Photonic crystal fluorescence enhancement of protein and miRNA biomarker microarray assays.

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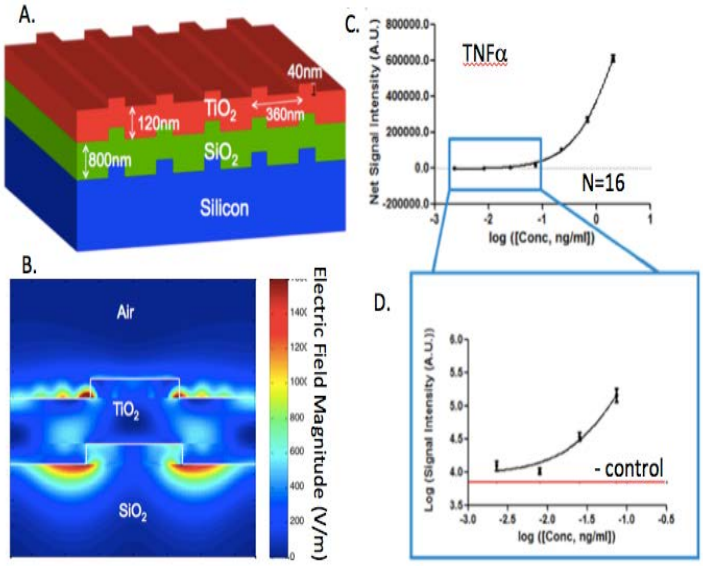
Enhancement of the fluorescent output of surface-based fluorescence assays by performing them upon nanostructured photonic crystal (PC) surfaces has been demonstrated to increase signal intensities by >8000x. Using the multiplicative effects of optical resonant coupling to the PC in increase the electric field intensity experienced by fluorescent labels ("enhanced excitation") and the ability of a photonic PC optics ("enhanced extraction"), PC enhanced fluorescence (PCEF) can be adapted to reduce the limits of detection of disease biomarker assays, and to reduce the size and cost of high sensitivity detection instrumentation.

In this work, we demonstrate the first silicon-based PCEF multiplexed biomarker assay. The silicon-based PC structure (Figure A), comprised of a SiO_2 grating that is overcoated with a thin film of high refractive index TiO_2, is produced in a semiconductor foundry for low cost, uniform, and reproducible manufacturing. We have also designed, built, and demonstrated a compact detection instrument that efficiently couples fluorescence excitation from a semiconductor laser to the resonant optical modes of the PC, resulting in elevated electric field strength that is highly concentrated within the region <100 nm from the PC surface (Figure B). The instrument utilizes a cylindrically focused line to scan a microarray in <1 minute. The microarrays used in this work were produced by spotting capture antibodies and miRNA by Dip Pen Nanolithography, enabling arrays to be small enough to be performed easily with a 10 μL test sample. To demonstrate the capabilities of the system, microspot fluorescent sandwich immunoassays using secondary antibodies labeled with Cy5 for two cancer biomarkers (TNFα and IL-3) were performed. Biomarkers were detected at concentrations as low as 0.1 pM (Figure C-D for TNFα). In a fluorescent microarray for detection of a breast cancer miRNA biomarker, the miRNA was detectable at a concentration of 0.6 pM.



A. Cross section of linear grating device structure of the Si-based PC surface on 8-inch diameter Si wafers at a semiconductor foundry. B. Computer simulation of electric field distribution within one period of the PC structure when illuminated with a laser at the resonant coupling condition. C. Dose- response characterization of 10 ml droplets of buffer spiked with multiple biomarkers. Data shown only for TNFα capture spots. Values represent fluorescent spot intensities obtained with our custom detection instrument. D. Log-log plot of lowest four concentrations of the assay. The lowest concentration of 2 pg/ml is observed above the background fluorescence and negative control spots (red line).

Measurement of anti-erythropoiesis-stimulating agent IgG4 antibody as an indicator of antibody-mediated pure red cell aplasia.

Weeraratne DK, Kuck AJ, Chirmule N, Mytych DT. Amgen Inc., Thousand Oaks, CA.

Patients treated with erythropoietin-based erythropoiesis-stimulating agents (ESAs) can develop a rare but life-threatening condition called antibody-mediated pure red cell aplasia (amPRCA). The antibody characteristics in a nephrology patient with am-PRCA include high antibody concentrations with neutralizing activity and a mixed IgG subclass including anti-ESA IgG4 antibodies. In contrast, anti-ESA IgG4 antibody is generally not detected in baseline samples and antibody-positive non-PRCA patients. Therefore, we validated a highly sensitive immunoassay on the ImmunoCAP 100 instrument to quantitate anti-ESA IgG4 antibodies using a human recombinant anti-epoetin alpha (EPO) IgG4 antibody as a calibrator. The biotinylated ESA was applied to a streptavidin ImmunoCAP, and bound anti-ESA IgG4 antibodies were detected using a β-galactosidase-conjugated mouse anti-human IgG4 antibody. The validated assay was used to detect anti-ESA IgG4 in amPRCA and non-PRCA patients. The immunoassay detected 15 ng/mL of human anti-EPO IgG4 antibody in the presence of a 200M excess of human anti-ESA IgG1, IgG2, or IgM antibody and tolerated 2 µg/mL of soluble erythropoietin. All patient samples with confirmed amPRCA had measurable anti-ESA IgG4 antibodies. In addition, 94% (17/18) of non-PRCA patient samples were antibody negative or had below 15 ng/mL of anti-ESA IgG4 antibodies. This novel immunoassay can measure low-nanogram quantities of human anti-ESA IgG4 antibodies in the presence of other anti-ESA antibodies. An increased concentration of anti-ESA IgG4 antibody is associated with the development of amPRCA. We propose that the measurement of anti-ESA specific IgG4 antibodies may facilitate early detection of amPRCA in patients receiving all ESAs structurally related to human erythropoietin.

Palladium colloid for immunochromatography marker.

Watabe M. Winered Chemical Corporation, Tokyo, Japan.

Syntheses of precious metal colloids

By chance, we discovered a reddish solution that contained nano-size gold colloids during the course of preparation of a gold complex containing oligopeptides. Since then, various methods to synthesize precious metal nanoparticles were developed by us. Here we describe a method to produce new precious gold or palladium colloids, and immunochromatography tests of the new palladium colloid.

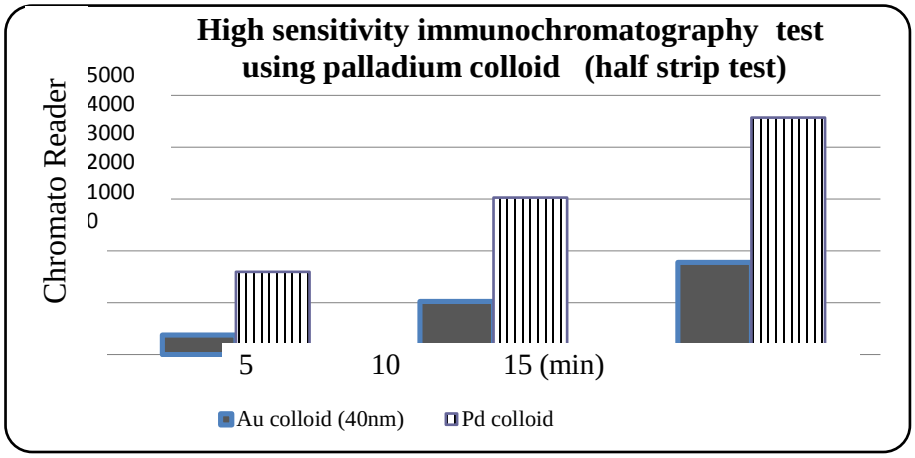
The colloidal particles of gold are extremely stable and do not condense at a high concentration even after half a year at room temperature. The synthetic method is simple. We added amino acids (or peptides, or glucosamine) and alkaline solutions to an aqueous solution of precious metal ions, and then applied heat to the solution for several minutes. Gold, palladium, and platinum colloids were synthesized. It is known that peptides attach strongly to gold colloids and prevent the colloids from agglutination. The colloidal particles are protected with an oligopeptide oxide.

