## REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

# Amplification Free Digital Crispr-cas13a For Absolute Quantification Of Hiv-1 Rna

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Acquired immunodeficiency syndrome (AIDS) has led to millions of deaths worldwide since its origin. Early awareness of infection enables treatment for exposed patients and prevents further transmission. While RT-PCR has been the gold standard for the quantification of HIV-1, external references are needed for quantification. Digital assays concepts could overcome this issue where absolute quantification without external references could be achieved. In addition, the recent discovery of Cas proteins has led to the emergence of CRISPR-mediated biosensors. This project aims to develop a digital CRISPR-Cas13a system for amplification-free absolute quantification of viral particles HIV-1. This system would offer a specific and sensitive test for quantifying HIV-1 for early awareness of infections. The objectives of this paper are three-fold. First, testing the digital assay with synthesized HIV-1 RNA and confirming the absolute quantification capability of the system. Second, with synthesized plasma extracted RNA to evaluate the effect of plasma background on the quantification. Third, testing with clinical samples to evaluate the system performance for testing the real samples. This system not only improves the performance of viral load quantification of HIV-1 RNA by utilizing the concept of digital assay but also achieves an amplification-free CRISPR. Most CRISPR assays need an amplification step which complicates the testing process. While this study has been conducted using HIV-1 RNAs as a target, this system could be readily utilized for other virus and pathogen detection by changing the crRNA design. This system's sample-to-answer time, including the sample preparation, would be less than 1 hour, which could be crucial for fast and accurate pathogen testing in the field.

In this work, a commercial track-etched polycarbonate (PCTE) membrane was utilized for digitalization. To fill the membrane reliably, we developed a stamping technique. The membrane was sandwiched between a polymethyl methacrylate (PMMA) holder and double-sided tape in this technique. Afterward, the membrane was filled by pressing the stamp system on top of the assay solution containing HIV-1 RNA, Cas13a, and crRNA complex, fluorophore quencher (FQ)-labeled single-stranded RNA reporters. To test the quantitative performance of our system, a series of synthetic HIV-1 RNA dilutions from 10aM to 1pM were quantified. In the range from 50aM to 1pM, the measured concentrations correspond very well to the expected concentrations (Fig. 1).

Fig.1 Comparison of measured RNA concentrations to the expected concentrations

In the next step, we evaluated the system performance for quantifying HIV RNAs in a plasma background. Fig. 2 shows the measured concentration for a series of dilutions from 1000 to 3000 copies/ml of plasma.

Fig.2 Measured concentration for serially diluted spiked HIV-1 RNA samples and one no-target control sample

In conclusion, we presented a membrane-based digital CRISPR-Cas13a system for amplification-free absolute quantification of viral particles HIV-1. The quantitative performance of our system was confirmed when a series of synthetic HIV-1 RNA was quantified accurately from 50 aM to 1 pM. Finally, a test with spiked samples showed that our system is capable of quantifying spiked samples with the resolution of 1000 copies per 1 ml of plasma.

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