Clinical Microbiology and Infection 32 (2026) 181–184

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.org

Research note

European pilot interlaboratory comparison study on Mpox virus whole genome sequencing

Jonas Fuchs [1], Claire Bertelli [2], Trestan Pillonel [2], Rita Cordeiro [3], Jacques Izopet [4], Christophe Pasquier [4], Kuiama Lewandowski [5], Anastasija Maks [1], Janine Michel [6], Belen Rodriguez-Sanchez [7], Maria Paz Sanches-Seco [8,9], Juan Ledesma [8,9], Daniel Sobral [10], Koen Vercauteren [11], Tessa de Block [11], Antonio Mauro Rezende [11,12], Annika Brinkmann [6], Andreas Nitsche [6], Gilbert Greub [2], Marcus Panning [1,*]

[1] *Institute of Virology, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany*
[2] *Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*
[3] *Emergency Response and Biopreparedness Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal*
[4] *Department of Virology, Toulouse University Hospital, Toulouse, France*
[5] *United Kingdom Health Security Agency, Research & Evaluation Services, Porton Down, United Kingdom*
[6] *Highly Pathogenic Viruses, Centre for Biological Threats and Special Pathogens, German Consultant Laboratory for Poxviruses, Robert Koch Institute, Berlin, Germany*
[7] *Servicio de Microbiología Clínica y Enfermedades Infecciosas Hospital Gregorio Marañón and Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*
[8] *Laboratory of Arboviruses and Imported Viral Diseases, National Center of Microbiology, Institute of Health "Carlos III", Ministry of Science, Innovation and Universities, Madrid, Spain*
[9] *Center for Networked Biomedical Research in Infectious Diseases (Ciberinfec), Institute of Health "Carlos III", Ministry of Science, Innovation and Universities, Madrid, Spain*
[10] *Genomics and Bioinformatics Unit, Department of Infectious Diseases, National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal*
[11] *Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium*
[12] *Research Group of Biotechnology Applied to Pathogens, Instituto René Rachou, Fundação Oswaldo Cruz (Fiocruz), Belo Horizonte, Minas Gerais, Brazil*

ARTICLE INFO

Article history:
Received 12 May 2025
Received in revised form
11 September 2025
Accepted 12 September 2025
Available online 22 September 2025

Editor: C. Moore

Keywords:
Interlaboratory comparison study
Mpox virus
Whole genome sequencing
Poxvirus
Prepardness

ABSTRACT

Objectives: Since 2022, distinct Mpox virus (MPXV) clades have been spreading across different geographic regions, causing a challenging epidemiological situation. Whole genome sequencing (WGS) proved to be instrumental for patient management and global public health. We report a pilot interlaboratory comparison study for MPXV WGS.

Methods: We distributed noninfectious DNA samples, including the main MPXV clades I and II, to eight European laboratories. We included one cowpox (CPXV) sample as a specificity control. Participants were free to choose their WGS pipeline of choice to mimic a real-world scenario and were asked to report on the sequencing pipeline used, average genome coverage, and MPXV species, clade, and subclade assignments.

Results: Seven of the eight invited laboratories reported results back. All participants largely identified the MPXV clades and reported high-quality genomes with minimal variations, specifically for MPXV clade IIb 2022 outbreak strains. However, reconstructed genomes showed high variability for nonclade IIb MPXV strains. The CPXV sample was correctly identified by three laboratories.

Conclusions: Although results for MPXV clade IIb 2022 outbreak strains are reassuring, the inclusion of MPXV clade I and IIa strains highlights pitfalls for targeted sequencing approaches and subsequent bioinformatic analyses. Our findings underscore the need for standardized external quality assessment studies. **Jonas Fuchs, Clin Microbiol Infect 2026;32:181**

* Corresponding author: Marcus Panning, Institute of Virology, Medical Centre – University of Freiburg, Faculty of Medicine, University of Freiburg, Hermann-Herder-Str. 11, 79104 Freiburg, Germany.
E-mail address: marcus.panning@uniklinik-freiburg.de (M. Panning).

