A Rapid, Multiplexed Diagnostic Platform for Detection of Proteins and Nucleic Acids

Beacon Biotechnology
Small company. Big science.

M Cull; T Roark; T West; F Mitchell

Beacon Biotechnology LLC, Bioscience Park Center, 12635 E. Montview Blvd., Aurora, CO 80045

Introduction

The BrightSPOT™ device is a highly sensitive, multiplexed rapid diagnostic micrroarray platform that can be used in a POL or other resource-limited setting to perform virtually any diagnostic test currently performed by microtiter plate. At the heart of the technology is an inexpensive, ultra-sensitive CMOS detector array and extremely-bright *Gaussia* luciferase-based diagnostic assays. Because light generation occurs via a chemical reaction, all the power needed to perform analysis can be supplied by a USB port.

The extraordinary sensitivity allows small samples (5-10 µl) to be applied to the surface of the array and can therefore be used with a fingerstick sample. The small sample volume also allows rapid annealing and detection of nucleic acid capture probes with target DNA or RNA. The sensitivity of the BrightSPOT platform combined with our luciferase assays eliminates the need for active mixing or amplification of the DNA or RNA target.

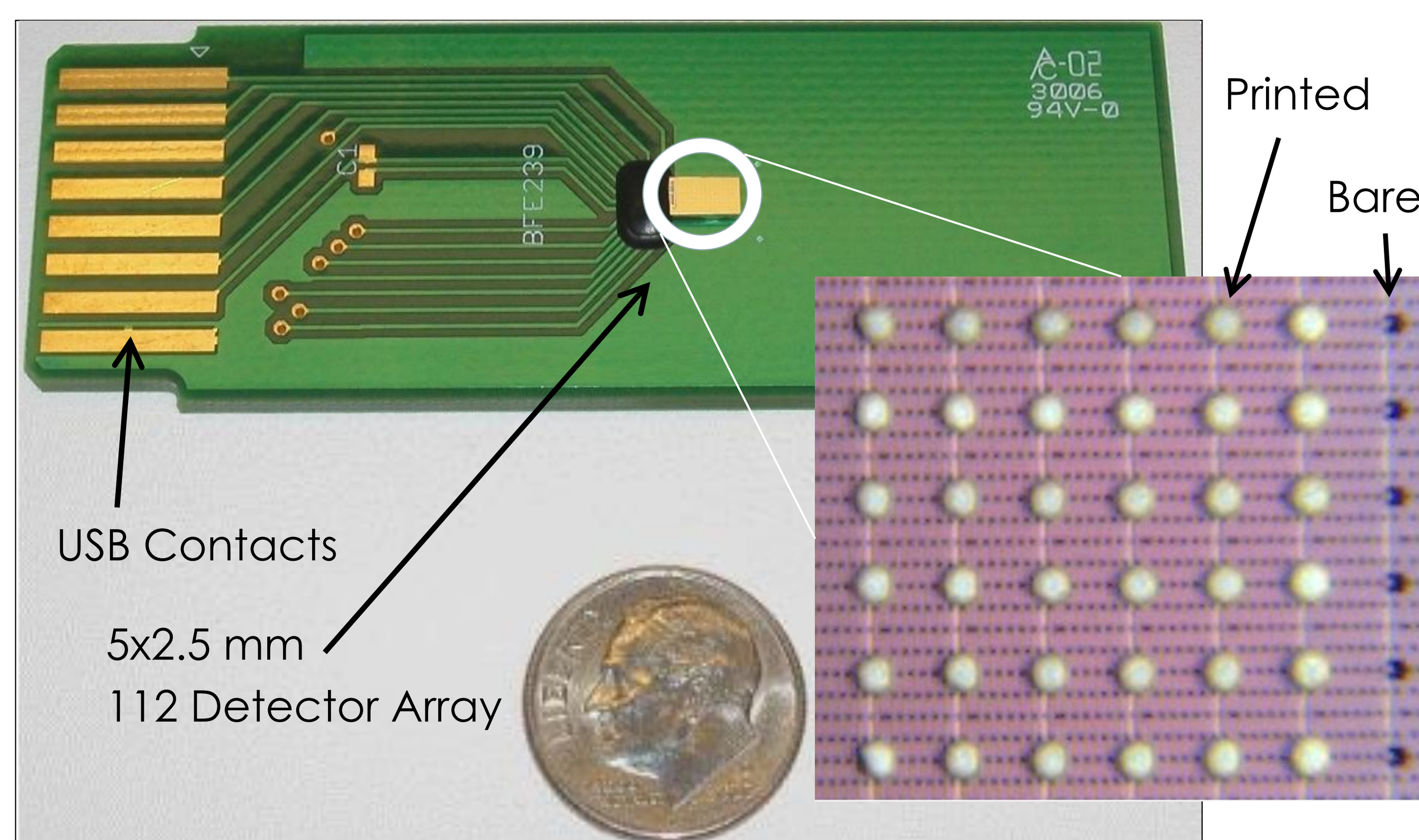

Figure 1. The BrightSPOT disposable The 5 x 2.5 mm optical detection array is the rectangle in the center of the slide. The array contains 112 individually and simultaneously addressable detectors. Each can perform a different diagnostic test. The card edge (left side) interfaces with a USB adapter.

The 8 x 14 array on the BrightSPOT can be thought of as a miniature microtiter plate, with each detector a 1µm deep well that uses picoliter volumes of reagents.

Contact

Millard Cull, CTO
Telephone: (720) 859-6112
Email: mcull@beaconbiotechnology.com
web: www.beaconbiotechnology.com

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Methods

As proof of concept, we demonstrate the BrightSPOT device on three common diagnostic formats: 1) antigen-down detection of antibody, 2) antibody sandwich assays, and 3) nucleic acid duplex detection. We present brief information on protein based methods and focus on NA method results due to space limitations.

Antigen-down: We previously showed an antigen-down test that detected HIV antibodies against the multiplexed HIV antigens p24 and p51 in a 13 minute test (Oak Ridge poster, 2009) that is more sensitive than a validated commercially available ELISA (BioRad) that takes three hours.

In a collaboration with the Barbara Davis Center for Childhood Diabetes Research (Aurora, CO), the BrightSPOT platform was able to detect GAD65 auto-antibodies, a T1DM marker in 1.5 hr and outperformed a traditional liquid phase RIA requiring 2 days with 16X better sensitivity. We later performed the test in whole blood, as HIV above, in 11 minutes and then successfully demonstrated the test on saliva. (data on file)

Antibody Sandwich: The BrightSPOT platform differentiated between phosphorylated and non-phosphorylated kinases with an anti-phospho- capture and protein-specific secondary antibody. (data on file)

Figure 2. Summary of Protein Data

Diagnostic Test	Sample Type	Demonstrated Detection	Time to Result
HIV (multiplex)	Whole Blood	> 1:1,000,000 dilution	13 min
T1DM (GAD65)	Whole Blood Saliva Whole Blood	> 1:1,000,000 dilution 1:2 dilution 1:10 dilution	1.5 hrs 1.5 hrs 11 min

