IVD.08 - Point-of-Care Testing Based on RNA Aptamers: A New Strategy for Yellow Fever Rapid Diagnostic

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Introduction: The resurgence of Yellow Fever poses a serious threat to human health because of its clinical severity and high potential for dissemination. Thus, control strategies aimed at the early detection of virus circulation in rural and urban areas are crucial for monitoring areas at risk. Point-of-Care (POC) diagnostics are used for on-site testing, leading to accelerated clinical and diagnostic judgment. Side flow devices are of special interest because of their convenience and ease of use. One of the most promising techniques for the development of rapid diagnostic tests is the use of aptamers, which are single-stranded DNA or RNA molecules that can adopt specific three-dimensional conformations to specifically link targets of interest.

Objective: To develop POC's based on RNA aptamers for the rapid diagnostic of wild Yellow Fever for virus detection in human and non-human primates.

Methodology: For the *in vitro* selection of aptamers, SELEX will be performed from an RNA library with a random internal region composed of 40 nucleotides. The selection of the aptamers will be performed in microwells using envelope protein of the Yellow Fever domain III envelope as target. Negative selections will be performed with domain III proteins from the envelope Dengue type 2 and Zika. The progress of the selection will be monitored by screening through RT-PCR, also evaluating the effectiveness of protein immobilization. When the library has selective enrichment, cloning and sequencing will aid in the identification of specific clones. For the construction of the POC's the methodology described by Ahmad Raston, Nguyen, & Gu, 2017. After the construction of the POCs, the specificity of the test will be evaluated, where the aptamers conjugated to AuNPs will be incubated with samples of dengue type 1, type 2 and type 3 dengue positive sera, in dose-dependent conditions.

Results: Ten rounds of selection were performed. Negative selection steps using protein DIII-type EDIII 2 and Zika EDIII proteins were introduced in cycles 5 and 10. When verifying the enrichment of the selection by RT-PCR, 100% enrichment was verified when comparing round 5 with the initial library, mainly due to the inclusion of negative selection in this round, and the enrichment progressively progressed until round 10, reaching an approximate total of 200% enrichment. The efficiency of protein immobilization was verified by RT-PCR using Round 10 without protein immobilization, enrichment below the threshold of the initial library was evidenced.

Conclusion: It is verified that the methodology used for selection of RNA aptamers for domain III of the Yellow Fever envelope protein is efficient, showing enrichment in the selection rounds, being possible to follow the steps of identification of the possible clones, conjugation with AuNPs and construction of POCs.

Keywords: RNA Aptamers; SELEX; Rapid Diagnostic