Ongoing MPXV infections in the Berlin MSM cohort – Multiplex PCR for differentiation between MPXV clade I; Ib and clade II

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AIM

Infections with MPXV declined sharply after the pandemic in summer 2022 but have continued to occur sporadically in the Berlin area with its large MSM scene to this day. With the increased occurrence of variant Ib in the Democratic Republic of Congo and the spread in Central Africa, a worldwide spread is likely. Until today only two imported cases of MPXV calde Ib were reported in Germany. For this there is a need for the differentiation of MPXV strains in the routine laboratory.
We adapted a multiplex PCR into our routine diagnostics to differentiate MPXV clade I, clade Ib and clade II.

METHODS

The standard screening for MPXV in our multiplex PCR includes a generic MPXV-specific probe, an OPXV-specific probe, a cell control (ß-globin) and an internal control. In order to differentiate between MPXV clade I and II, we tested the PCR according to Shuting Huo et al. 2022, which differentiates OPXV generic, clade I and clade II a/b as a 3-plex PCR. As a further method and for further differentiation in case of positive screening, the methods of Y. Li et al. 2010 (MPXV generic, west african (clade II), congo basin (clade I)) and Leonard Schuele et al. 2024 (MPXV clade Ib) were combined into a multiplex PCR (4-plex). 16 randomly selected positive samples from the period Nov. 2022 to Nov. 2024 were retested with both multiplex PCR methods.

RESULTS

All samples were confirmed positive with the generic MPXV/OPXV probes and were positive for clade II (West African specific). No clade I or clade Ib positive sample was detected, only the positive controls were detected here. 30 positive routine samples from the end of November 2024 to the end of March 2025 also all tested positive as clade II.