dsDNA Detection

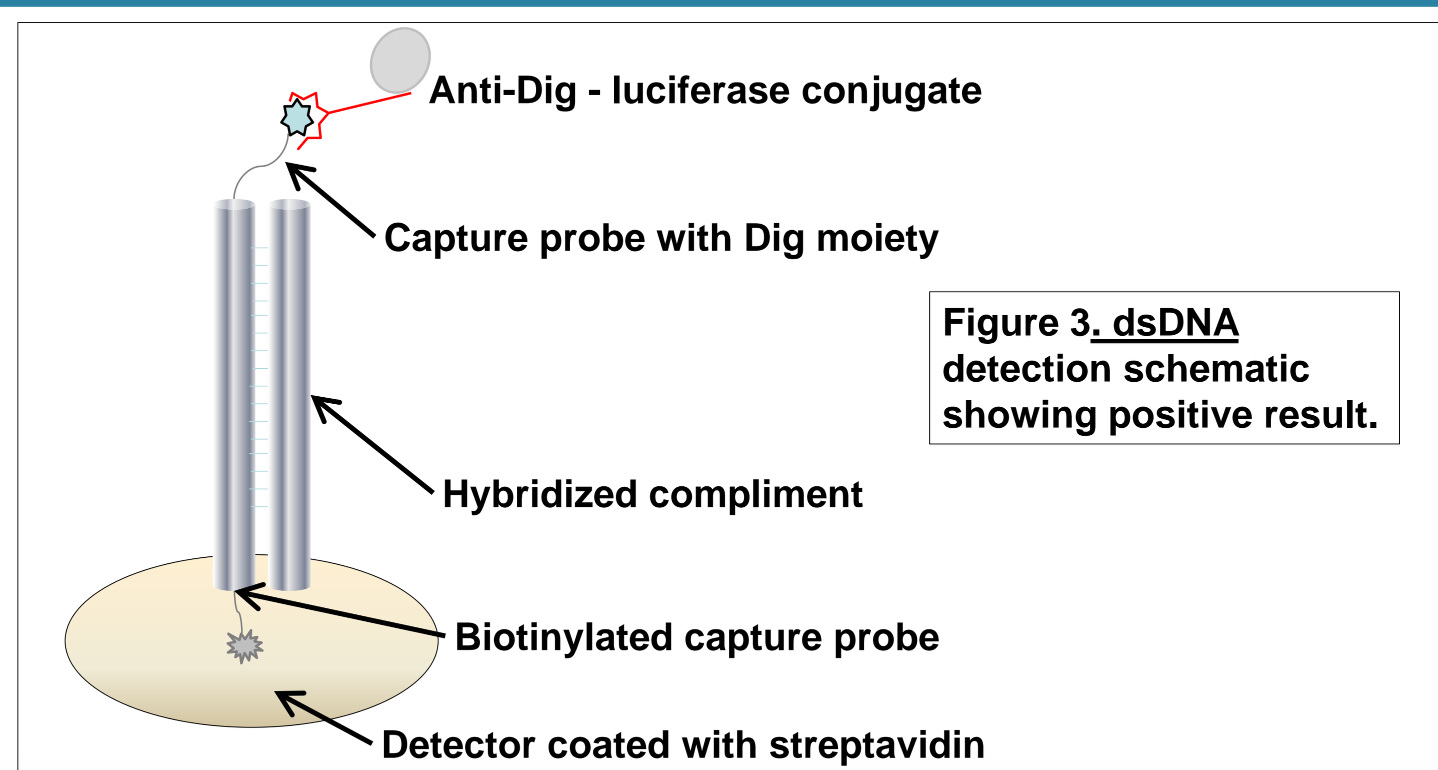
