A novel aptamer-based colorimetric test for point-of-care malaria diagnosis

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Global efforts against malaria are in need for better point-of-care diagnostic methods to combat the disease in resource-poor settings. Antibody-based rapid diagnostic tests (RDTs) have greatly helped such efforts but still incur issues with cost and stability. Aptamers are single-stranded oligonucleotide sequences which specifically bind to targets and show potential for incorporation into RDT technologies due to their specificity, affordability, and stability. We present a new aptamer-tethered enzyme capture (APTEC) test which uses aptamers specific for the malaria biomarker \textit{Plasmodium falciparum} lactate dehydrogenase (\textit{Pf}LDH). The test works by tethering \textit{Pf}LDH from blood samples then harnesses the enzyme’s intrinsic activity to produce a colorimetric response via the reduction of a tetrazolium salt. In a 96-well plate format, APTEC successfully detected recombinant \textit{Pf}LDH (limit of detection = 4.9 ngmL\(^{-1}\)) and diagnosed malaria patient blood samples with a sensitivity consistent with commercially available antibody-based RDTs. APTEC was also found to be reusable with no significant decrease in response after six regenerations. Aptamers were subsequently immobilized onto magnetic beads and could tether \textit{Pf}LDH in the APTEC assay as before. The beads were incorporated into 3D-printed fluidic devices to improve the usability of the APTEC assay. The aptamers were also immobilized onto paper by biofunctionalizing microbeads which are large enough to be trapped in the cellulose matrix. With further development, the fluidic-based and paper-based adaptations of the APTEC assay could produce highly cost-effective and reliable point-of-care malaria diagnostics for the developing world.

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**Figure 1.** APTEC assay which detects the malaria biomarker \textit{Pf}LDH. Aptamers capture the antigen, non-bound analytes are washed away, and then the enzymatic activity of \textit{Pf}LDH is coupled to the reduction of a tetrazolium salt to give a blue colorimetric response.

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