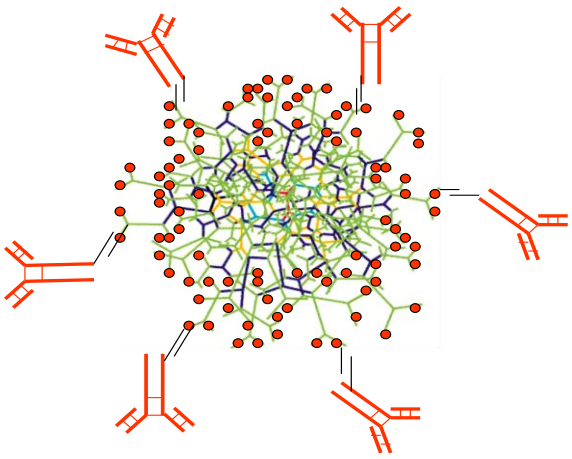
Improving the Limit of Detection of Lateral Flow Assays using 3DNA® Dendrimers

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 (2, 3) 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 (4-7).

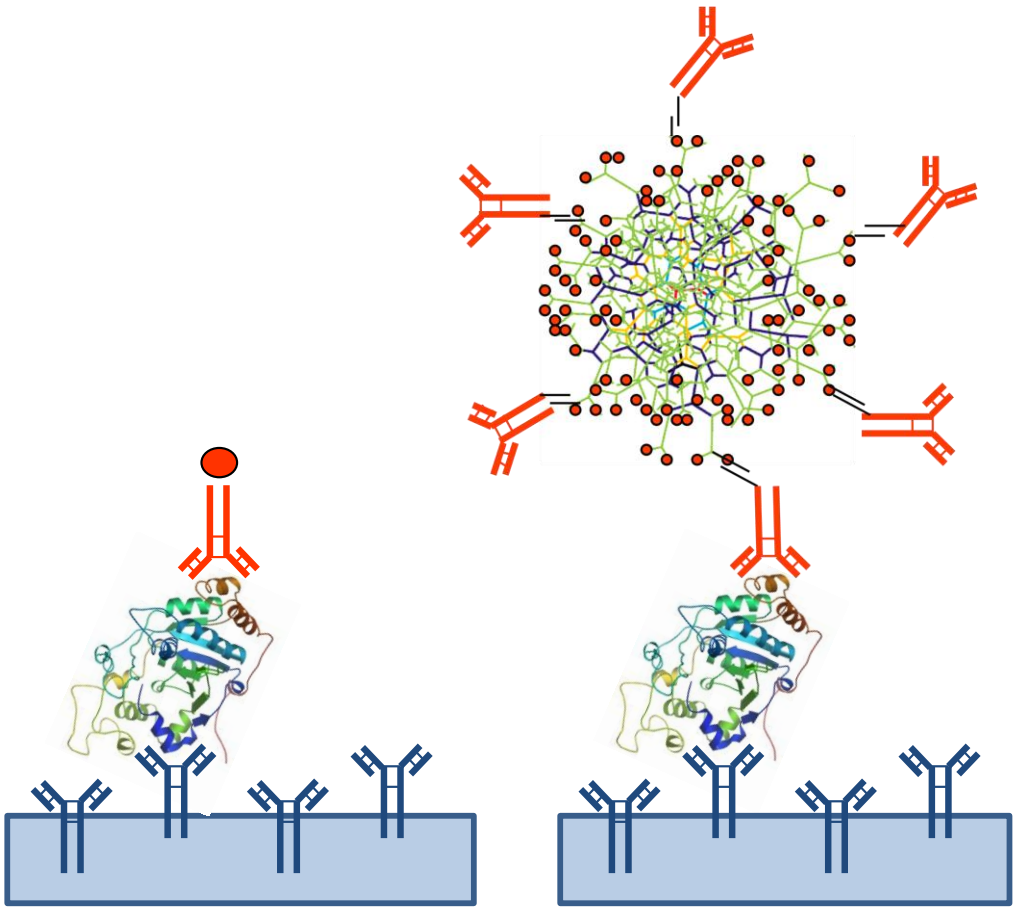


The 3DNA® Dendrimer consists of a core of double stranded DNA with hundreds of single stranded peripheral sites for the attachment of labels, therapeutics and/or targeting agents. An example of a 3DNA® Dendrimer with antibody targeting agents and multiple label molecules attached is shown in the cartoon. 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, using both gold particle detection and fluorescence detection. Then, we partnered with other institutions to run collaborative experiments testing a variety of additional analytes.