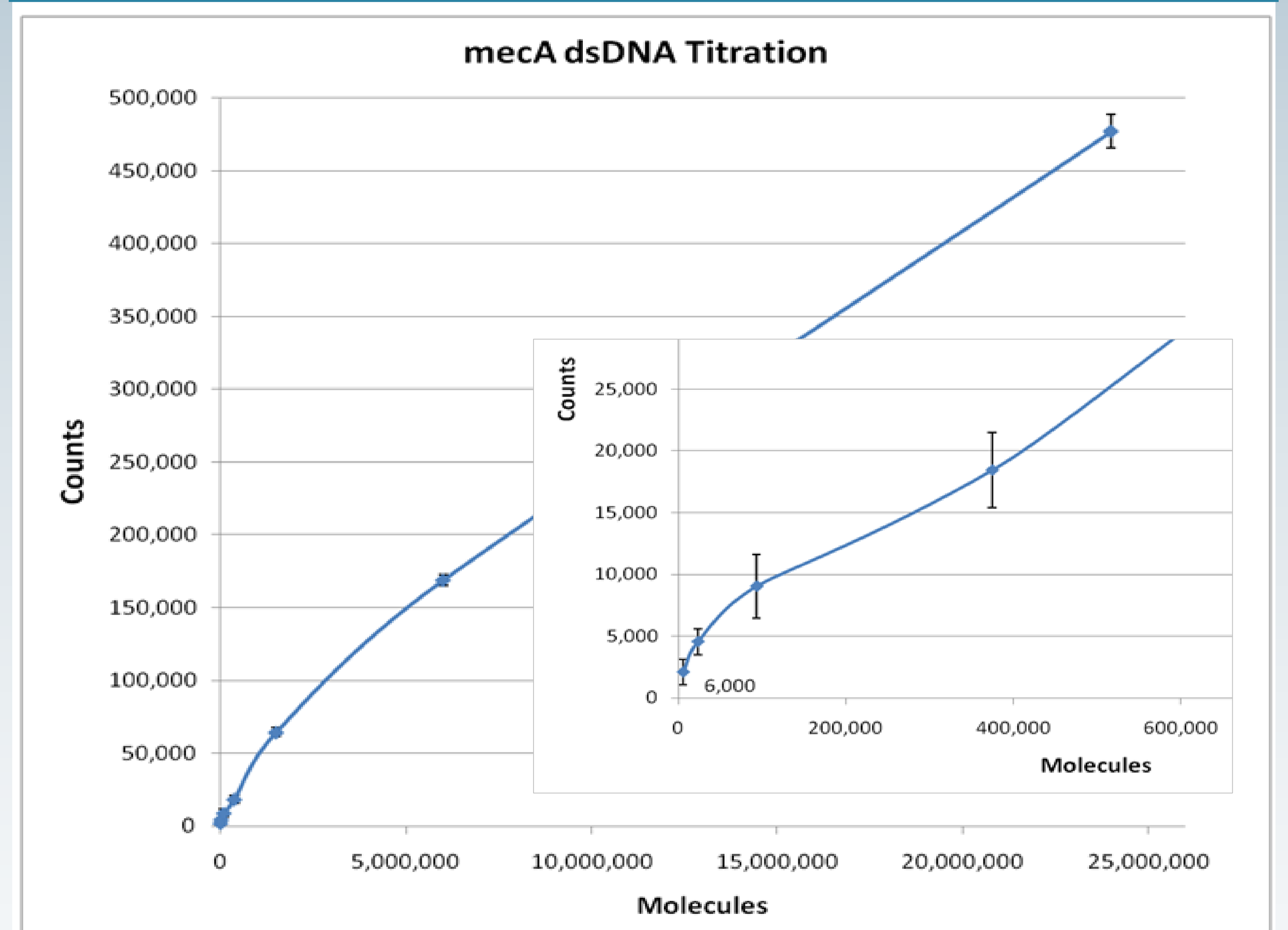


