing epidemiology of human monkeypox—a potential threat? A systematic review. *PLoS Negl Trop Dis*. 2022;16(2):e0010141. doi: [10.1371/journal.pntd.0010141](https://doi.org/10.1371/journal.pntd.0010141).
- [2] Adler H, Gould S, Hine P, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect Dis*. 2022;22(8):1153–1162. doi: [10.1016/S1473-3099\(22\)00228-6](https://doi.org/10.1016/S1473-3099(22)00228-6).
- [3] Thornhill JP, Barkati S, Walmsley S, et al. Monkeypox virus infection in humans across 16 Countries – April–June 2022. *N Engl J Med*. 2022;387(8):679–691. doi: [10.1056/NEJMoa2207323](https://doi.org/10.1056/NEJMoa2207323).
- [4] Mitjà O, Alemany A, Marks M, et al. Mpox in people with advanced HIV infection: a global case series. *Lancet*. 2023;401(10380):939–949. doi: [10.1016/S0140-6736\(23\)00273-8](https://doi.org/10.1016/S0140-6736(23)00273-8).
- [5] Tarín-Vicente EJ, Alemany A, Agud-Dios M, et al. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study. *Lancet*. 2022;400(10353):661–669. doi: [10.1016/S0140-6736\(22\)01436-2](https://doi.org/10.1016/S0140-6736(22)01436-2).
- [6] Girometti N, Byrne R, Bracchi M, et al. Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis. *Lancet Infect Dis*. 2022;22(9):1321–1328. doi: [10.1016/S1473-3099\(22\)00411-X](https://doi.org/10.1016/S1473-3099(22)00411-X).
- [7] World Health Organization. Laboratory testing for the monkeypox virus: interim guidance. [23 May 2022; accessed 28 December 2025]. Available from: <https://www.who.int/publications/i/item/WHO-MPX-laboratory-2022.1>.
- [8] Palich R, Burrell S, Monsel G, et al. Viral loads in clinical samples of men with monkeypox virus infection: a French case series. *Lancet Infect Dis*. 2023;23(1):74–80. doi: [10.1016/S1473-3099\(22\)00586-2](https://doi.org/10.1016/S1473-3099(22)00586-2).
- [9] Vivancos-Gallego MJ, Sánchez-Conde M, Rodríguez-Domínguez M, et al. Human monkeypox in people with HIV: transmission, clinical features, and outcome. *Open Forum Infect Dis*. 2022;9(11):ofac557. doi: [10.1093/ofid/ofac557](https://doi.org/10.1093/ofid/ofac557).
- [10] Tsai Y-L, Wang H-C, Lo C-F, et al. Validation of a commercial insulated isothermal PCR-based POKKIT test for rapid and easy detection of white spot syndrome virus infection in *Litopenaeus vannamei*. *PLoS One*. 2014;9(3):e90545. doi: [10.1371/journal.pone.0090545](https://doi.org/10.1371/journal.pone.0090545).
- [11] Tsai J-J, Liu L-T, Lin P-C, et al. Validation of the Pockit dengue virus reagent set for rapid detection of dengue virus in human serum on a field-deployable PCR system. *J Clin Microbiol*. 2018;56(5):e01865–17. doi: [10.1128/JCM.01865-17](https://doi.org/10.1128/JCM.01865-17).

- [12] Liu L-T, Chen P-C, Lin P-C, et al. Defining the clinical utility of dengue RT-iiPCR: stratification by days post-onset and primary vs. secondary infection during the 2023 Taiwan outbreak. *Travel Med Infect Dis*. 2026;69:102945. doi: [10.1016/j.tmaid.2025.102945](https://doi.org/10.1016/j.tmaid.2025.102945).
- [13] Yu Z, Zhang W, Fu H, et al. Genomic analysis of Poxviridae and exploring qualified gene sequences for phylogenetics. *Comput Struct Biotechnol J*. 2021;19:5479–5486. doi: [10.1016/j.csbj.2021.09.031](https://doi.org/10.1016/j.csbj.2021.09.031).