https://doi.org/10.1016/j.cmi.2025.09.011
1198-743X/

Introduction

Mpox virus (MPXV), a species within the orthopoxvirus (OPXV) genus, is endemic in several African countries [1]. It is of zoonotic origin and causes Mpox in humans, which usually presents as a febrile illness with a typical papular rash and locoregional adenopathies. MPXV forms two genetically distinct clades. Clade I is endemic in Central Africa and historically associated with more severe disease, and clade II is prevalent in Western Africa and tends to be clinically milder [1]. Since May 2022, MPXV clade IIb has emerged globally in multiple previously unaffected regions, prompting the World Health Organization to declare a Public Health Emergency of International Concern (PHEIC). The number of notified MPXV clade IIb cases has now declined considerably, and the PHEIC was lifted in May 2023, although Mpox cases continue to occur on a sporadic level globally outside endemic countries. Of recent concern, MPXV clade Ib cases are increasingly reported in the Democratic Republic of Congo (DRC) since December 2023, prompting the declaration of a novel PHEIC in August 2024 [2]. Related to this upsurge, travel-associated MPXV clade Ib cases were identified in Europe and the United States since 2024 [3,4]. Furthermore, Kinshasa, the capital of the DRC and a major hub for international travel, is currently facing unprecedented parallel MPXV outbreaks driven by human-to-human transmission of both subclade Ib as well as Ia [5].

During the 2022 Mpox upsurge, diagnostics, including real-time PCR and whole genome sequencing (WGS), were rapidly established by many laboratories worldwide [6]. WGS provides valuable information, e.g. identifying transmission pathways and analysing genetic markers associated with virulence and host adaptation [7,8]. However, international experience and sequencing approaches specifically optimized for non-2022 MPXV outbreak strains are lacking. External quality assessment (EQA) is an integral part of diagnostic laboratories and instrumental in evaluating the quality of laboratory testing [9]. Although EQA for MPXV real-time PCR detection showed promising results [10], EQA for MPXV WGS has not been reported. We anticipated that European laboratories are well-accommodated for genomic surveillance within ongoing outbreaks, but less prepared for other emerging OPXV. To test this hypothesis, we implemented a pilot interlaboratory comparison (ILC) study for MPXV WGS under the auspices of the European Society of Clinical Microbiology and Infectious Diseases study group on Genomic and Molecular Diagnostics.

Methods

We invited several European laboratories to participate in a pilot ILC study. Participants comprised university hospital-based laboratories, national reference centres, and public health institutions. We propagated MPXV representing different clades (MPXV clade Ia; MPXV clade IIa; and MPXV clade IIb 2022 outbreak strains) under biosafety level 3 conditions using VeroE6 cells (Table 1). In addition, we included one cowpox virus (CPXV) isolate as specificity control (sample 2). We produced high-titre virus stocks and subjected them to DNA isolation, sequencing library preparation, and Illumina WGS [11]. To generate a high-quality ground truth, we performed nontargeted Illumina DNA sequencing and mapped sequences to closely related reference genomes for the respective isolates, yielding highly covered consensus sequences with minimal amount of detected variants (Fig. S1a, Supplementary Material). Importantly, sample six had a known, large deletion of 1000 base pairs as shown by the coverage drop between reference positions 11 325 to 12 238, which we subsequently masked in our consensus sequence. We confirmed their phylogenetic relationships in the context of a global MPXV tree (Fig. S1b). We distributed a panel of seven anonymized viral DNA preparations (samples 1–7, Table 1) to the participating laboratories. All samples proved to be noninfectious as determined by cell culture prior to shipment (Supplementary Material). There was no predetermined specification for the WGS pipeline and subsequent bioinformatic analyses to be used by the participants to mimic a real-world diagnostic scenario. Table S2 shows an overview of the instruments and methodologies of individual participants (Supplementary Material). We accompanied samples with a questionnaire asking for information on the sequencing pipeline used, average genome coverage, and species, clade, and subclade assignments. We did not specifically request a deadline for submitting results since this was a pilot ILC. We shipped samples frozen and in accordance with current biosafety regulations.

Results

Eight laboratories from Belgium, France, Germany, Portugal, Spain (2 ×), Switzerland, and the United Kingdom agreed to participate in the pilot ILC. Of these, one subsequently withdrew its participation. All laboratories provided results, including raw sequence files, on a secure website provided by the organizers. Most laboratories employed targeted approaches, whereas centres 2 and 7 used nontargeted sequencing approaches, which required a higher read count per sample (Fig. 1a). As expected, base-quality scores (Phred) depended on the sequencing platform, with 10 to 15 for labs using Oxford Nanopore Technologies (ONT) and around 25 for labs using Illumina platforms (Fig. 1b). Reported mean coverage per centre ranged from >10 × to >1000 × per sample (Fig. 1c). Centres 2 and 5 uniformly reported lower mean coverages across samples. However, centre two was the only participant who reported close to 100% genome recovery across all samples (Fig. 1d). Two of seven laboratories (28%) correctly identified all MPXV samples at the subclade level and correctly reported the CPXV sample 2 (Fig. 1e). Another two laboratories (28%) correctly