Figure 3. dsDNA detection schematic showing positive result.

dsDNA Demonstration Method

dsDNA Detection: A simplified assay was used to measure the potential limit of detection for dsDNA on the BrightSPOT platform. Pre-formed hybrids (5:1 target:capture) were printed onto a streptavidin coated BrightSPOT chip. The mecA capture strand had a biotin at one end for attachment to the surface, and a DIG moiety at the other for detection with anti-DIG/luciferase-antibody. The negative control was an un-related capture (BRCA1) and target DNA (BRCA1 complement) where the capture strand contains a biotin at one end, but **lacked** the DIG moiety for attachment of the luciferase-labeled antibody. A constant amount of dsDNA was printed on each detector, but the target was titrated downward within each printed sample. Detection was accomplished when anti-DIG luciferase-labeled antibody was added, the chip washed, and signal generated by the antibody-bound duplexes with the addition of the luciferin coelenterazine.

Results



The BrightSPOT platform demonstrated detection of 24 million molecules down to 6,000 molecules (10 zeptomoles) of mecA, the gene for Methicillin resistance from MRSA.

Conclusion

The low-cost BrightSPOT platform is capable of performing a wide variety of IVD methods at performance levels equal to and better than current instrumented equivalents. The sensitivity of the platform for detecting duplexed nucleic acid may allow the target nucleic acids to be detected rapidly and without amplification.