- [14] Li Y, Zhao H, Wilkins K, et al. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods*. 2010;169(1):223–227. doi: [10.1016/j.jviromet.2010.07.012](https://doi.org/10.1016/j.jviromet.2010.07.012).
- [15] Chang H-FG, Tsai Y-L, Tsai C-F, et al. A thermally baffled device for highly stabilized convective PCR. *Biotechnol J*. 2012;7(5):662–666. doi: [10.1002/biot.201100453](https://doi.org/10.1002/biot.201100453).
- [16] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013;30(4):772–780. doi: [10.1093/molbev/mst010](https://doi.org/10.1093/molbev/mst010).
- [17] Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. 2020;37(5):1530–1534. doi: [10.1093/molbev/msaa015](https://doi.org/10.1093/molbev/msaa015).
- [18] Zhu J-H, Tai C-H, Ping C-F, et al. Evaluation of a sample-to-result POKKIT central SARS-CoV-2 PCR system. *Diagnostics (Basel)*. 2023;13(13):2219. doi: [10.3390/diagnostics13132219](https://doi.org/10.3390/diagnostics13132219).
- [19] Yang H, Xie X, Zeng M, et al. Clinical characteristics, viral dynamics, and antibody response of monkeypox virus infections among men with and without HIV infection in Guangzhou, China. *Front Cell Infect Microbiol*. 2024;14:1412753. doi: [10.3389/fcimb.2024.1412753](https://doi.org/10.3389/fcimb.2024.1412753).
- [20] Yang Y, Niu S, Shen C, et al. Longitudinal viral shedding and antibody response characteristics of men with acute infection of monkeypox virus: a prospective cohort study. *Nat Commun*. 2024;15(1):4488. doi: [10.1038/s41467-024-48754-8](https://doi.org/10.1038/s41467-024-48754-8).
- [21] Gigante CM, Korber B, Seabolt MH, et al. Multiple lineages of monkeypox virus detected in the United States, 2021–2022. *Science*. 2022;378(6619):560–565. doi: [10.1126/science.add4153](https://doi.org/10.1126/science.add4153).
- [22] Huo S, Chen Y, Lu R, et al. Development of two multiplex real-time PCR assays for simultaneous detection and differentiation of monkeypox virus IIa, IIb, and I clades and the B.1 lineage. *Biosaf Health*. 2022;4(6):392–398. doi: [10.1016/j.bsheal.2022.10.005](https://doi.org/10.1016/j.bsheal.2022.10.005).
- [23] Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. 2018;34(23):4121–4123. doi: [10.1093/bioinformatics/bty407](https://doi.org/10.1093/bioinformatics/bty407).
- [24] Vakaniaki EH, Kacita C, Kinganda-Lusamaki E, et al. Sustained human outbreak of a new MPXV clade I lineage in eastern Democratic Republic of the Congo. *Nat Med*. 2024;30(10):2791–2795. doi: [10.1038/s41591-024-03130-3](https://doi.org/10.1038/s41591-024-03130-3).
- [25] World Health Organization. Mpox – Democratic Republic of the Congo; 2024 [accessed 28 December 2025]. Available from: <https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON522>.
- [26] Chen W-Y, Cheng S-H, Chen C-P, et al. Risk factors for mpox infection among MSM with STIs in northern Taiwan, 2022–2024. *J Infect Chemother*. 2025;31(8):102750. doi: [10.1016/j.jiac.2025.102750](https://doi.org/10.1016/j.jiac.2025.102750).
- [27] Cordeiro R, Caria J, Sobral D, et al. Mpox in people living with HIV: clinical challenges, preventive strategies and public health implications. *Viruses*. 2025;17(12):1558. doi: [10.3390/v17121558](https://doi.org/10.3390/v17121558).
- [28] Yu C, Zuo L, Miao J, et al. Development of a novel loop-mediated isothermal amplification method for the rapid detection of monkeypox virus infections. *Viruses*. 2022;15(1):84. doi: [10.3390/v15010084](https://doi.org/10.3390/v15010084).