Table 1
Description of the interlaboratory comparison panel including cycle threshold-values of cell culture isolates, their origin, and classification

Sample	Cell culture isolate	Ct (orthopoxvirus)	Ct (MPXV)	% mapped reads (nontargeted)	Note	Classification
1	MPXV Freiburg_1	35	27	11.94	2022 outbreak isolate	MPXV/II/B.1
2	Cowpox isolate (GuWi)	30	negative	15.39	cowpox isolate	CPXV
3	MPXV BNI Gabon 1987	31	24	61.72	Non-2022 outbreak isolate	MPXV/Ia
4	MPXV Taï National Park	36	27	16.98	Non-2022 outbreak isolate	MPXV/IIa
5	MPXV Freiburg_2	33	27	12.72	2022 outbreak isolate	MPXV/II/B.1.1
6	MPXV Germany/2022/RKI18	30	25	39.16	2022 outbreak isolate, large deletion	MPXV/II/B.1.15
7	MPXV Germany/2022/RKI01	32	25	36.61	2022 outbreak isolate	MPXV/II/B.1.1

CPXV, cowpoxvirus; Ct, cycle threshold; MPXV, Mpoxvirus.

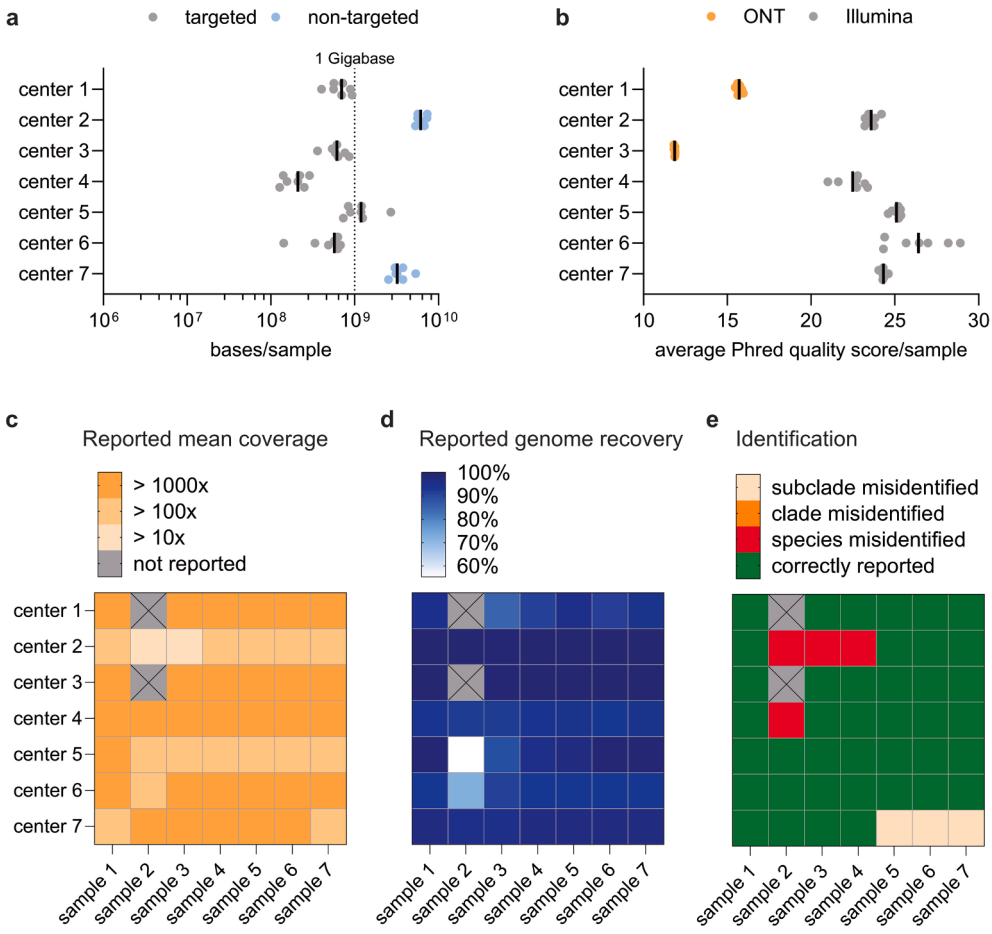

Fig. 1. Sequence quality and self-reporting. Statistics for raw read data was generated with SeqKit. The (a) average produced bases per sample and centre and (b) base-quality Phred score are displayed. The self-reporting was evaluated and displayed as heatmaps for (c) mean coverage, (d) genome recovery, and (e) species, clade, and subclade identification. Shown are the median (a) or mean (b).

reported all MPXV samples at the subclade level but did not report data for the CPXV sample. Only three laboratories (42%) correctly identified CPXV sample 2, two misidentified the species, and another two did not report data.

