Cambridge Consultants
XEN Biofluidx
OAK RIDGE CONFERENCE
Oak Ridge Conference
Capturing Innovation: The Impact of Emerging Diagnostic Technologies, April 22-23, 2010
San Jose, CA

Novel Time Resolved Fluorescence Platform for Near Patient Diagnostics

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Abstract

A new universal immunoassay platform for a variety of biomarkers integrating a novel time resolved fluorescence (TRF) label and a low cost portable detection technology is reported. We have previously used this platform to demonstrate a sensitivity improvement of orders of magnitude over gold-label based assays, with excellent repeatability. The platform has several benefits, in addition to its high sensitivity, the core components are low-cost and compact, which offers the potential to place the platform at the core of highly commercially attractive low-cost rapid diagnostic systems. Furthermore, the platform can be applied retrospectively to traditional gold-based lateral flow systems to realise significant performance enhancements.

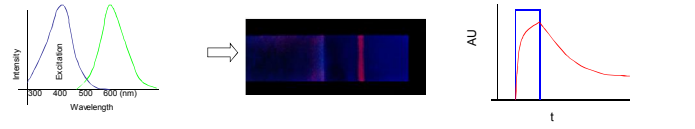
New TRF Label

At the core of this platform technology is a novel highly sensitive label, developed by XenBio. Because of the time resolved properties this label has a low background signal and exhibits very low non-specific binding due to its proprietary surface. Designed with flexibility in mind, proteins can be covalently bound or physically adsorbed to the surface. This label, when integrated with Cambridge Consultants detection technology, is an ideal label for high sensitivity assays at low cost diagnostic tests, providing the precision, accuracy and sensitivity expected of a clinical laboratory in a near patient setting.

Important characteristics of the label include

- Label is inert to biological fluid
- Particles are mono-dispersed to improve precision, sensitivity and accuracy
- Can be functionalized covalently with no physical adsorption
- Concept has been demonstrated on a bio-threat, cardiac and fertility assay
- Surface chemistry is scalable to manufacturing quantities (million assays per lot)
- Demonstration of lateral flow assays is complete and readily adaptable to a variety of tests
- High sensitivity and low cost instrument, manufactured in volume suitable for less than $1/ test

Figure 1: Integrated low cost reader with rich UI and wireless connectivity. Target CoGs, $50



Excitation and emission — Fluorescence — Time resolved fluorescence

Figure 2: At the core of the platform is a high sensitivity label and detection technology that can measure both fluorescence and phosphorescence. The unique way in which the measurement is made enables significant sensitivity enhancements over both traditional labels and amplification techniques

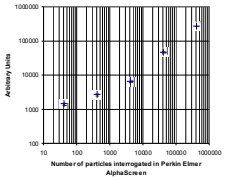


Figure 3: TRF particles interrogated in a Perkin Elmer AlphaScreen. Data plotted is the result from three runs. Background measurement was 1109 AU

To determine how mono-dispersed the label was, a serial dilution was run through a Perkin Elmer instrument. A total of three runs were completed. Results showed that just 40 particles could be detected with a c.v. of 5.9% illustrating firstly the high Quantum efficiency of the label and also effectiveness of the surface properties in ensuring the mono-dispersion is maintained

New Platform

We have developed a new platform that can be rapidly tailored to a range of analytes and capable of being applied to a number of substrates, arrays, planar surfaces as well as being capable of being retrofitted to a range of lateral flow assays. The current development of the instrument uses a scanning feature to increase the sensitivity of the detection but it can be run in a static read format. Based on low cost electronic components, a simple PoC reader is targeted to have a CoGs of <$50 and a disposable OTC device for <$5, where some performance is traded for cost.

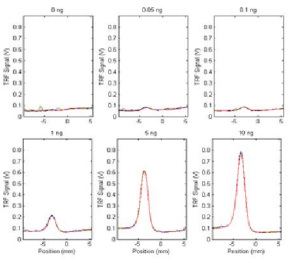


Figure 4: Results from scanning three lateral flow strips with the CC prototype reader. Results show excellent repeatability

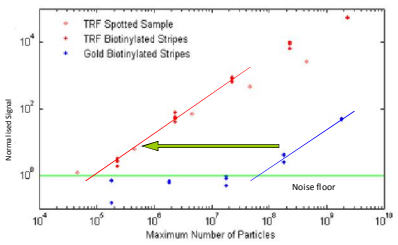


Figure 5: Comparison of TRF label to a 40nm gold Colloid label. TRF label read with CC low cost reader, gold colloid read using Hamamatsu ICA1000 reader. Substrate used was lateral flow membrane

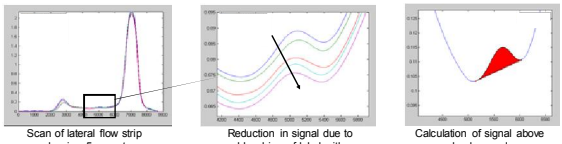
At the core of the reader is a sophisticated detection unit and front end analogue electronics, reliant upon a proprietary modulation technique. Incorporated in the reader is a dynamic mode which allows the sensitivity of the reader to be extended over at least five orders of dynamic range, Figure 5. At the same time results have shown the reader capable of detect 10000 beads, Figure 5, on a lateral flow membrane and encouraging tests have also been shown on a planar substrate. When comparing the performance of the TRF label to the gold label, at least three orders improvement in sensitivity can be seen.

