Technical Note

Adaptation of 3DNA® Dendrimers to Point of Care Assays

Introduction

Lateral Flow (LF) and similar assays represent a unique growing class of Point of Care (POC) tests designed to rapidly diagnose patients within clinically actionable timeframes. These assays have increased in both number and diagnostic focus, representing an easy alternative to classic immunoassay techniques (1). While consumers may perceive these assays to be low tech, the materials inside the device and how they are combined to signal the outcome, represent a combination of sophisticated technology and reagents including antibodies, modified membranes, impregnated buffer formulations, etc. This complex association of materials in an easy to use format has yet to reach its full potential, despite its incredible value as an effective tool to the health care industry (1). In addition, some POC tests are limited in sensitivity and quantitative accuracy.

Genisphere has developed and commercialized a diverse catalog of products based on its 3DNA® Dendrimer signal amplification technology, designed to improve the sensitivity of protein and nucleic acid detection. 3DNA® Dendrimers are extremely versatile by custom design, and may be applied to a wide variety of bioassay formats including microarrays, ELISAs, Luminex® bead-flow assays, LF POC tests, and others. Depending on the assay platform and analyte, detection may be improved up to greater than 100 fold (Figure 1).

Figure 1: Examples of fold improvement in sensitivity observed when using 3DNA® Dendrimers adapted to various applications *http://www.genisphere.com/signal_amp_app_notes.html, **http://www.genisphere.com/array_detection_900.html, ***data taken from this Technical Note
The 3DNA® Dendrimer consists of a core of double stranded DNA with hundreds of single stranded peripheral sites for the attachment of therapeutics and targeting agents (2-4). An example of a 3DNA® Dendrimer with antibody targeting agents and multiple label molecules attached is shown in Figure 2. To test the utility of our technology in LF POC tests, we chose to use an established LF assay for human chorionic gonadotropin (hCG) as a model system. Here we describe improved sensitivity and quantitative utility in a model LF POC test, using customized 3DNA® Dendrimers.

Methods and Materials

**Custom 3DNA® Dendrimer preparations.** Since the hCG LF assay is a classic sandwich ELISA format, 3DNA® Dendrimers were custom manufactured with multiple anti-hCG antibodies and approximately 1000 biotins or Oyster®-650 fluorescent dyes (similar to Cy™5) per 3DNA®.

**Lateral Flow assays.** Lateral flow strips were prepared containing immobilized anti-hCG capture antibody. A second and different anti-hCG antibody was either bound to a single 40nm gold particle and dried to the conjugate pad (standard assay), or was conjugated to a biotin-labeled 3DNA® Dendrimer and dried to the conjugate pad, along with streptavidin coated 40nm gold particles (direct 3DNA® assay). Samples representing 2 fold dilutions of hCG were prepared and run side by side in both the standard assay and direct 3DNA® assay (Figure 3), then scored visually using a 1-10 color grading scale. In parallel, fluorescent dendrimers were dried down on the conjugate pad and the same hCG dilution samples were run, and compared to a standard anti-hCG antibody conjugated with 3-5 fluorescent dyes. Fluorescence was captured using an Axon scanner.

**Results**

In order to compare the performance of 3DNA® Dendrimers in a model LF assay we compared the standard hCG assay to an adapted system designed to include custom 3DNA® reagents. Our initial experiments were designed to address the potential improvement in sensitivity (by 3DNA®) of the classic gold conjugate detection. The dendrimer reagent used for gold detection was designed as a
biotinylated reagent to capture streptavidin gold conjugates and produce signal in the model assay. While two separate reagents were used for the dendrimer gold detection method, no additional procedural steps were required since all reagents used were dried onto the conjugate pad. The overall assay was designed to compare a dilution series of hCG using both the standard and direct 3DNA® methods run side by side. When run in a standard lateral flow assay procedure using dendrimer reagents dried down on the strip’s conjugate pad, the dendrimer assay achieved 64 fold improvement of sensitivity over the standard non-dendrimer format when comparing limits of detection (LOD) (Figure 4).

![Figure 4: Comparison of standard hCG detection to direct 3DNA® hCG detection using gold particle detection](image)

In order to determine if fluorescent 3DNA® Dendrimers can also be adapted as reagents for use in a fluorescent LF assay, we replaced the biotinylated 3DNA® Dendrimer and streptavidin coated 40nm gold particles with a single Oyster®-650 fluorescent reagent and ran the same hCG dilution series, compared to a standard anti-hCG antibody conjugated with 3-5 fluorescent dyes. Similar sensitivity to the visual, gold 3DNA® detection was observed when using fluorescent 3DNA® reagents (Figure 5).

![Figure 5: Comparison of standard hCG detection to direct 3DNA® hCG detection using fluorescent detection](image)
Summary

3DNA® Dendrimers are powerful and adaptable molecular devices capable of improving the sensitivity of a wide variety of assays including lateral flow, immunoassays and nucleic acid hybridization. High profile and high value tests offered as Point of Care assays are adaptable to include 3DNA® reagents to improve the limit of detection. Here we report a 64 fold improvement of sensitivity in a model Lateral Flow POC test. Similar improvements in sensitivity have been observed in other LF assays, and we envision the utilization of this technology will reduce the false negative rate and significantly improve the accuracy of the otherwise subjective reading of the outcome. The stability of dendrimer reagents in solution is known to exceed 12 months, and preliminary experiments studying the stability of dried dendrimer reagents suggest a similar stability profile. Improved fluorescent detection will further enable POC tests to become more quantitative and better predictive of disease, condition and outcome. Because it is a passive amplification technology (unlike PCR, for example), the 3DNA® target-specific probe may be used to improve the sensitivity of existing bioassays without significant modification. Utilization of 3DNA® Dendrimers can be achieved with minimal additional manufacturing cost and without altering end user protocols.

References