Conjugation of the oligopeptide-based metal colloid with an antibody

During conjugation using these colloids, the oligopeptide is released from the metal particles and an antibody (large peptide) attaches to the colloid metal.

Immunochromatography tests of the new palladium colloid

The antibody-palladium colloid conjugate was easily obtained in a high yield, as was the conjugated gold colloid. The conjugated palladium colloid is as stable as the conjugated gold colloid. In an immunochromatography test, the palladium colloid lines can be detected more clearly than the gold colloid lines.



Novel sandwich immunoassay for quantification of 25-hydroxy vitamin D in human blood.

Omi K, Ando T, Sakyu T, Sato H, Oka A, Hotta Y, Horiike M, Shirakawa T, Goishi K. Fujirebio Inc., Tokyo, Japan.

Vitamin D is well known for its role as an important regulator of calcium homeostasis and bone remodeling, and also known to affect the development of several non-bone diseases. 25-hydroxy vitamin D (25OH-D) is considered to be the best vitamin D marker, and 25OH-D can be measured by using competitive immunoassay, HPLC, and liquid chromatography tandem mass spectrometry. As the number of tests increases, demand for an automated 25OH-D assay continues to grow, but the automated immunoassays available from various diagnostic manufacturers reportedly fail to meet accuracy and specificity requirements.

Here we show a novel sandwich immunoassay for 25OH-D using an antibody that specifically recognizes an immunocomplex consisting of 25OH-D and anti-25OH-D antibody. Immunocomplex was formed by mixing specimens with anti-25OH-D antibody-conjugated magnetic beads in treatment solution. The immunocomplex was quantified using an alkaline phosphatase-labeled second antibody against the complex. All reactions were executed on fully automated chemiluminescence analyzer (LUMIPULSE, Fujirebio Inc.). The assay specifically detected 25OH-D immunocomplex in a dose-dependent manner, and demonstrated significant correlation with the commercially available Total Vitamin D RIA assay (Pearson's correlation coefficient, $r > 0.98$). Total assay CVs for 14 ng/mL, 39 ng/mL, and 97 ng/mL samples were 2.2%, 1.9%, and 3.3%, respectively.

Assay performance was significantly improved by converting the immunoassay principle from competitive to sandwich. Using two antibodies, our assay showed improved specificity against immunoreactive derivatives such as 24,25(OH)2-D, which can be present in human serum and known to cross-react with antibodies used in most commercially available immunoassay kits. Our novel sandwich immunoassay platform would provide high-throughput, accurate and specific immunoassay for 25OH-D, leading to the breakthrough in routinely performed 25OH-D assay.

Novel chemiluminescence immunoassay platform for small molecules using immuno-complex antibodies: proof of concept in E2, FT3 and vitamin D assay with high sensitivity and specificity.

Sakyu T, Ando T, Oka A, Takemura F, Shirakawa T, Goishi K, Omi K. Fujirebio Inc., Tokyo, Japan.

Small molecules immunochemically classified as haptens are measured with competitive immunoassay, which is theoretically inferior to noncompetitive sandwich immunoassay in sensitivity and specificity. Developing sandwich immunoassay format for haptens would lead to the breakthrough in small molecule analyses, enabling ultra-sensitive and more specific and accurate assays. However, conventional sandwich immunoassay cannot be applied for measuring haptens, because haptens are too small for two antibody molecules to bind simultaneously. Although some reports have been made on the sandwich immunoassay for haptens using antibodies that react with hapten-antibody complex, it is virtually impossible to obtain such antibodies systemically by conventional methods. Moreover, no automated assays for haptens using such antibodies have been reported so far.

Here we demonstrate a novel sandwich immunoassay format for haptens using ADLib (Autonomously Diversifying Library) antibodies. ADLib system, developed by Chiome Bioscience Inc., is an in vitro antibody generation system using antibody library generated by activating immunoglobulin gene diversification of chicken-derived DT40 cell. Using ADLib antibodies that specifically recognize hapten-antibody immunocomplex, we have successfully established sandwich immunoassays for haptens including estradiol, triiodothyronine and 25-hydroxyvitamin D with high sensitivity and specificity. These assays exhibited >100-fold sensitivity as compared to the conventional competitive assay, and cross reactivity with immunoreactive derivatives has been drastically improved. Furthermore, these assays are applicable to fully automated chemiluminescence analyzer (LUMIPULSE, Fujirebio Inc.). These results suggested that our platform enables the systematic establishment of high-throughput sandwich immunoassays for haptens with highly improved sensitivity and specificity. Our hapten sandwich immunoassay platform should be the simplest and most practical approach for routine assays of haptens including hormones, vitamins, drugs and toxins, leading to the breakthrough in analytical/clinical chemistry.

Analysis of multiplex assays using a common bead and slide surface.

Jennins D, Chung E, Cooper SJ, Fontanelle BT, Gao Y, Koudijs MM, Yang L, Ling T, Vukovic P, Wong A, Maeji NJ. Anteo Diagnostics Ltd., Brisbane, Australia.

To simplify protein binding for immunoassays and bioseparations, metal polymer solutions call Mix&Go™ were developed. These aqueous polymer solutions bind to any surfaces having electron-donating potential to form an activated surface within minutes. In turn, this activated surface will bind proteins in minutes, or this activated surface can be stored for years with no loss of protein binding activity. Mix&Go represents a “one-size-fits-all” surface chemistry approach in situations where maximum protein performance and uniformity at the surface interface is of importance.

Mix&Go can activate particles such as iron oxide nanoparticles (IONPs), gold colloids, latex, and many other particles while minimizing their tendency for aggregation. As well, Mix&Go can activate planar surfaces such as bare silica, ceramics and polystyrene surfaces commonly used in array applications. Regardless of the underlying material or whether the format is a particle, film, or slides, Mix&Go creates a common chelating surface for protein binding.

To determine whether antibodies bound to Mix&Go surfaces behave the same regardless of the underlying material, multiplex assays were tested on both Mix&Go activated Luminex beads and glass slides. Each exemplifies the two most common approaches to multiplexing but with one, the underlying surface is latex polymers with antibody coupled beads stored in solution. The other is glass, a material that usually needs to be well-coated to protect protein stability, and antibodies are stored dried on the surface. There are other differences between the two formats so with a common set of capture antibodies, the influence of these differences were investigated to determine whether the patterns of outcomes of a multiplex assay were affected by the underlying material. Since it is well known that the performance of antibodies are affected by the mode of binding, this multiplexed data from Mix&Go beads and glass surfaces were compared to conventional amide (Luminex beads) and epoxy (glass slides) chemistries.

Development of a wireless-enabled point of care platform for biomarker detection using Surface Acoustic Wave biosensors.

Lawson V[1], Athey D[1], Yatsuda H[2], Kogai T[2]. [1]OJ-Bio Ltd., Newcastle-upon-Tyne, UK, [2]Japan Radio Co. Ltd., Tokyo, Japan.

We describe a novel platform for the measurement of a variety of biomarkers (protein, virus, bacteria) in various sample types using Surface Acoustic Wave (SAW) biosensors.

The platform allows for measuring single or multiple targets on disposable biochips using a hand-held bio-electronic device that can be connected to a smartphone. This enables immediate communication of the results to healthcare professionals for clinical management decisions and patient treatment selection. The technology has many applications for rural or remote settings, and is equally applicable to use in high street or walk-in clinics and consumer diagnostic applications.

The biosensors combine traditional immunoassay formats and novel biotechnology with SAW technology to create a quantitative analyte-specific biochip. Patient sample (nasal swabs, serum, whole blood, urine, saliva or wound fluid) is added directly to the biochip surface and the signal recorded by the hand-held reader. The versatile biosensor converts recognition events (equivalent to analyte concentration) on the biochip to an electronic signal. Results are provided automatically at the end of the test (typically ≤ 5 minutes) along with ID, date/time and location. The individual test steps are customised for each analyte, depending on assay requirements and sensitivity. The self-contained SAW biochip, low cost, quick result time and wireless connectivity make the hand-held biosensor ideally suited for rapid testing at the point of use.

