

An in silico analysis of PCR-based monkeypox virus detection assays: a case study for ongoing clinical surveillance

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Abstract

The 2022 global mpox outbreak swiftly introduced unforeseen diversity in the monkeypox virus (MPXV) population resulting in numerous Clade IIb sublineages. This propagation of new MPXV mutations warrants thorough re-investigation of previously recommended or validated primers designed to target MPXV genomes. In this study, we explored 18 PCR primer sets and examined their binding specificity against 5,210 MPXV genomes, representing all established MPXV lineages. Our results indicated that only five primer sets resulted in almost all perfect matches against the targeted MPXV lineages, and the remaining primer sets all contained 1-2 mismatches against almost all MPXV lineages. We further investigated mismatch primer-genome pairs and revealed that some of the primers overlaid with poorly sequenced and assembled regions of the MPXV genomes that are consistent across multiple lineages. However, we identified 173 99% genome-wide conserved regions across all 5,210 MPXV genomes representing 30 lineages/clades with at least 80% lineage-specific consensus for future primer development and primer binding evaluation. This exercise is crucial to ensure current detection schemes are robust and serves as a framework for primer evaluation in clinical testing development for other infectious diseases.

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