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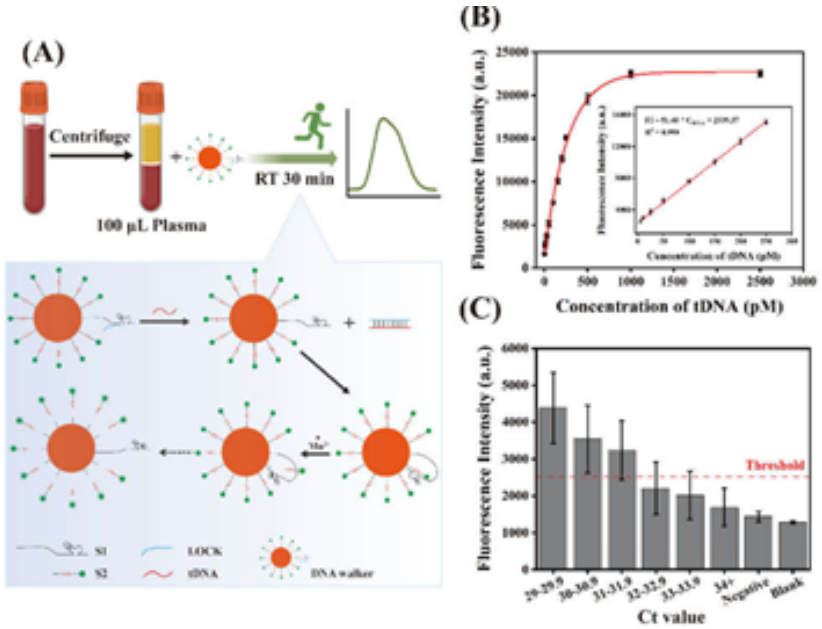
A robust DNA walker for simplified, rapid and sensitive detection of HBV DNA in blood donors

ABSTRACT

Background: Although preventive vaccine is available, HBV infection continues to be a global health challenge, especially in rapid and accurate screening in resource-limited regions due to underdeveloped infrastructures.

Aim: This study aimed to address the pressing need for efficient and user-friendly diagnostic technologies in resource-limited regions as a point-of-care testing (POCT).

Method: We developed a self-powered DNA walker system integrating AuNPs and DNA strands for direct detection of HBV DNA in the blood.



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The lock strand (LOCK) was first hybridized with the walking strand (S1) to a S1-LOCK duplex, which blocked the DNAzyme activity on S1. Then the S1-LOCK and fluorophore-labeled substrate strand (S2) were modified onto AuNP surfaces, constructed a DNA walker system. Upon the target HBV DNA (tDNA) presence, strand displacement reactions between tDNA and S1-LOCK were triggered to release DNAzyme, which subsequently mediated cleavage of S2 with Mn2+ cofactors autonomously and progressively, generating amplified fluorescent signals.

Figure 1. (A)Principle of HBV DNA detection by DNA walker in the plasma. (B) Response fluorescence intensity at series concentrations of target. Inset: Linear calibration range (5–250 PM). (C) Clinical performance evaluation using HBV DNA-positive (n=22) and negative (n=3) plasma samples.

Result: This DNA walker system enabled rapid detection within 30 minutes at room temperature, demonstrating a sensitivi- ty of 3.6 PM with a linear range of 5-250 pM (R2 = 0.999, Fig. 1B). Clinical validation using 22 HBsAg-/HBV DNA+ (OBI) plasma samples from blood donors revealed a strong negative correlation (r = -0.86) between fluores- cence signals and qPCR Ct values, with a detection limit of Ct <32 (approximately 20,000 IU/mL, Fig. 1C), that con- sistent with the WHO-recommended viral load threshold for initiating nucleos(t)ide analogue therapy.

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