Rapid viral load monitoring of HIV-1 using CRISPR technology

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Background:
Over 31 million people receiving antiretroviral therapy for HIV require viral load monitoring every 3–6 months to ensure viral control. Current standard approaches rely on real-time PCR performed in centralised laboratories, requiring specialised equipment, and turnaround times of days to weeks. This limits accessibility, especially in resource-limited settings. These logistical barriers, alongside emotional burdens of delayed results, contribute to gaps in sustained viral load suppression. Emerging CRISPR-Cas technologies offer a rapid and highly specific alternative. This study aimed to develop a novel CRISPR-based test capable of distinguishing unsuppressed (>200 copies/mL) from suppressed (<200 copies/mL) viral loads.

Methods:
Isothermal amplification and CRISPR-Cas detection were used to determine viral load in an immortalized human T-cell line with an integrated HIV-1 provirus (J-Lat). In-house bioinformatic pipelines were used to design primer/guide sets targeting the HIV *gag* region. Nine sets were interrogated by serially diluting J-Lats to determine the limit of detection (LoD). Two best performing sets were tested against clinically relevant pathogens to examine specificity. Sensitivity was compared to real-time PCR. The assay was validated using HIV-1 positive plasma samples.

Results:
The assay was performed at 37ºC within 60 minutes. The best performing primer/guide sets demonstrated an LoD of 60 copies/µL of HIV RNA. A 100% specificity was observed when tested against clinically relevant pathogens. The assay was successfully correlated with standard of care, real-time PCR. Finally, the assay was able to detect viral load in a range of subtypes in clinical samples.

Conclusion:
We demonstrate the feasibility of a novel CRISPR-based, rapid viral load monitoring assay. Beyond improving user experience and care, particularly in resource-limited settings, accessible viral load testing may enhance participant safety and engagement in clinical trials. Future efforts will focus on transferring the assay to a

portable device, working toward decentralised implementation in global HIV cure research.

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