We evaluated the quality of reporting by comparing the reported consensus genomes to our quality-controlled consensus sequences (Fig. S1a). Multiple sequence alignment showed little variation for MPXV clade IIb 2022 outbreak strains (Fig. S2a). Most differences were masked regions and missing information at the 3′ and 5′ prime ends, likely due to terminal repeats in the reference, which are sometimes not targeted in amplicon sequencing or can cause common mapping quality issues in bioinformatic pipelines. Importantly, only centres 1 and 7 did not detect the deletion of sample 6 (MPXV/II), and instead of masking or deleting the respective region, they included reference nucleotide information into their consensus sequences. For the MPXV nonoutbreak 2022 strains and the CPXV genome, reconstructions were highly variable, as shown by the high number of masked regions and variations compared with our ground truth consensus genome. Therefore, we calculated the genome recovery defined as nucleotide information over the ungapped reference sequence irrespective of mismatches, and compared it with the reported genome recovery (Fig. S2b). Our calculation showed little to no difference from the reported recovery. However, a detailed analysis of the percentage of masked regions and the number of detected variants compared with our reference showed a high percentage of masked regions and a high number of nucleotide differences for sample 2 (CPXV), sample 3 (MPXV/Ia), and sample 4 (MPXV/IIa) (Fig. S2c, d). The lower overall quality of the reported consensus sequences for samples 2 to 4 likely originated from targeted sequencing approaches that are not designed to recover all MPXV strains and CPXV. This resulted in an overall lower genome recovery and a higher number of masked regions. Second, bioinformatic analyses can also have pitfalls. For example, the usage of a distantly related reference sequence can result in a high number of single nucleotide polymorphisms (SNPs) if, instead of masking low coverage regions, the reference nucleotide information is incorporated into the consensus sequence.

Discussion

We report the results of the first pilot ILC trial for MPXV WGS. High-quality genome reconstruction is of particular importance for outbreak cluster analysis and correct classification within the global phylogeny [8]. In this context, our pilot ILC trial shows overall reassuring results for MPXV clade IIb 2022 outbreak strains for which high-quality genomes with minimal variations were reported for all participants. However, the included Ia, IIa, and the CPXV strains reveal pitfalls for targeted sequencing approaches

and/or subsequent bioinformatic analyses. This demonstrates the critical role of EQA programmes to explore preparedness for new or emerging pathogens and strains [12]. Our study highlights the need for optimized approaches allowing accurate OPXV genomic surveillance beyond the MPXV clade IIb 2022 outbreak strain. This is of particular relevance in the backdrop of unprecedented MPXV spreading in DRC and the broader central African region [13], which is characterized by sustained human transmission of multiple MPXV subclades in parallel [14]. In addition, there are rare reports on cocirculation of different OPXV species [15]. Limitations of our study include the small number of participating laboratories from European countries only, and its implementation by a non-ISO (International Organization for Standardization) 17043 accredited EQA provider. Follow-up EQAs, including larger panels and more laboratories worldwide, are therefore highly desirable and will strengthen future pandemic preparedness.

CRediT authorship contribution statement

Jonas Fuchs, Gilbert Greub, and Marcus Panning: Conception and design of the study. Jonas Fuchs, Annika Brinkmann, and Marcus Panning: Analysis and interpretation of the data. Acquisition of data: All authors. Drafting or revision of the article: All authors. Final approval of the manuscript: All authors.

Transparency declaration

Potential conflict of interest

All authors declare no conflicts of interest.

Financial report

The European Society of Clinical Microbiology and Infectious Diseases study group on Genomic and Molecular Diagnostics (ESGMD) supported this study. Institute of Tropical Medicine Structurele Onderzoeksfinanciering (ITM's SOFI) programme supported by the Flemish Government, Science & Innovation cosupported this study. This study was cofunded by the European Union project "Sustainable use and integration of enhanced infrastructure into routine genome-based surveillance and outbreak investigation activities in Portugal"—GENEO (project number 101113460) on behalf of the EU4H programme (EU4H-2022-DGA-MS-IBA-01-02).

Acknowledgements

We are grateful to Prof Dr Georg Kochs, Freiburg, Germany for providing Mpox virus (MPXV) isolates and to Dr Toni Rieger, Hamburg, Germany for providing the MPXV/Ia isolate. Current MPXV/IIA and the orthopoxvirus isolates were kindly provided by German Consultant Laboratory for Poxviruses, Berlin, Germany.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.09.011.

References

[1] Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox-A potential threat? A systematic review. PLOS Negl Trop Dis 2022;16:e0010141. https://doi.org/10.1371/journal.pntd.0010141.
[2] World Health Organization (WHO). Emergency Committee. WHO Director-General declares mpox outbreak a public health emergency of international concern. Geneva: WHO; 2024. Available from: https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern
[3] Treutiger CJ, Filen F, Rehn M, Aarum J, Jacks A, Gisslen M, et al. First case of mpox with monkeypox virus clade Ib outside Africa in a returning traveller, Sweden, August 2024: public health measures. Euro Surveill 2024;29:2400740. https://doi.org/10.2807/1560-7917.ES.2024.29.48.2400740.
[4] Levy V, Branzuela A, Hsieh K, Getabecha S, Berumen R, Saadeh K, et al. First Clade Ib monkeypox virus infection reported in the Americas—California, November 2024. MMWR Morb Mortal Wkly Rep 2025;74:44–9. https://doi.org/10.15585/mmwr.mm7404a1.
[5] Bangwen E, Diavita R, De Vos E, Vakaniaki EH, Nundu SS, Mutombo A, et al. Suspected and confirmed mpox cases in DR Congo: A retrospective analysis of national epidemiological and laboratory surveillance data, 2010-23. Lancet 2025;405:408–19. https://doi.org/10.1016/S0140-6736(24)02669-2.
[6] Michel J, Targosz A, Rinner T, Bourquain D, Brinkmann A, Sacks JA, et al. Euro Surveill 2022;27:2200816. https://doi.org/10.2807/1560-7917.ES.2022.27.45.2200816.
[7] Hammerschlag Y, MacLeod G, Papadakis G, Adan Sanchez A, Druce J, Taiaroa G, et al. Euro Surveill 2022;27:2200411. https://doi.org/10.2807/1560-7917.ES.2022.27.22.2200411.
[8] Kabir F, Plaisance E, Portman A, Marfo A, Cirrincione K, Silva D, et al. J Infect Dis 2024;229:S163–71. https://doi.org/10.1093/infdis/jiad504.
[9] Benschop KSM, Staines H, McKloud E, McCulloch E, Montgomery D, Sutton G, et al. J Med Virol 2024;96:e70103. https://doi.org/10.1002/jmv.70103.
[10] Ehmann R, Donoso Mantke O, McCulloch E, Yousef A, Ricketts A, Staines H, et al. J Clin Virol 2024;175:105741. https://doi.org/10.1016/j.jcv.2024.105741.
[11] Fuchs J, Nekrutenko A, Kohl A-K, Technau-Hafsi K, Hornuß D, Rieg S, et al. Roloff. 2022. Available from: https://virological.org/t/travel-associated-monkeypox-virus-genomes-from-two-german-patients-and-of-a-derived-virus-isolate-all-closely-related-to-a-us-sequence-2022/844
[12] Wegner F, Roloff T, Huber M, Cordey S, Ramette A, Gerth Y, et al. J Clin Microbiol 2022;60:e0169821. https://doi.org/10.1128/JCM.01698-21.
[13] Yinda CK, Koukouikila-Koussounda F, Mayengue PI, Elenga RG, Greene B, Ochwoto M, et al. Lancet 2024;404:1815–22. https://doi.org/10.1016/S0140-6736(24)02188-3.
[14] Wawina-Bokalanga T, Akil-Bandali P, Kinganda-Lusamaki E, Lokilo E, Jansen D, Amuri-Aziza A, et al. Euro Surveill 2024;29:2400592. https://doi.org/10.2807/1560-7917.ES.2024.29.38.2400592.
[15] Kalonji T, Malembi E, Matela JP, Likafi T, Kinganda-Lusamaki E, Vakaniaki EH, et al. Emerg Infect Dis 2024;30:761–5. https://doi.org/10.3201/eid3004.231413.