OJ-Bio is developing biochips to detect multiple markers for a variety of disease states. Here we demonstrate detection of viruses, bacteria, and protein biomarkers. The performance of the biosensor is compared with laboratory based methods and commercially available POC tests.

A handheld, rapid, sensitive electrochemical immunoassay point of care system.

Porter R, Hutchinson E, Chard M, Wilson P. AgPlus Diagnostics, Bedfordshire UK.

ELISA and clinical lab analyzers are routinely used for carrying out immunoassays where robust, reliable results are required for clinical decisions to be made. With the changing landscape in healthcare provision, the need for a more mobile, rapid and responsive diagnostic tool that can be used in a range of environments has become much greater, whilst still delivering results that can be used for clinical treatment decisions, as provided by the current laboratory-based systems.

We have addressed the challenge of developing a point of care (PoC) system that will meet the sensitivity of central laboratory analysers in its assay system alongside being a portable, rapid diagnostic tool. The work has resulted in the development of an electrochemical metalloimmunoassay based on silver nanoparticles. The assay developed is run on a single-use fluidic chip in conjunction with the portable device to deliver clinical results in 10 minutes or less.

As stated, the system employs silver nanoparticles as an electrochemical label and magnetic particles as the solid phase, with the assay run on a fluidic chip. The chip contains all required assay reagents. The silver and magnetics conjugates are dried down on the chip's reaction chamber, and the wash buffer and silver reading solution held in fluid filled blisters, deployed by the actuation system contained in the handheld device during the assay sequence. The non-optical detection system removes the need for sample preparation prior to analysis.

A troponin I sandwich assay has been demonstrated on the system. In serum the assay is showing results of 1 ng/mL-50 ng/mL with CV<20% in un-optimised assay. The improvement that the system brings to the clinical environment for a PoC system comes from the combination of the electrochemical detection system with the sliver nanoparticles.

Ammonium thiocyanate is the active agent for the silver to be measured, giving a greater increase in signal amplification in small sample volumes. Ammonium thiocyanate cleaves the silver nanoparticles from the complex and forms a negatively charged monolayer around the silver nanoparticle, which is drawn down to the sensor under a positive electrical potential. The nanoparticle are converted to metal ions that are electro-active, for each 40 nM nanoparticle it can be converted to $1x10^{12}$ ions thus allowing for signal amplification. The amount of silver particles is directly proportional to the amount of analyte in the sample.

The electrochemical detection system only requires low power consumption and much lower sample volumes can be utilised for the assays, combining the benefit of the system to be developed in a compact nature needed for PoC systems

The system has been developed as a platform technology and has wide applications in a range of settings where rapid, accurate diagnostics can allow for quicker clinical decision to be made. The platform also shows promise for single chip multiplexing with other metallic particles, such as gold and copper. The technology also has potential applications in molecular diagnostics given its sensitivity of detection.

Next generation handheld / point of care diagnostics platform.

Duer R, Chang YP, Sabet L. PLC Diagnostics Inc., Chatsworth, CA.

Challenges in measuring proteins for clinical diagnostics at the point of care include high sensitivity, reliable results and turnaround time. Existing technologies often deliver one attribute at the expense of the other. In addition, a majority of the existing point of care technologies are inherently incompatible with multiplexing. PLC Diagnostics is finalizing the development of a revolutionary detection platform based on the innovative and proprietary "FENT" technology. FENT has exquisite sensitivity, offers inherent multiplexing, has 5-15 minute turnaround time, and can run proteins and nucleic acids. It is easy to use, compact, portable and inexpensive.

The FENT platform is a waveguide-based optical sensing platform, where excitations of fluorescent molecules and collection of corresponding fluorescent signals occurs on waveguides fabricated on a silicon chip. The waveguides form microarrays of 10 channels or 100 wells that allow for testing of 10 or 100 different analytes from the same sample. With FENT technology, the rate of increase in the fluorescent signal can be monitored in real time. The signal comes from the binding of analytes to the capture layer on the waveguide, and is proportional to the concentration of target analyte in the sample.

The two main components of the FENT technology are a small disposable cartridge containing a specially designed silicon chip, and a cell-phone size reader. A small volume of sample (blood, urine etc.) is added to the cartridge where it is mixed with on-board reagents and delivered to the surface of the silicon chip. Unlike most other assays there is no need for washing steps. The results can be quantitative or qualitative. In 5-15 minutes, results are displayed on the screen of the reader and can also simultaneously be transmitted to "the cloud" to be made available for patients, physicians and other relevant service providers.

The handheld prototype has been tested using clinically relevant analytes such as cTnI, IL-1β, *C. Difficile* toxin A, and S100B. Performance characteristics of the platform along with results from these biomarkers are discussed in the presentation.

On the path to a random access LC/MS workflow - A novel approach to calibration.

Cooper DP, Molloy BJ. Waters Corporation, Manchester, UK.

Introduction: Quantitative LC/MS usually requires the analysis of multiple matrix-matched calibrators before the concentration of analyte in a sample can be determined. This consumes valuable instrument time and resources and accounts for a large proportion of the time to first result. Conventional calibration also limits LC/MS to a batch mode of operation. A new method of calibration is proposed (Internal Calibration) that can overcome these limitations by using stable isotope labeled (SIL) and/or analogue calibrators that are added directly to the sample. A quantitative result can be obtained from a single analysis, thereby allowing the possibility of random access operation.

Aim: To demonstrate the feasibility of Internal Calibration for routine LC/MS/MS analysis.

Methods: Internal Calibrator (IC) mix was added to the samples with each IC representing a different known concentration of the target analyte (Table 1). The responses of the ICs and the analyte were measured in a single LC/MS/MS analysis and an individual calibration curve constructed for each sample using those responses, thereby allowing the analyte concentration to be determined from the data obtained from that single analysis.

Results: Forty-five de-identified individual serum samples and one pooled sample were analysed using both a conventional quantitative LC/MS/MS procedure and using the Internal Calibration approach with 3 SIL calibrators. There was good agreement between the results (slope = 0.966, $R^2 > 0.99$, mean bias -0.09 ng/mL). Replicate analyses of the pooled sample suggested within-day imprecision < 3% at 2.3 ng/mL (N=5).

Table 1: Internal Calibrator characteristics

Analyte or Internal Calibrator	**Concentration (ng/mL)**	**MRM Transition**
Analyte: testosterone	-	289.25 > 96.9
IC1 testosterone-d2	0.2	291.25 > 98.9
IC2 testosterone-d3	4.0	292.25 > 96.9
IC3 testosterone-d5	10.0	294.25 > 99.9

Conclusion: These preliminary data suggest that Internal Calibration may be a viable alternative to the traditional external calibration method. The benefits may include simplified calibrator preparation, improved assay throughput, perfect matrix matching, reduced time to results and the potential to develop routine random access LC/MS platforms.

For Clinical Research purposes, not for use in Diagnostic Procedures.

Direct detection of bacterial DNA and viral RNA at subfemtomolar concentrations using single molecule arrays (Simoa).

Song L, Shan D, Zhao M, Pink BA, Minnehan KA, York L, Gardel M, Sullivan S, Duffy DC.
Quanterix Corporation, Lexington, MA.