Methods and Materials

Custom 3DNA® Dendrimer reagent preparations. 3DNA® Dendrimers were custom manufactured with ~960 biotins or Oyster®-650 fluorescent dyes (similar to Cy™5) per 3DNA®. For each analyte tested, its appropriate detection antibody was conjugated to a short oligo, complementary to the 3DNA dendrimer.

Lateral Flow assays. Lateral flow strips were prepared containing immobilized capture antibody striped as a control or test line. Samples containing titrated amounts of each analyte were prepared. Detection antibodies were either directly labeled for standard assays, or conjugated to short oligos for 3DNA® assays, then dried to the conjugate pad. For 3DNA® assays, streptavidin coated 40nm gold particles were also dried to the conjugate pad. The lateral flow procedures were run per standard or recommend protocols. Experiments were designed as side-by-side comparisons of the standard assay to the 3DNA® assay (see cartoon). All data points were performed in triplicate. Gold detection assays were scored visually using a 1-10 color grading scale or scored electronically using the ESEQuant reader (QIAGEN®). For fluorescent detection assays, the signal 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. No additional procedural steps were required for 3DNA® assays since all reagents used were dried onto the conjugate pad. In a dilution series of hCG, the dendrimer assay achieved 64-fold improvement of sensitivity over the standard format in both gold detection (Figure 1) and fluorescent detection (Figure 2) formats. (LOD = Limit Of Detection)