Possibility of detecting kinetics or end point
True quantatitive test/ Repeatability – assay/ Variation in buffer and plasma

Exploring the repeatability of the instrument we repeatability removed and re-inserted a 1.18pM NT-proBNP test strip in to the reader five times, Figure 6. From these measurements we found that:

(i) The reader shows good repeatability in signal profile when scanning a strip.
(ii) 'bleaching' resulting from repeat scanning can be seen, a small reduction in peak magnitude is observed

In this configuration the measurements are made using a scanning configuration of the reader. It is possible to further cost reduce the instrument to < $5, for use in an OTC setting or in a disposable format but we would expect to see some degradation in performance.



Scan of lateral flow strip showing 5 repeat measurements — Reduction in signal due to bleaching of label with successive reads — Calculation of signal above background

Figure 6: Repeatability measurements made using the CC prototype instrument. Five repeat measurements show the excellent repeatability of the measurement, while also illustrating bleaching effect of the label

Results

The results show that the platform is capable of detecting NT-proBNP and Botulinium concentrations of less than 1pM and under certain conditions as low as 0.01pM. The platform can be used for monitoring heart failure and cancer biomarkers and has the potential to achieve a high sensitivity within an optimized system. The low cost of the platform and its suitability for the Point-of-Care and Rapid Diagnostic Test markets make it an attractive candidate for the near patient markets.

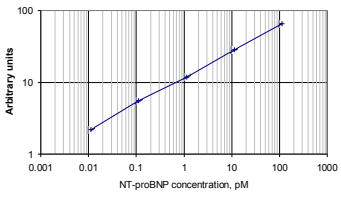


Figure 7: NT-proBNP assay read using low cost reader – single repeat - 10µl sample – 5 minutes to result

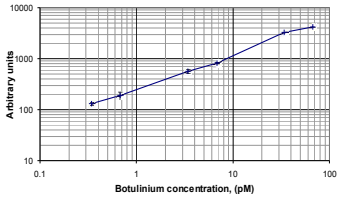


Figure 8: Botulinium assay read using low cost reader – 3 repeats, 10µl sample – 5 minutes to result

hCG assays were run in plasma and buffer to determine the effect of a biological matrix. The results in Table 1 show that the signal running the assay in plasma is identical to buffer. Assay repeatability was measured by running an hCG standard curve with three replicates for each point. 10 replicates of 0.075mIU/mL hCG were then measured and plotted on the curve and the CV calculated to be 7.5%, Table 1. Although assay optimisation is still to be performed, where we expect improvements in both precision and sensitivity, the dynamic range of the NT- proBNP assay was measured to be 0.1pM to 100pM. In addition we have demonstrated sensitivity for the NT-proBNP assay to be up to 0.1pM in a 20uL sample. To improve the clinical precision of the system (currently the clinical precision of (hCG at 0.19 pM is 8%) particle mono-dispersity should be tightly controlled and monitored to remove or disrupt any doublets or triplets during the preparation. We expect tight control of particle deposition to be necessary before connecting with the solid phase to limit variability of particle/reagent reconstitution. Careful selection of both the pore size and homogeneity of solid phase to improve strip to strip variability, as will blocking of solid phase surface to remove any non-specific interaction.

hCG Level (mIU/mL)	Buffer	Plasma
0	47	32
0.02	1971	1886
0.08	7428	7418
0.16	11752	10773

Table 1: Effect of plasma vs buffer on an hCG assay

	AU		AU
Repeat 1	6155	Repeat 6	5586
Repeat 2	6356	Repeat 7	5536
Repeat 3	5569	Repeat 8	5119
Repeat 4	6477	Repeat 9	5543
Repeat 5	5800	Repeat 10	5497

Table 2: Effect of plasma vs buffer on an hCG assay

Conclusions

Cambridge Consultants, together with Xenbio, has developed a class leading technology platform that can be adapted for a variety of assays. By combining a novel label and detection technology we have demonstrated pM sensitivity with a low cost of goods. The platform is applicable to a range of fields and offers, due to its maturity, a rapid transition to a product. We are seeking companies who are aiming to launch class leading diagnostic products to tailor technology platform.

About XenBio

Xen Biosciences, Inc (XenBio) is an early stage biotechnology company located at the heart of San Diego's Golden Research Triangle. It was founded in 2005 to develop and commercialize novel in vitro diagnostic (IVD) technologies to allow accurate answers to health questions at the doctor's office, home, mall, and other point-of-use locations. XenBio's proven and patented portable detection platform is poised to address the need for real time, low cost, point-of-use solutions for the heart disease and breast cancer diagnostics markets. XenBio presently offers development services on behalf of clients with access to other validated markers. Contact: Victor Manneh, Victor.manneh@hotmail.com

About CC

Cambridge Consultants, Ltd. (www.cambridgeconsultants.com) is a leading technology and innovation company renowned for its ability to solve technical problems and provide creative, innovate solutions to business needs. CC has offices in the UK and USA and employs the some of the world's leading scientists and engineers. Contact: Simon Burnell, simon.burnell@cambridgeconsultants.com