We describe a method capable of direct detection of genomic DNA and RNA extracted from bacteria or viruses at subfemtomolar concentrations with no necessary target amplification using polymerases. This approach includes first generating short nucleic acid fragments from large genomic DNA/RNA molecules either via random sonication or digestion using specific restriction enzymes. For DNA molecules, heat-denaturation is used to generate single-stranded fragments for capture. The method then involves the formation of hybridized complexes through capturing the fragmented target DNA/RNA on magnetic beads via sequence-specific capture probes, and hybridizing to multiple biotinylated detection probes, followed by labeling the resulting hybridized DNA/DNA or DNA/RNA complexes with streptavidin-conjugated beta-galactosidase. Signals from single labeled nucleic acid molecules on beads are generated by converting the substrate of beta-galactosidase, resorufin-β-D-galactopyranoside, into a fluorescent product in single molecule arrays (Simoa). The resulting signal is reported as average number of enzymes per bead (AEB) that is correlated to the concentration of nucleic acid in a sample. We have demonstrated the detection of genomic DNA purified from *S. aureus* with an average limit of detection (LOD) of 0.07 fM, equivalent to 2,100 DNA molecules per 50 µL sample; and genomic RNA purified from Sendai virus with an average LOD of 0.09fM, equivalent to 2,700 RNA molecules per 50 µL sample. We also applied this Simoa technology to clinical and environmental samples for bacteria detection. An average sensitivity of 2,200 bacteria per 50 µL sample (0.074fM) and 1,300 bacteria per 50 µL sample (0.042fM) were obtained for *S. aureus* spiked into human whole blood and river water, respectively. The capability of detecting single enzyme molecules using Simoa enables the direct detection of target DNA and RNA without requiring target amplification, and provides a highly sensitive alternative approach to polymerase chain reactions (PCR) that is much less susceptible to carryover.

Stimuli-responsive reagents enable rapid biomarker capture and separation.

Nehilla BJ[1], Lai JJ[2], Stayton PS[2], Schulte TS[1]. [1]Nexgenia Inc., Seattle, WA; [2]University of Washington, Seattle, WA.

Stimuli-responsive polymers change their properties (i.e., from hydrophilic to hydrophobic) in response to environmental triggers such as temperature, solution ionic strength or pH. Biomolecules and nanoparticles modified with these polymers exhibit similar stimuli-responsiveness. We developed a novel reagent system that combines stimuli-responsive polymers with magnetic nanoparticles and biomolecules for immunoassays and other bioseparation applications. The new reagent system enables rapid recognition of target molecules in the hydrophilic state and rapid magnetic separation (less than 30 seconds) of the captured molecules in the hydrophobic state.

Here we demonstrate several strategies to synthesize stimuli-responsive polymer-biomolecule conjugates, including end-functionalization and new di-block copolymer compositions. New conjugation strategies provide control over the grafting density and functionality of the polymer-biomolecule conjugates. The resultant conjugates isolated clinically relevant targets such as protein antigens and oligonucleotides from spiked serum samples with approximately 95% capture efficiencies. In serum samples containing 150 pg/mL p24 HIV-1 antigen, the stimuli-responsive reagent system captured 90% of p24 within 1 minute of binding at 37°C following the addition of an appropriate aggregating stimulus. This is a significant improvement over the commercial magnetic microparticle reagents, which required more than 15 minutes to achieve the same level of p24 binding.

We therefore conclude that the stimuli-responsive reagent technology can potentially be utilized in existing clinical immunoassay analyzers to increase both the speed and sensitivity of immunoassays by enabling rapid and efficient biomarker separation.

A novel multiplex molecular detection technology for use at the point-of-care.

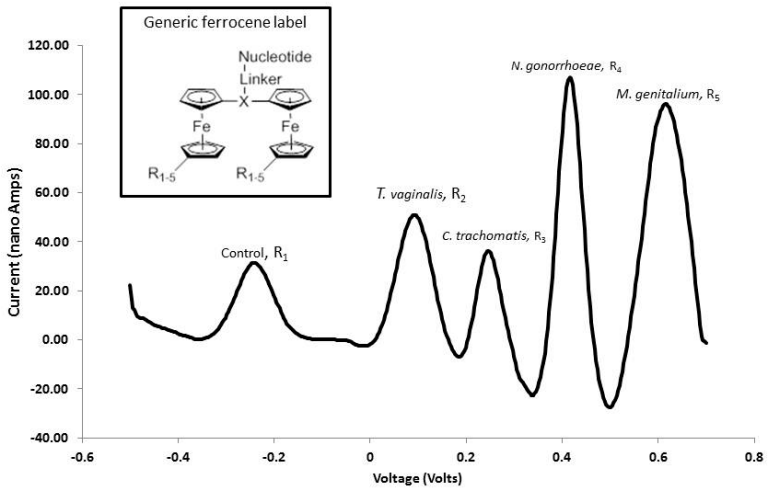
Pearce DM, Marsh BJ. Atlas Genetics Ltd., Trowbridge, UK.

Our challenge was to develop a novel, universal molecular assay methodology that would be rapid, low-cost, robust, and suitable for use at the point of care. Emphasis was placed on achieving multiplex analyte detection, as well as providing both qualitative and quantitative results. To meet these fundamental criteria, we have developed a detection technology that uses electrochemically-active compounds as labels, combined with specific exonuclease cleavage, to detect target DNA extracted from clinical samples within 30 minutes.

A range of novel electrochemically-active labels has been synthesised, each of which oxidases at a discrete voltage over a wide potential sweep when coupled to oligonucleotide probes. After target DNA amplification by PCR the electrochemical probes hybridise to the amplified DNA followed by double-strand specific DNA exonuclease cleavage of the probe/target duplex. The electrochemical label, bound to the released terminal nucleotide, is specifically detected on screen-printed electrodes without interference from any unbound labeled probe.

This novel detection technology has been applied to the multiplex detection of sexually transmitted infections (STIs) in patient samples. Five electrochemical probes have been used to demonstrate simultaneous detection in less than 30 minutes of four clinically important STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and *Mycoplasma genitalium*) and a control. Detection of fewer than 10 genome copies has been routinely observed for this series of analytes. The methodology has been shown to be capable of both qualitative and quantitative performance over a range of targets and analyte concentrations. While demonstrated here for an STI panel, this unique high-sensitivity technology is universally applicable for the detection of any nucleic acid target.

The technology is being developed commercially to operate within a fully-integrated fluidic cartridge combining nucleic acid extraction, amplification and detection. The cartridge, containing all assay reagents, will be run on an associated instrument platform and requires no user interaction following sample addition.



Five-multiplex electrochemical detection of four STI targets plus control from a single sample

Ultra-high sensitivity POCT system based on Optimiser technology.

Han J[1], Kai J[1], Puntambekar A[1], Lee S[1], Santiago N[1], Ahn C[1,2]. [1]Siloam Biosciences, Cincinnati, OH; [2]University of Cincinnati, Cincinnati, OH.

This abstract reports the development of the first-ever "open source" point-of-care test (POCT) system based on Siloam's Optimiser™ technology platform. The POCT system and microfluidic test cartridge have been recently used to demonstrate:

1. High-sensitivity cardiac Troponin I (hs cTnI) 20 minute assay with detection limit of **0.04 ng/mL** – making this one of the most sensitive POCT instruments for hs cTnI detection.
2. Wide-dynamic range β-hCG (total) 15 minute assay with operating range of **5 IU/L to 68,500 IU/L** – the first POCT to report such broad dynamic range.

The newly developed POCT system uses two key components – a microfluidic reaction cartridge and a modular analyzer system. The analyzer contains a precision liquid-handler, high-sensitivity chemiluminescence detection module and linear stages for cartridge positioning. Test-specific assay reagents (antibodies/controls) are stored in dry form on the cartridge and all assay buffers are placed in liquid form in the system. The assay buffer pack is designed to allow for a full day of operation.

As part of preliminary validation, for both hs cTnI and β-hCG; multi-day calibration curves were generated showing excellent reproducibility confirming that stored calibration curves are suitable. Separate calibration curves were developed for plasma/serum and whole blood samples. 18 patient plasma samples were tested for β-hCG in correlation study against Siemens ADVIA Centaur system. Excellent correlation and linearity were achieved (R^2=0.99, slope = 0.98). Spiked whole blood samples were also tested at six different concentration levels against plasma extracted from spiked whole blood samples to demonstrate consistent offset [Conc.(plasma) = 1.81xConc. (whole blood)]. Twelve patient plasma samples were tested for cTnI in a correlation study against results from Siemens VISTA dimension system. Good correlation was achieved (R^2 = 0.97) and the bias offset indicates that Siloam's POCT reads out samples ~2.7 times higher than Siemens system (bias-adjusted cTnI sensitivity on Siloam's POCT is 0.014 ng/mL). The sensitivity of Siloam's POCT assay can be "tuned" further to <0.01 ng/mL by using the innovative "repeat sample load" feature (where multiple aliquots of sample are sequentially incubated for increased sensitivity).