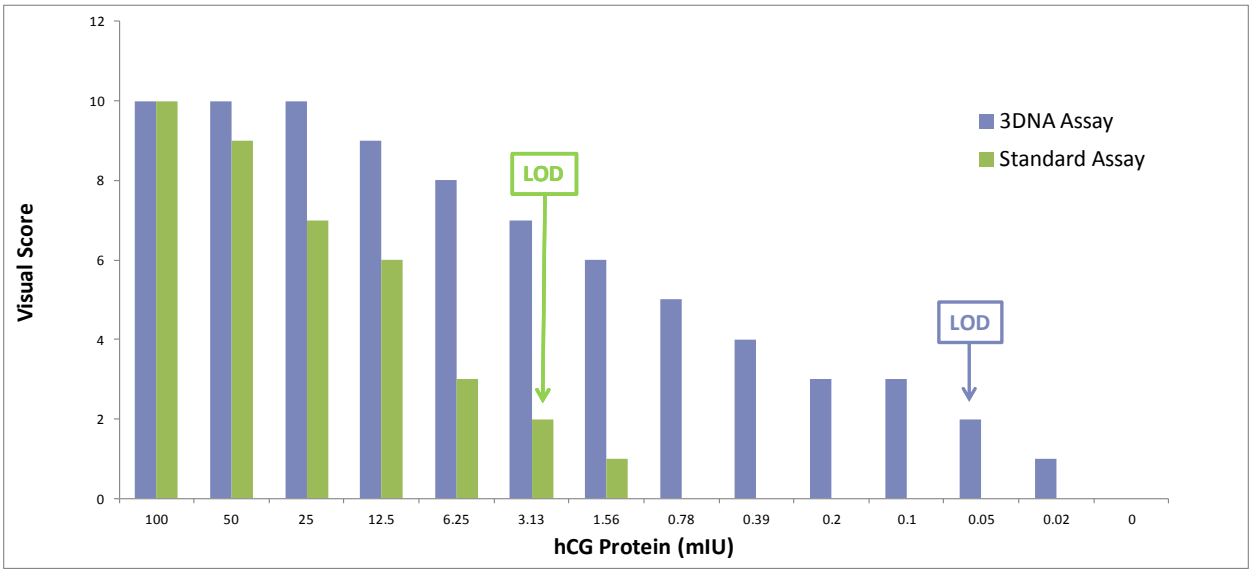


Figure 1: Comparison of hCG LOD
using standard and 3DNA® assays - gold detection

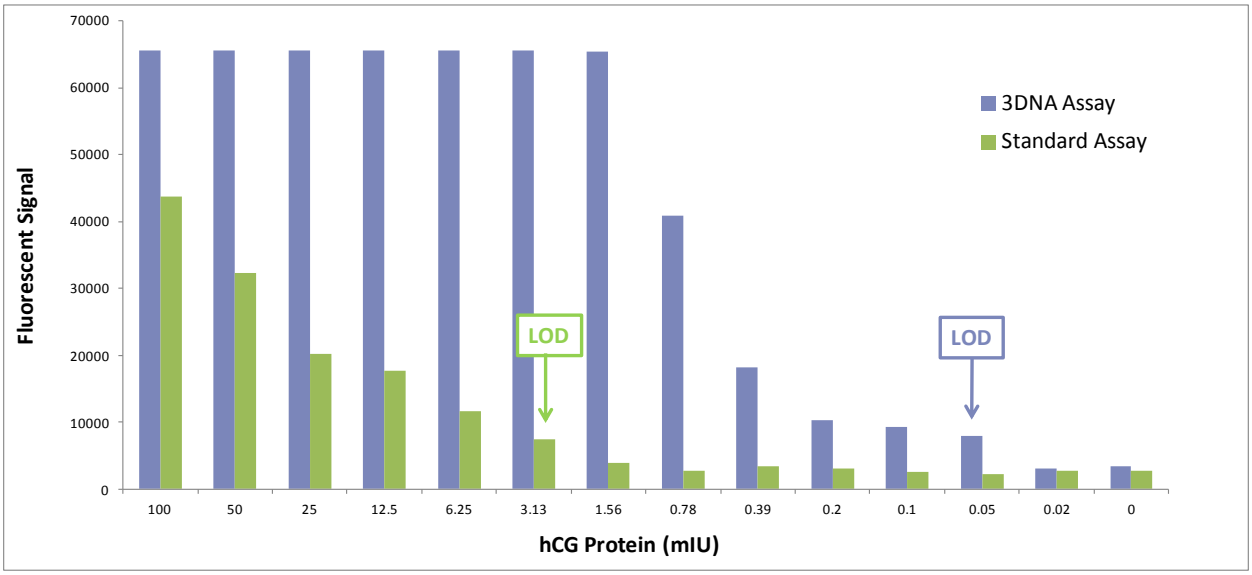


Figure 2: Comparison of hCG LOD
using standard and 3DNA® assays - fluorescent detection

We established collaborations with academic and industrial partners to customize our reagents for lateral flow assays detecting additional analytes. 3DNA® Dendrimer assays improved the limit of C-Reactive Protein detection by 50-fold (Figure 3) and improved the limit of Influenza A detection by 64-fold (Figure 4).

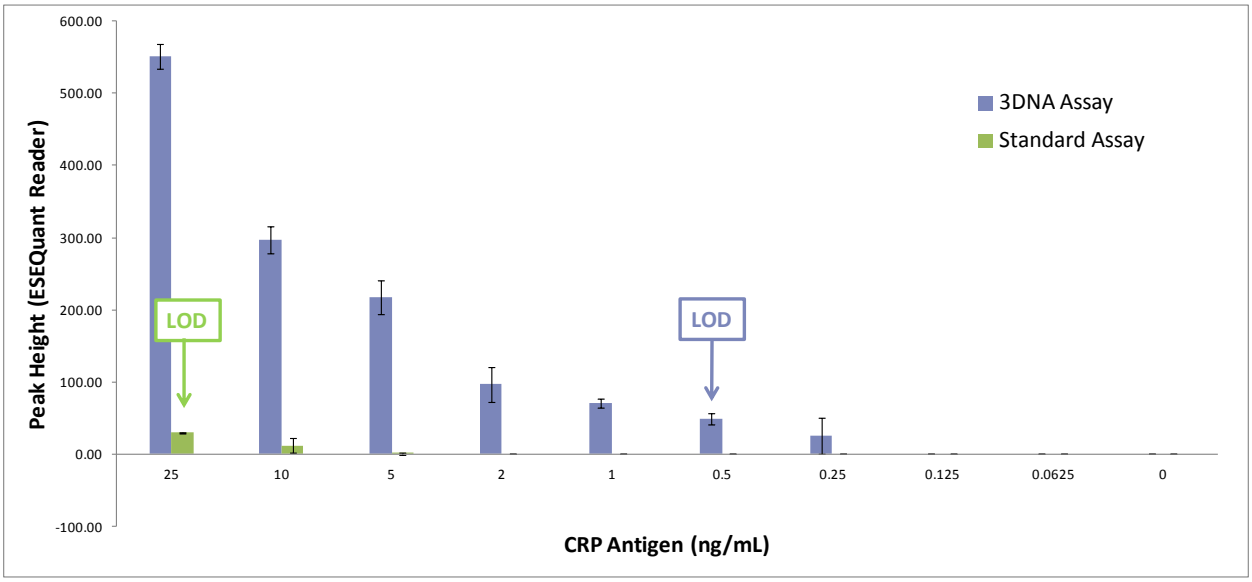


Figure 3: Comparison of C-Reactive Protein LOD using standard and 3DNA® assays - gold detection

