CRISPR-Cas Mediated Differential Diagnoses of Three Genital Ulcerative Diseases – Syphilis, Herpes and Mpox

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Background:
Genital ulcerative diseases (GUDs), caused by *Treponema pallidum*, herpes simplex virus (HSV), and mpox virus (MPXV), present overlapping clinical features, complicating diagnosis. Syndromic assessment often guides sequential single-pathogen testing based on clinical suspicion, which is unreliable and can delay appropriate treatment. Current gold-standard diagnostics – serology for *T. pallidum* and PCR for HSV and MPXV – are not integrated within a single multiplex framework, limiting differential diagnosis. 10% of primary syphilis cases are seronegative, and molecular detection of *T. pallidum* from primary lesions remains restricted to centralised laboratories. At the point-of-care, *T. pallidum* tests are primarily serological, with no approved tests for HSV and MPXV, let alone simultaneous detection of all three pathogens. This study developed a world-first, rapid triplex CRISPR-Cas assay to address this diagnostic gap.

Methods:
Target-specific genomic regions were amplified using recombinase polymerase amplification (RPA), followed by CRISPR-mediated detection using distinct Cas enzymes and fluorescent reporters: LbaCas12a (FAM) for *T, pallidum*, LwCas13a (HEX) for HSV and PsmCas13b (Cy5) for MPXV, each paired with pathogen-specific guide RNAs. Analytical sensitivity was evaluated using serial dilutions of target DNA/RNA to determine limits-of-detection (LoDs). Assay specificity was evaluated using clinically relevant bacterial, protozoan and viral pathogens. Clinical evaluation was conducted in a blinded concordance study comparing the assay to real-time PCR and previously developed point-of-care tests.

Results:
The assay was performed within 60 minutes at 37°C, achieving LoDs of 10 copies/µL for *T. pallidum* and HSV and 70 copies/µL for MPXV, with 100% specificity against

non-target pathogens. Assay performance on clinical samples was comparable to real-time PCR.

Conclusion:
A novel CRISPR-based triplex assay was developed, enabling simultaneous differential diagnosis of all three GUDs. The assay has potential to mitigate GUD misdiagnosis in clinical and low-resource settings. Future work will entail translating the assay to a point-of-care platform and undertaking clinical implementation studies.

Disclosure of Interest Statement:
The authors declare no competing interest.

Key Words:
Diagnostics, Sexually Transmitted Infections, Biomedical sciences, Clinical sciences, Reproductive health