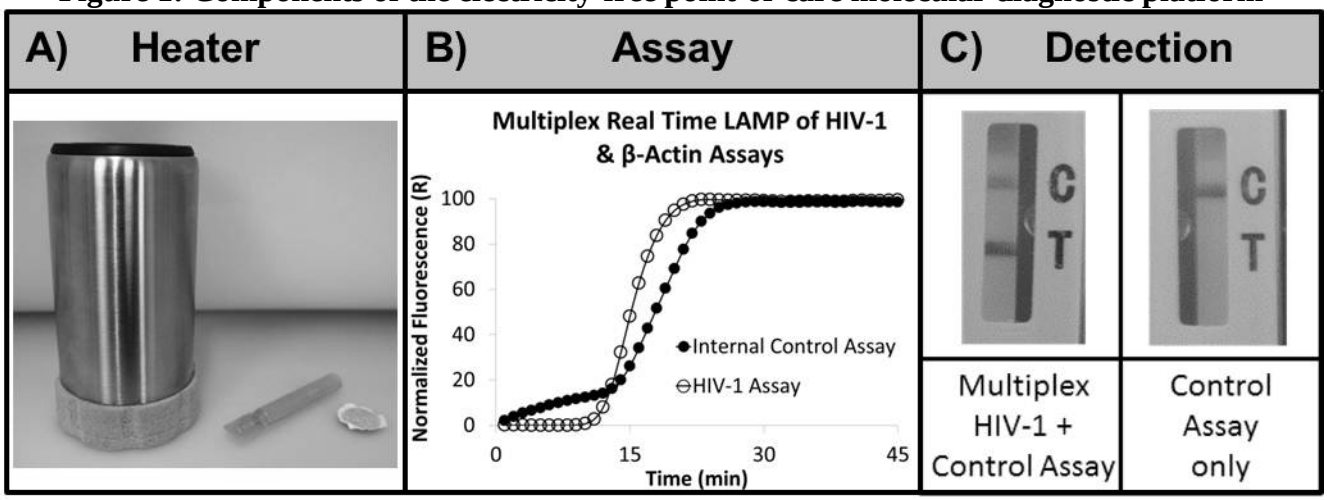
A key advantage of the system is the "open source" architecture that allows for new ELISA tests to be developed rapidly on this system. The microfluidic reaction cell in the POCT test cartridge is exactly identical to the microfluidic reaction cell on the OptiMax™ 96-well microplate (commercialized). We have demonstrated over 50 assays on the OptiMax plate with superior sensitivity and/or assay time over the same assays on conventional microplates. Hence, assays can be developed and exhaustively validated using lab-automation systems and optimized assays are then migrated with minimal effort to the open-source POCT system. The identical reaction cell ensures that the same performance can be achieved with the POCT system. This allows new POCT assay development time to be reduced from 12-18 months to less than three months (applicable to ALL existing conventional microplate based assays).

Point-of-care multiplexed molecular amplification and visual detection for HIV-1 and beta-actin without electricity.

Osborn JL, Singleton J, Hawkins K, Guelig D, Price W, Johns R, LaBarre P. PATH, Seattle, WA.

The lack of point-of-care (POC) molecular in vitro diagnostic (IVD) tests is a critical barrier to timely diagnosis and treatment of infectious diseases such as HIV in the developing world. Molecular HIV IVD tests enable acute case detection and early infant diagnosis, unlike immunodiagnostic tests commonly employed for HIV detection in the developing world. Isothermal nucleic acid amplification methods are amenable to POC molecular IVD tests since they significantly reduce device complexity and power requirements. PATH has developed a robust device for POC nucleic acid amplification that provides controlled isothermal heat by thermally coupling an exothermic reaction and phase change material in a low-cost, compact, and easy-to-use design. Herein, we demonstrate electricity-free isothermal amplification of HIV-1 and an internal amplification control—beta-actin, a housekeeping gene—followed by electricity-free signal detection using a nucleic acid lateral flow (NALF)-detection cassette (BioHelix Corporation). We modified the primer design of an HIV-1 LAMP assay developed by Hosaka, et al (*1*) to incorporate two tagged primers, which in the presence of a target amplify to form dual-tagged amplification products capable of specific downstream detection in a lateral flow format. Additionally, we designed an internal beta-actin control LAMP assay using the same tagged primer approach for multiplex amplification of both the HIV-1 and beta-actin targets in less than 30 minutes. The multiplex reaction can be detected in a single NALF-detection cassette (BioHelix, BESt™ type II), a fully enclosed cassette that prevents environmental amplicon contamination and contains two distinct amplicon capture lines. The combination of LAMP amplification coupled to NALF detection enables POC electricity-free nucleic acid amplification to detection in less than 40 minutes, with a degree of robustness not previously demonstrated. This work is a substantial advancement towards realizing an electricity-free POC molecular IVD test appropriate for use in low-resource settings.

Figure 1: Components of the electricity-free point-of-care molecular diagnostic platform



(a) Heater device design, pre-filled saline tube and fuel pouch required for a single run; (b) real time LAMP amplification plots for HIV-1 (○) and beta-actin (●); and (c) NALF cassette with dual amplicon capture lines (Test [T] and Control [C]). The image on the left shows multiplex HIV-1 (T) and beta-actin control (C) detection and on the right, the beta-actin control (C) detection only.

1. Hosaka N, Ndembi N, Ishizaki A, et al. Rapid detection of human immunodeficiency virus type 1 group M by a reverse transcription-loop-mediated isothermal amplification assay. *J Virol Methods.* 2009;157:195-9.

Development of Minicare point-of-care cardiac troponin-I test.

Nieuwenhuis JH[1], de Theije FK[1], Immink AHJ[1], Dekkers DWC[2]. [1]Philips Handheld Diagnostics, Eindhoven, the Netherlands; [2]Future Diagnostics BV, Wijchen, the Netherlands.

Cardiac troponin I (cTnI) testing with a recommended turnaround time (TAT, time from blood draw to reporting of result) of ≤1 hour is a key element in the evaluation of patients with chest pain and suspected acute coronary syndrome. We aim to develop a point-of-care (POC) test for cTnI that works from a single droplet of whole blood or plasma with a TAT of less than 10 minutes.

The Minicare cTnI POC test will be a one-step sandwich immunoassay that takes place in a compact plastic disposable cartridge with on-board dry reagents. The assay is based on the Magnotech technology [*1*] which uses actuated magnetic nanoparticles as labels; detection is based on frustrated total internal reflection. The disposable cartridge was shown to be suitable for both whole blood and plasma samples [*2*].

cTnI measurements were performed on a panel of 70 plasma samples comparing our test with the Beckman Coulter Access AccuTnI. The outcome demonstrates a good correlation (R=0.98) between the two systems. All measurements were performed in duplicate to estimate the precision profile. From this curve the limit of quantification at 20% and 10% could be estimated at 35 ng/L and 112 ng/L respectively. Preliminary results show the potential to develop a Minicare cTnI POC test with competitive specifications.

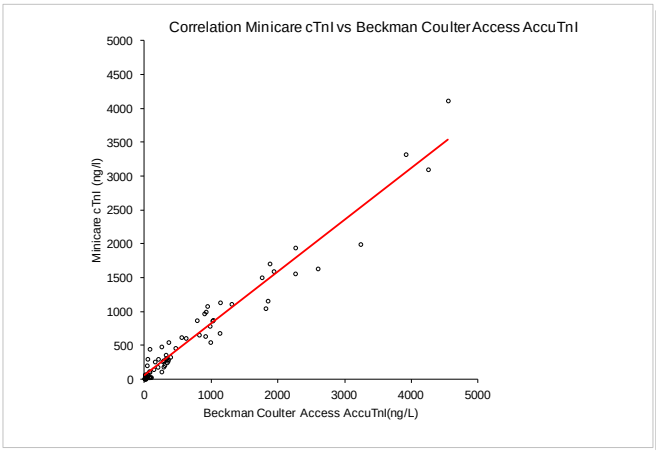


Figure 1. Correlation with the Beckman Coulter AccessTnI

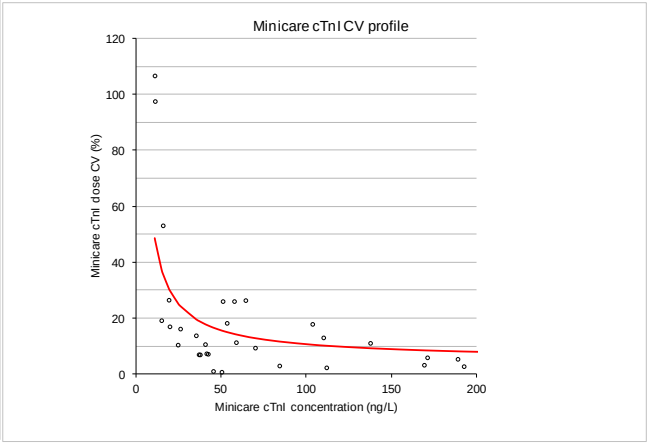


Figure 2. CV profile for the Minicare cTnI test

References:
1. Bruls et al. *Lab Chip* 2009;9:3504-10.
2. Nieuwenhuis et al. 'Handheld analyzer based on Magnotech Poster presentation at 43rd AACC Oak Ridge Conference 2011.

Optimization and characterization of on-board dispensing and mixing on batch-fabricated lab-on-a-chip devices.

Ehrlich PS, Podczerviensky J, Levine LM. ALine Inc., Rancho Dominguez, CA.

Microfluidics-based lab-on-a-chip strategies are increasingly important for the development of quantitative, multiplexed diagnostic tools that can be used near the patient and deliver actionable results within thirty minutes. The development of lab-on-a-chip products is often challenged by the high-cost and long turnaround times associated with traditional injection molded tooling. A non-tooling process, the integrative polymeric platform (IPP) lowers the barrier to commercialization by shortening the design/build/test cycle from months to days. The design strategies and processes are scalable for high-volume, roll-to-roll manufacture. Understanding performance variability that results from alignment tolerances and process variability is important for quality control, and knowing the limits of operation of a particular design. In this paper, we describe the performance of our valves, vents, and pumps using a pneumatic instrument platform to optimize actuation routines and measure pumping and mixing performance and variability.

IPP incorporates a variety of standard commercially available materials to build layered devices that incorporate on-board operations such as venting, filtering, pumping, and mixing, blister packs and reagents. The valves and pumps are controlled through the ADEPT, an instrument developed for pneumatic control that runs on house air (or vacuum) with eight independent outputs for pressure or vacuum. The outputs are routed to a manifold that interfaces with the chip and can be observed under a video camera or measured on an optical comparator.

Measurement of on-board pump variability was performed by recording the displaced volumes over several repetitions. Time optimization was accomplished by varying the time period of the actuation routines until the displaced volume varied significantly from the expected volume, or a significant increase in pumping variability was measured. Mixing was characterized by observing the mixing of a coloring changing dye, cresol red, when combined with sodium hydroxide, shifts from yellow to purple. The change in the absorption spectrum was measured using an Ocean Optics USB spectrometer.

Pumps and valves were operated at pressures of positive and negative eight psi. The average pumped volume was 4.8 µL/stroke with a standard deviation of less than two percent. Metering channels (graduated at 0.2 µL/mm) measured pump variability. Inter- and intra-device variability were measured across three devices and both were found to be lower than two percent. Valve actuation time was reduced to 50 milliseconds without performance losses.

Upon combination of 1mM cresol red with 1 mM NaOH into one chamber, a single stroke of the on-board mixing element resulted in complete mixing. Mixing was also aided by turbulent flow upon entry into the detection chamber through the device vial. Further studies with more viscous solutions will be explored to reduce the diffusion coefficient.

In this poster, we report on the inter- and intra device variability of a lab on a chip test device that performs metering, mixing and dispensing using standard designs, fabrication processes, and actuation routines.

A novel infrared imaging spectroscopy applicable to diagnostics.

Tao R. Kagawa University, Takamatsu, Japan.

Fourier transform infrared spectroscopy (FTIR) has been one of the most powerful tools and has quite a few applications to the diagnostics, both in-vivo and in-vitro. They include the identification of tumor cells in human organs and analysis of the components in blood, urine, and other interstitial fluids. So far, FTIR has been equipped with a Michelson interferometer where light from the polychromatic infrared source is split and coupled again. The varying difference in the optical path length between the two split light beams results in the interference when they are coupled again. Although FTIR is so powerful, the Michelson interferometer makes its equipment less practical to obtain infrared spectra and carry out diagnostic tests in a mobile manner. Nowadays, these tests can be carried out in laboratories.

We have developed a novel infrared imaging spectroscopy technology which, implemented in a hand-held instrument or so, can be applied to many diagnostic tests carried out not only in laboratories but also in a mobile manner. The spectrophotometer, consisting of no moving parts but of just a beam slit, an objective lens, a phase shifter and an imaging lens, generates an interferogram of the light coming from every point all at once along a line on the focal plane in the object.

The parallel light beam collimated by the objective lens of the light from the imaging line in the object plane partly goes through the wedge glass and, together with that through the cuboid glass, focused again by the cylindrical imaging lens. The different light path length between the light beam going through the wedge glass and that going through the cuboid glass results in an interferogram generated along the line in the detector perpendicular to the imaging line. Each interferogram is Fourier transformed to the spectrum.

In this spectrophotometer, no interferograms are recorded of the lights from the objects located in the plains other than the focal one, because the coupled light beams interfere only when they are focused and the interferogram can be detected only when the interference takes place on the detector. This feature should help ensure high signal-to-noise ratio spectra of the light coming from a tiny part of the organs like blood vessel thoroughly separated from the lights originated from the other parts.

An additional mechanical scanning can make it generate a two dimensional infrared spectrum within several seconds. In addition, when equipped with the vertical moving capability, a three dimensional infrared spectrum can be obtained within a minute or so. The potential applications of this novel infrared imaging spectroscopy include non-invasive glucose monitoring, three dimensional spectroscopic funduscopy, and spectroscopic endoscopy.

In conclusion, we have developed a novel infrared imaging spectroscopy technology which is applicable to diagnostic tests carried out not only in laboratories but also in a mobile manner.

An increase in the sensitivity of HIV-1 p24 ELISA using a photochemical signal amplification.

Bystryak S, Santockyte R. Allied Innovative Systems LLC, Hillsborough, NJ.

The industry standard technology enzyme-linked immunosorbent assay (ELISA) is widely used in biology and medicine for detection of viruses, bacteria, cancer markers, and other physiologically active substances. It is used routinely in research, clinical and pharmaceutical laboratories. However, in many cases the sensitivity of ELISA is inadequate. Thus, increasing the sensitivity, accuracy and general performance of these assays offers a significant opportunity to address unmet clinical needs. Our AmpliFlux technology improves the sensitivity and accuracy of numerous diagnostic assays.

Based on the combination of its patented and proprietary technologies, Allied Innovative Systems (ALLIS) has developed the AmpliFlux method for drastically increasing the sensitivity of ELISA-based assays. AmpliFlux is based on the use of a unique photochemical amplification reaction, and consists of two steps. The first step is a conventional ELISA. In the second step, AmpliFlux Reagent solution is added to the substrate solution and the mixture is irradiated by visible light (*1*). Illumination of the samples leads to a drastic increase in the final signal.

In order to demonstrate that the AmpliFlux method allows one to increase the sensitivity of determination of HIV-1 p24 antigen, the reagents from the Alliance® HIV-1 p24 ELISA kit (Perkin Elmer Life Sciences, Boston, MA) for detection and quantification of the major structural core component of HIV-1 virus were used. This kit is used to carry out p24 quantification assays in two formats: ICD and non-ICD. In the immune complex disruption (ICD) assay format, the immobilized monoclonal antibody captures both free HIV-1 p24 and that which has been released upon disruption of immune complexes in serum or plasma. In the non-ICD format, serum/plasma samples do not undergo immune complex disruption. The non-ICD assay format is used for detecting early infection during the earlier part of the window period due to its high analytical sensitivity, whereas the ICD assay format is used to detect cases during the later part of the window period.

