Demonstration and Optimization of Multiple Aptamer-ELISA “ELASA” Assays with Novel DNA Aptamers

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Abstract
While the ELISA method is generally very sensitive with specificity depending on the quality of primary detection antibody, protein-based antibody reagents are not very stable in non-refrigerated (i.e. point-of-care) applications. In contrast, DNA aptamers are extremely stable in both hydrated and lyophilized form and therefore amenable to field-deployed assays. Towards using such reagents, we are demonstrating their suitability in several modified ELISA formats termed enzyme-linked aptamer/sorbent assays or “ELAS”. Using novel aptamers, here we demonstrate both direct and sandwich ELASA approaches. For each of the approaches we determine their apparent limit of detection.

Introduction
Aptamers have a number of potential advantages over conventional, single-target antibodies [1], especially for point-of-care or “deployed” assays in which antibodies require a cold-chain of custody to prevent degradation. Nevertheless, the extensive use of enzyme linked immunosorbent assays or “ELISAs” has resulted in well-established methods, enzymes, and substrates for sensitive detection of antigens/analytes. Thus, we are demonstrating aptamers as modular replacements for antibodies in convenient “ELISA-like” formats familiar to many potential users. Others have rather naturally termed such assays “enzyme linked aptamer sorbent assays” or “ELASAs” [2].

Aptamers are single-stranded DNA or RNA oligonucleotides selected to have unique three-dimensional folding structure for binding to a variety of targets such as proteins, peptides, and even small molecules with affinity and specificity rivaling that of antibodies. They are typically selected in vitro by a process commonly referred to as “SELEX” as depicted in Figure 1.

Methods

Methods preparation: PVDF-coated slides (ArrayIt) were pre-wetted by 100% methanol and allowed to dry for 60 min. For the “direct” or dot-blot method, 0.5 – 2.0 µl spots of varying concentration were spotted and dried for 60 min. Arrays are then blocked with 1 mg/ml non-fat dry milk before aptamer binding. For the indirect methods, neutravidin (Pierce) or analyte-specific antibody, 0.5 – 2.0 µl were similarly spotted and dried for 60 min. at room temperature. For “Indirect Method 1”, 1 – 2 µl of aptamer was then spotted directly over the neutravidin spot. Arrays thusly prepared were then blocked with non-fat dry milk prior to offering varying concentrations of protein analyte.

Results

The direct approach (Figure 3A.) “Method 1” has the advantage of small analyte consumption. Here, the protein (or eventually, serum sample) is spotted in a small (~1 µl) volume. Because of this small volume, the ultimate sensitivity is reduced. In contrast, both of the indirect methods (Figures 3B and 3C) capture protein analyte from a much larger volume (~400 µl). The resulting sensitivity or limit-of-detection is on the order of fmoles/µl as shown below.

References

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