# EXTENDING THE CAPABILITY OF CRISPR-DIAGNOSTICS TO DEVELOP A POINT-OF-CARE-TEST FOR SYPHILIS AND HERPES SIMPLEX VIRUS DETECTION

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**Background:**
Left untreated, syphilis results in substantial disease and morbidity, and is often misdiagnosed as herpes simplex virus (HSV) due to similar clinical presentations. Annually, syphilis, caused by *Treponema pallidum* (TP) leads to over 300,000 avoidable stillbirths and neonatal deaths worldwide. However, early diagnosis in pregnancy can prevent almost 100% of mother-to-child transmission. As current diagnostics rely largely on centralised laboratory testing, we aimed to develop a point-of-care test (PoCT) to detect and distinguish between TP and HSV infections.

**Method:**
A multiplexed CRISPR-Cas test with isothermal pre-amplification (TP-HSV-CRISPR) was developed for the detection of TP and HSV using Cas12a and Cas13a, respectively, targeting two genes for each pathogen. Where relevant, reflex testing is performed to distinguish HSV-1 from HSV-2. Candidate primer and guide RNA sets were designed using an in-house bioinformatic pipeline, and multiplexed sets producing the strongest signal using a fluorescence-based readout were selected. TP-HSV-CRISPR was evaluated for analytical sensitivity and specificity on a portable isothermal fluorimeter using serial dilutions of target gDNA and gDNA from a panel of viral and bacterial pathogens. Additionally, clinical validation was performed on gDNA from 400 specimens obtained from multiple body sites.

**Results:**
Fully integrated onto a PoC device, the time from sample to answer is approximately 35 minutes. The test can detect single copies per µL of bacterial gDNA and displays 100% analytical specificity against a panel of relevant bacterial and viral pathogens. Results from the clinical validation align with the World Health Organisation target product profile for syphilis PoCTs.

**Conclusion:**
We have developed a novel CRISPR-based multiplexed PoCT for the detection of TP and HSV. The useability of the test presents a potential solution to increase testing access across high-risk communities and settings, anticipated to mitigate the risks of syphilis being underdiagnosed.

**Disclosure of Interest Statement:**
Authors declare no competing interest.