Perkin Elmer and our results show that the limit of detection of HIV-1 p24 for the conventional non-ICD assay equals 3.3 pg/mL whereas the detection limit for the same non-ICD ELISA + AmpliFlux is approximately 0.08 pg/mL at 12 min illumination. Thus, the analytical sensitivity of the assay increases more than 40-fold using AmpliFlux. Even more impressive, a greater than 50-fold increase in the analytical sensitivity was achieved using AmpliFlux for two modified ICD assay formats.

AmpliFlux technology allows one to increase sensitivity, accuracy, dynamic range, and signal-to-noise ratio of ELISA assays, while saving reagent costs and reducing time of analysis. Similar outstanding results have been achieved for other analytes, such as hepatitis B surface antigen, prostate specific antigen (PSA) and other physiologically active substances.

Reference:

1. Bystryak S, Santockyte R. Methods for improving analyte detection using photochemical reactions. Patent App No. 61/656336.

Highly sensitive nanotube-based point-of-care test platform

Wang F, Chen Q. BioMedomics Inc., Research Triangle Park, NC.

Introduction: Nanotechnology-based testing assays hold the potential for point-of-care (POC) test applications. Recent advances in controlled growth of nanotubes and separation of the nanotubes according to their electronic properties provides a possibility for nanotube field-effect transistor (FET) biosensing devices. There are several significant advantages for using nanoscale FET as a sensing platform compared to fluorescent sensors and other optically detected sensors, including real-time response, direct electronics detection, device simplicity and continuous monitoring. BioMedomics developed a patented POC platform which is a label-free, real-time detection of biomarkers with high selectivity and sensitivity using antibody-functionalized, carbon nanotube FET sensors coupled to lateral flow immunoassay technologies. It would work in the same way as a common and easy-to-use compact blood glucose meter.

Method: The principle of nanotube based lateral flow tests is similar like normal lateral flow assay. The only difference is that nanotube array lines are used as detection mechanism as a form of immunoassay in which the test sample flows along a solid substrate via capillary action. After the sample is applied to the test, it passes through sample processing steps, and transits the substrate encountering nanotube array lines or zones which have been pretreated with an antibody or antigen. These testing lines or zones are structured as nanotube based biosensors with specific antibodies or antigens on its surface. Depending upon the analytes present in the sample, the biomarker associated components can become bound at the test line or zone. When analytes drop into lateral flow strip, through capillary flow force, the analytes (antigen) will bind to the testing line antibody which generates an electronic signal change for the detection.

Results: We have designed and performed a number of detection experiments using our nanotube based lateral flow test device. In these experiments, we established the detection sensitivity and specificity for biomarker C-reactive protein (CRP). They were very important experiments to demonstrate our nanosensing platform utility with nanotube based lateral flow immunoassay technologies. We purchased human CRP antibodies and antigens. We established our experimental protocols and carried out experiments with picogram/mL and sub-picogram/mL range of human CRP antigens. By measuring resistance of electrical signals of nanotube based sensors, we are able quantitatively determine the concentrations of CRP biomarkers.

Conclusions: We successfully developed early version nanotube based lateral flow immunoassay platform. The platform with test strips and detection device utilized an immune reaction as a mechanism to detect biomarkers, which possessed excellent sensitivity with capability to detect biomarker CRP antigen at a concentration as low as 0.1 pg/mL. The salient features of our novel nanotube based diagnostic platform are: ultrasensitive, easy to operate, and fast to get results, and can be easily made at low cost. With future funding, we will establish other nanotube-based lateral flow immunoassay platforms and develop test strip packaging and a compact reader device for ultra-sensitive and multiplex biomarker tests. With this compact and all electrical detection platform, we anticipate broad clinical diagnostic applications which can be easily deployed as current easy-to-use compact blood glucose meters.

Selective patterning of bacteria in microfluidic channels using biphasic parallel flow.

Mu X, Zheng Z. Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China.

Background

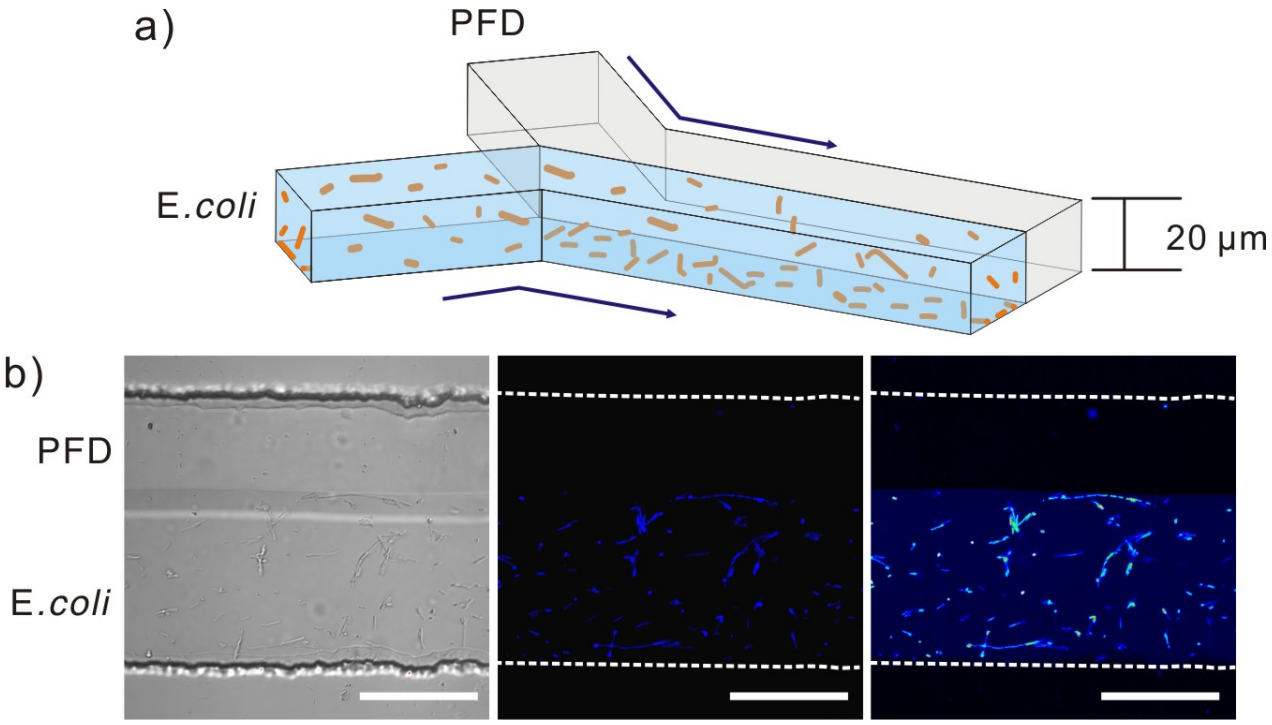
Bacteria are among the most common pathogens to cause various diseases such as typhoid fever and tuberculosis. Microfluidic patterning is a very convenient way to pattern bacteria having fluidic shape. However, it suffers from the transverse diffusion, thereby obscuring an organism's boundary and distorting its shape.

Methods

Here we develop a method to employ biphasic parallel flow to pattern bacteria with distinct geometry. A biphasic flow employing perfluorodecalin (PFD) and water is inherently immiscible, and thus has a very distinct boundary. PFD is biocompatible, which is suitable for patterning living bacteria. The schematic of selectively patterning of *E. Coli* is demonstrated in Figure A. The PFD and *E. Coli* suspension (3-4×10^8 cells per mL) from two inlets were driven by vacuum at one outlet, forming biphasic parallel flow in the main channel. *E. Coli* stained in blue fluorescence were patterned on one half of a microfluidic channel in Figure B. From left to right are bright field, fluorescence and pseudo-color images of patterned bacteria. The pseudo-image reflected the auto-fluorescence of LB broth, which indicates interface between two immiscible liquids. After patterning, the bacteria would adhere to the specific area of bottom wall of microfluidic channels.

Conclusions

The patterning of bacteria in designed geometry may be particularly useful to interrogate the mechanisms of, for example, chemotaxis and drug resistance.



Next generation multiplexed protein screening.

Nelson J, Sloan D, Votaw G, Ure D. Inanovate, Inc., Research Triangle Park, NC.

While a variety of technologies exist to measure proteins (e.g., ELISA, mass spec, and 2D gel), the most promising for screening multiple proteins are biochip or microarray based technologies. However, even with biochip devices, measuring specific interactions between multiple protein combinations remains problematic. Proteins are complex and fragile bio-molecules and often interact in complex and unpredictable ways with other proteins and/or protein analysis equipment, causing non-specific (false) signals. Furthermore, many proteins of interest to the life science industry are present in samples at very different concentrations, limiting which proteins can be screened in a single multiplex test and sample dilution.

To address these problems, Inanovate has developed a new multiplexed protein quantification technology call Longitudinal Assay Screening (LAS)^{TM}, combining high sensitivity confocal imaging and microfluidics alongside protein based microarrays. Inanovate has recently completed testing and benchmarking of the first platform integrating LAS technology across a range of demonstration assays. Instead of depending on a 96-well micro-titer plate, the new LAS platform utilizes a glass slide based protein microarray and microfluidics for dispensing and binding samples and detection antibodies. In its most basic form, the protein microarray is composed of capture antibodies for the proteins being measured, as well as positive and negative quality control features for ensuring sample to sample, run to run, lot to lot, and user to user consistency. Conceptually very similar to a real-time PCR reaction, the LAS platform iteratively flows small volumes of sample and labeled detection antibodies across the protein microarray (housed on Inanovate's fluidic cartridges) and fluorescently measures the formation of the sandwich between capture antibody, analyte of interest, and detection antibody in real-time.

Due to the time-resolved nature of the assay, the resulting data is a rate of reaction, instead of a simple final fluorescent reading. This 'rate of reaction' based analysis helps deliver the following core advantages of LAS technology, each of which has been demonstrated through the development and validation of a three-plex assay consisting of CRP, IL-6 and IL-1b.

1. Large detection range: LAS enables the accurate quantitation of protein concentrations across a 7-log range in a single multiplex test. This eliminates the need for serial dilutions, making multiplexing faster, cheaper and helping preserve precious samples.

2. Multiplexing flexibility: Due to its large detection range, LAS allows users to run virtually any assay of interest in one test, enabling the development of biologically relevant multiplexes.

3. Improved accuracy: LAS produces and analyses real-time kinetic data on protein interactions (rate of reaction data), improving identification and discrimination of background and non-specific signals, delivering more accurate quantitation at low analyte concentrations.

LAS technology holds the potential to become to proteomics what PCR was to genomics. It offers a new approach to multiplexed protein screening that helps address many of the problems presently affecting the utility of biochips in protein biomarker discovery, validation and clinical screening applications. Our poster will summarize the technical components of the new LAS-based platform and present data from the three-plex demonstration work.

New dry cartridge design of a point of care affinity chromatography glycohemoglobin test.
Saunders PA, Saunders AM. Chronomed Inc., San Carlos, CA.

Design: A dry boronate affinity chromatography resin is fixed in an enclosed, thin layer cartridge, and a dilute blood sample buffered at pH 9.1 is added. Hemoglobin A1c is captured as the sample passes along. A second affinity section captures the remaining hemoglobin in a tight band, and excess buffer is adsorbed into porous paper. Active components are 0.5cm wide and 0.5mm thick and less than 2.5 cm long. The transparent cartridge fits easily onto a calling card.

Optics: A standard multicolor scanner is used. With a black background, the reflected light is converted to "reflex attenuance" (RA) as the inverse log, linear with amount. (K. Shibata, Reflection Methods, *Methods of Biochemical Analysis*, Vol. 9, 1962.) Optical aberrations are corrected by subtracting red RA from green RA.

Sample preparation: 2 μL of whole blood is diluted in 150mM ethanolamine, 50mM $MgCl_2$, 0.1% Triton X100, and pH 9.1 buffer.

Procedure: 200 μL of diluted sample is transferred to the cartridge port. The sample is pulled along a reservoir to the affinity resins. Developing the chromatogram from a dry start takes approximately 15 minutes. A 600 DPI image is acquired. The image is converted to a three-color numerical array by ImageJ (NIH), which is then analyzed by Microsoft Excel. Hemoglobin that has not yet arrived at the Ao trap is estimated and separated from A1c.

Status: To date, all cartridges are handmade, and are hand packed with affinity resins. Therefore, reproducibility is not yet at product quality. Both standards and a limited number of clinical samples have been analyzed.

Results: Linearity with standards gives a correlation of r = 0.997 over a range of 5% to 11.8% HbA1c. CV is 5.6% using an 8.2% A1c standard. Three-run averaged results produced a correlation coefficient of r = 0.96 on clinical samples with a range of 5% to 12% HbA1c compared to a reference method. The major sources of variation are in the handmade cartridges and imaging instability of a commercial scanner.

Advantages: The design is aimed at the untrained user, but the chemistry and analysis are equivalent to affinity chromatography. The combined dry-resin design is expected to be stable in the long term.

Conclusions:

1. The goal of designing a stable, easy-to-use POC device for HbA1c is achieved with dried chromatographic media.
2. With handmade cartridges and a simple scanner for instrumentation, averaging of data is required to show potential value of the system.
3. The system responds in a linear manner to a clinical range of samples and to commercial controls, thus validating the novel design principle.
4. Next steps are reagent assembly stabilization and an improved reader.
5. The inventors are searching for a partner with whom to develop this technology.

Measurement of lateral flow tests using a smartphone.

Polwart N, Lopes AD. Novarum Ltd., Linlithgow, UK.

Lateral flow tests are prevalent across a wide range of clinical diagnostic applications and are widely promoted as a tool for point of care testing. However some limitations restrict their use including ambiguous results close to the limit of detection, cost of instrumentation for quantitative tests and difficulties in ensuring test results are reliably recorded and reported to healthcare professionals. Smartphone technology has gained widespread acceptance and has put both high power processing and good quality cameras in the hands of a wide range of users. The objective of this study is to investigate using that processing power to overcome the limitations of visually read tests.

We have developed algorithms on commonly available smartphones that can, without any hardware modification, capture the results from lateral flow tests. Our novel approach uses the preview frames from the camera. Each frame is tested for a number of parameters such as focus, illumination and orientation of the test device such that when predetermined criteria are met the image is captured. By applying mathematical corrections to the image based on the known geometry of the test, misorientation of the device is managed. The corrected image is then subjected to a series of analyses which yield the intensity of test and control lines.

The methodology has been applied and adapted to a range of tests including sandwich and competitive assays, as well as both qualitative and quantitative tests. In the latter case simple calibration of the phone yields good correlation between devices, with pseudo-linear behavior which is consistent with data obtained by the ‘gold standard’ method. Reproducibility has been investigated and can be as low as 3% CV following optimisation of the algorithm to suit the test.

The limits of detection, quantitation and dynamic range have been assessed. These meet or exceed the range provided by other reader technologies commonly in use. The comparison to visual interpretation shows that the phone performs at least as well as an unskilled user (>95% CI), and by controlling the lighting conditions is able to prevent use of the test in poorly illuminated environments, or by controlling the phone’s LED flash enable use in situations where lateral flow tests would not normally be readable.

Lateral flow tests have been successfully read using both Android and iPhone devices showing substantial equivalence to visually read tests. Furthermore quantification of lateral flow tests using these devices has been demonstrated. Through the connectivity inherent in the phone these results can simply be communicated to healthcare professionals, or recorded to build up a long term trend, thus overcoming many of the limitations of lateral flow testing.

Performance of multiple enzymatic assays on dried blood spot samples for rapid newborn screening using digital microfluidics.

Sista RS[1], Wang T[1], