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Novel Time Resolved Fluorescence Platform for Point of Care Surgery

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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 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 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 to the surface. This label, when integrated with Cambridge Consultants detection technology and Xen Lateral flow 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

- 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

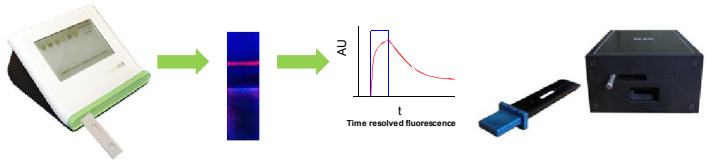

Figure 1: Second (left) and third (right) generation integrated low cost readers with rich UI and wireless connectivity. At the core of the platform is a high sensitivity label and detection technology that can measure both fluorescence and phosphorescence. The unique way in which the measurement is made enables significant sensitivity enhancements over both traditional labels and amplification techniques

To determine label sensitivity a serial dilution was run through a Perkin Elmer Instrument and our TRF Reader. A total of three runs were completed. Results showed that just 456 particles could be detected with Perkin Elmer and 732 particles with our TRF reader illustrating firstly the high Quantum efficiency of the label and also effectiveness of the surface properties in ensuring the mono-dispersion is maintained.

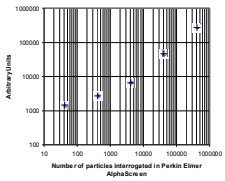

Figure 3A: TRF particles interrogated in a **Perkin Elmer AlphaScreen**. Data plotted is the result from three runs.

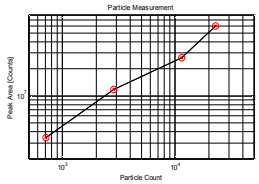

Figure 3B: TRF particles interrogated in a the **Third Generation TRF reader**. Data plotted is the result from three runs.

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.

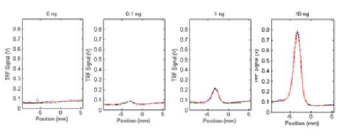

Figure 4: Results from scanning three lateral flow strips with the CC prototype reader. Results show excellent sensitivity and repeatability

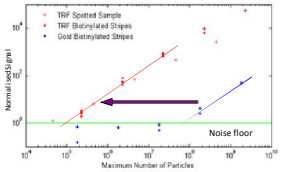

Figure 5: Comparison of TRF label to a 40nm gold Colloid label. TRF label read with CC low cost reader, gold colloid read using Hamamatsu ICA1000 reader. Substrate used was lateral flow membrane

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.

Application in Parathyroid Surgery

About 300,000 people in the United States per year are diagnosed with hyperparathyroidism and a majority will require the surgical removal of one or more of the parathyroid glands (PTG(s)). Intact parathyroid hormones (iPTH) levels must be monitored in patients undergoing unilateral or bilateral neck exploration. Most PTH monitoring is done by a central laboratory, resulting in the patient spending additional time under anesthesia, the surgical team remaining with the patient while the iPTH test is completed, and some question regarding the accuracy of the testing due to the half-life of iPTH being approximately 4 minutes.

Usually, the iPTH level is measured preoperatively, after manipulation of the PTG(s), and at 5, 10, 20, and/or 30 minutes after removal of the suspected hyperfunctioning PTG(s) in all patients. The associated cost for central laboratory iPTH testing is about $360 while placing an instrument in the surgery room requires a licensed medical technician and cost between $760 to $1,060. A large majority of the iPTH testing is currently done in the central lab. The annual cost associated with iPTH testing during the surgical removal of parathyroid gland(s) is approximately $150M per year in the United States.

There is a need for an inexpensive, intraoperative (point-of-care) iPTH diagnostic test. Our TRF iPTH point-of-care assay is perfectly suited for this use. The analytic sensitivity of a central laboratory test, which is currently 4 pg/mL, is well-within reach of the sensitivity of our assay. In addition, the assay will not require the use of a licensed medical technologist. The advantage of having this assay performed in the operating room is immediate feedback for (1) confirming the complete excision of all hyperfunctioning parathyroids; (2) differential jugular venous sampling for localization; (3) diagnosing suspected tissue without histopathology and (4) decrease cost **much less expensive than** compare to the central lab 5) decrease the possibility of infection.

Results

We first screened available antibodies for reactivity with iPTH in sandwich immunoassay format as shown in **Figure 6.** Latex particle pairs are formed in the assay through specific binding interactions. One particle contains photosensitizer and another – a chemiluminescer. Irradiation causes formation of a singlet oxygen , which migrates to a bound particle and activates the chemiluminescer, thereby initiating a delayed luminescence emission.

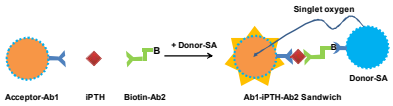

Figure 6: Screening antibody pairing using Luminescent Oxygen Channelling Immunoassay (Xen-LOCI). When two beads are brought closer together (<200nm) by the binding, singlet oxygen activates the chemiluminescent beads and the signal is read on PerkinElmer Fusion instrument.

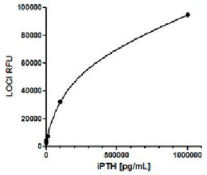

Figure 7: Binding isotherms for Ab1 vs. Ab2 antibody pairings by LOCI.

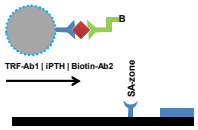

Figure 8: TRF Lateral Flow assay format. TRF-Ab1 and Ab2-bt form a sandwich with iPTH which is then captured by the streptavidin line.

[iPTH] pg/mL	Peak area
0	0
12.5	0.2772
25.0	0.5236

Table 1: Results of TRF scans on Gen 2 reader

[iPTH] pg/mL	LOCI Signal
0	0
1.0	461
10.0	1,241

Table 2: LOCI signal at low end of concentration range

In this case, the antibody pair was selected by Xen-LOCI out of 16 other pairings and integrated into TRF Lateral Flow assay format as shown in **Figure 8.** The results show that the platform is capable of detecting iPTH concentrations of at least 12.5pg/ml (1.3nM). The platform can be used for monitoring iPTH and has the potential to achieve even 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.

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. Here we demonstrated the use of this platform in a POC surgery situation for monitoring parathyroid surgery. Currently, most of intact parathyroid hormone (iPTH) monitoring is done in a central lab by a licensed medical technologist. This results in the patient spending additional time under anesthesia, the surgical team remaining with the patient while the iPTH test is done with some uncertainty remaining in regards to the accuracy of the test due to the short iPTH half-life. Our platform will be CLIA waived, placed in a surgery room, and the assay will be performed by any member of the surgical team with the results in less than 5 minutes.

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: Matthew Hayes, matthew.hayes@cambridgeconsultants.com