CRISPR-Cas mediated detection of Monkeypox Virus

Authors:
Low SJ*[1,2], O'Neill M*[1], Kerry W[1], Krysiak M[2], Papadakis G[3], Prestedge J[2,3], Whitehead W L[1], Prestedge[2,3], Williams L[1], Cooney P J[1], Tran T[3], Lim K C[2,3], Caly L[2,3], Towns M J[4,5], Bradshaw S C[4,5], Fairley C[4,5], Chow PF E[4,5,6], Chen Y M[4,5], Pellegrini M[1], Pasricha S[1,2 #], Williamson D[1,2,3 #]

[1] Walter and Eliza Hall Institute of Medical Research, [2] The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, [3] Victorian Infectious Diseases Reference Laboratory, [4]Melbourne Sexual Health Centre, Alfred Health, [5]Faculty of Medicine, Nursing and Health Sciences, Monash University, [6]Melbourne School of Population and Global Health, The University of Melbourne

Background: The 2022 outbreak of Monkeypox (MPX) presents a major public health concern. Previously considered rare and endemic to Africa, the zoonotic monkeypox virus (MPV) has spread to over 110 countries, demanding effective disease management and surveillance. Viral transmission occurs during close contact, largely amongst men who have sex with men. Current diagnostics rely on expensive equipment and specialized technicians. Therefore, using CRISPR-Cas technology, the objective of this study was to develop a rapid point of care assay for MPXV detection.

Methods: A portable isothermal amplification, CRISPR/Cas12a- based assay was developed for the detection of MPV. A panel of 22 primer/guide sets were interrogated for sensitivity and specificity using a fluorescence-based readout and lateral flow strips. The three best performing primer/guide combinations were selected, and the limit of detection (LOD) determined using serially diluted MPV gDNA. Assay specificity was confirmed using a panel of viral pathogens. Finally, a blind concordance study of 185 clinical samples was performed, comparing assay results to 'gold-standard' real-time PCR (RT-PCR). A sample was defined as MPV-positive by fluorescence readout if the fluorescence signal exceeded the threshold of 10 standard deviations above the mean fluorescence of the no template control. Lateral flow strips were interpreted visually and computationally based on the presence/absence of test bands.

Results: With a run duration of approximately 45 mins, the developed assay has a LOD of 1 copy/µL and 100% specificity against tested viral pathogens. Blinded concordance testing of 185 clinical samples resulted in 100% sensitivity and 99.36% specificity between the fluorescence-based readout and RT-PCR.

Conclusion: Overall, we have developed a novel point-of-care assay for the diagnosis of MPV. It's demonstrated useability presents a potential solution to MPV detection in low-income and remote settings, as well as a means of community based on-site testing.

Disclosure of Interest Statement: The authors declare no competing interest