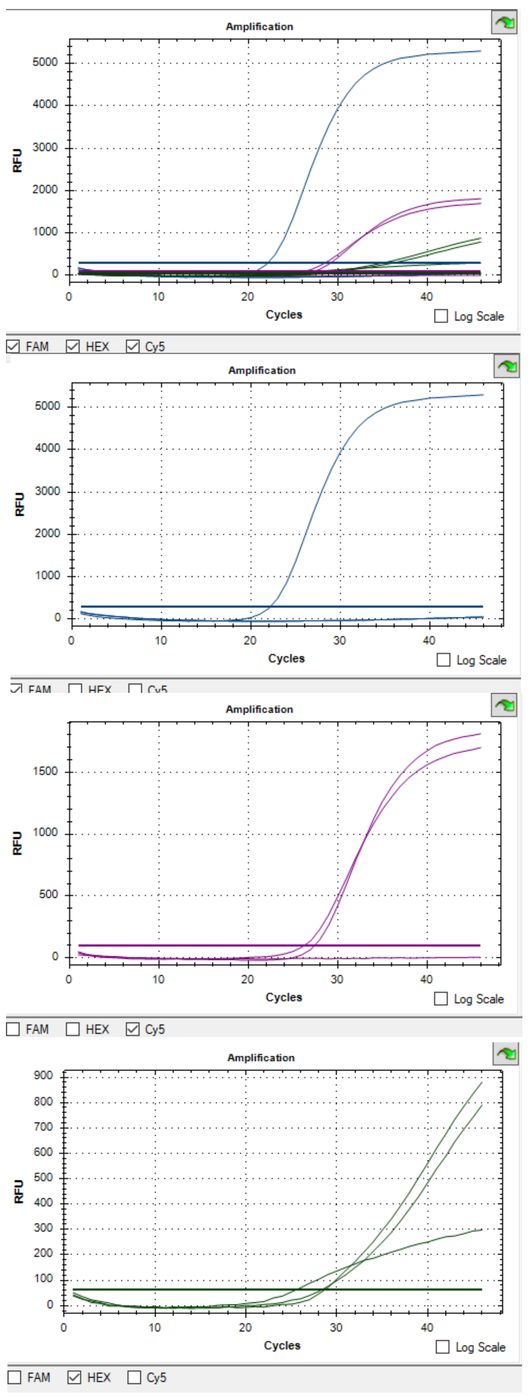

Fig. 2: Multiplex-PCR (Shuting et al.) with clade 1b differentiation

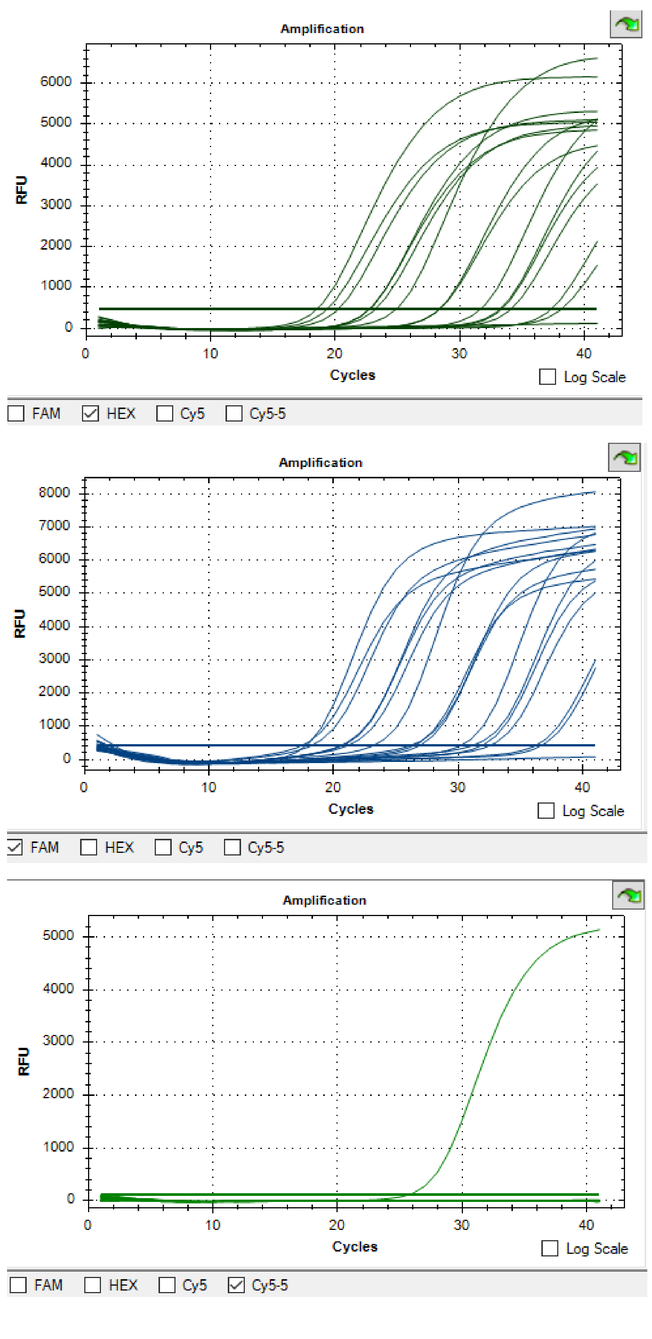

Fig. 2: Multiplex-PCR (Li et al. and Schuerle et al.) with clade 1b differentiation

Tab. 1: Primer and probes MPXV clade differentiation

	Shuting Huo et al. 2022 Biosafety and Health 4 (2022) 392–398	https://doi.org/10.1016/j.bsheal.2022.10.005
Mpox for	ATCCTCTCTCATTGATTTTTCGCGGGA	
Mpox rev	TGGAGAAGCGAGAAGTTAATAAAGC	
Probe 1 015c	CY5-TCGTCGGAACTGTACACCATAGTAC-BHQ2	Clade I Congo
Probe 2 UK P2	FAM-TCGTTGGAGCTGTAAACCATAGCAC-BHQ1	Clade IIa/b West Afrika
Probe 3 VACV	yak-TCGTCGGAGCTGTACACCATAGCAC-BHQ	OPXV

Tab. 2: Primer and probes MPXV clade and clade 1b differentiation

	Y. Li et al. / Journal of Virological Methods 169 (2010) 223–227
MPXV West African specific (G2R WA)	
G2R WA for	5′-CACACCGTCTCTTCCACAGA
G2R WA rev	5′-GATACAGGTTAATTTCCACATCG
G2R WA Probe	5′yy-AACCCGTCGTAACCAGCAATACATTT-3′BHQ
MPXV Congo Basin specific (C3L)	
C3L for	5′-TGTCTACCTGGATACAGAAAGC
C3L rev	5′-GGCATCTCCGTTTAATACATTGAT
C3L probe	5′Cy5-CCCATATATGCTAAATGTACCGGTACCGGA-3′BHQ
MPXV generic (G2R G)	
G2R_G for	5′-GGAAAATGTAAAGACAACGAATACAG
G2R_G rev	5′-GCTATCACATAATCTGGAAGCGTA
G2R_G probe	5′FAM-AAGCCGTAATCTATGTTGTCTATCGTGTCC-3′BHQ1
MPOX clade Ib	**Leonard Schuele et al.** ,Euro Surveill. 2024;29(32):pii=2400486. https://doi.org/10.2807/1560-7917.ES.2024.29.32.2400486
dD14 -16 for	AAGACTTCCAAACTTAATCACTCCT
dD14 -16 rev	GTTTGATATAGGATGTGGACATTT
dD14-16 probe	Cy5.5-ATATTCAGGCGCATATCCACCCACGT-BHQ-3

CONCLUSIONS

Clade I has apparently not yet arrived on the Berlin MSM scene. As soon as clade Ib spreads further, these multiplex PCR methods are well suited to differentiate infections with the far more virulent and more lethal MPXV clade I or Ib. If the incidence is low, the methods appear to be useful as add-on methods, and possibly also as a screening method if the incidence is higher.