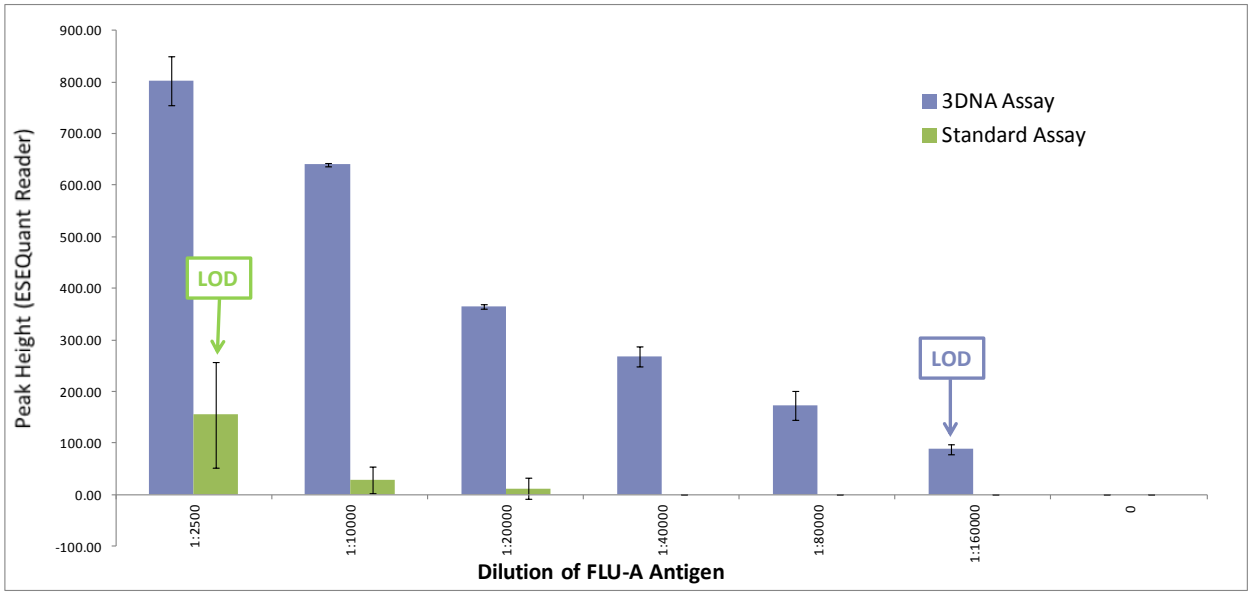


Figure 4: Comparison of Influenza A LOD using standard and 3DNA® assays - gold detection

Since each lateral flow platform is different, in terms of physical setup and samples tested, a range of 3DNA®-dependent signal amplification is both expected and observed. The current list of analytes tested comparatively in standard and 3DNA® lateral flow assays is summarized below, along with the corresponding sensitivity improvement.

Analyte	Sensitivity Improvement using 3DNA® Dendrimers
hCG	64 fold
C-Reactive Protein	50 fold
Influenza A Virus	64 fold
Influenza B Virus	>40 fold
Troponin-I	100 fold
Rabbit IgG	100 fold
Mouse IgG	20-100 fold
Human IgG	10-20 fold
Almond Protein Antigen	10 fold

Summary

3DNA® Dendrimers are powerful and adaptable molecular devices capable of improving the sensitivity of a wide variety of immunoassays and nucleic acid detection assays. 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 significant improvements in the Limit of Detection in a variety of lateral flow 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. Improved fluorescent detection will further enable POC tests to become more quantitative and better predictive of disease, condition and outcome. Utilization of 3DNA® Dendrimers can be achieved with minimal additional manufacturing cost and without altering end user protocols. The reproducibility of 3DNA® Dendrimer manufacturing, and the stability of dried down dendrimer and antibody-oligonucleotide conjugate, meet the requirements necessary for portable, rapid diagnostic tests to be clinically and commercially viable (data available upon request). To inquire about Genisphere's assay development collaboration program, please visit www.genisphere.com or send an email to partner@genisphere.com.

References

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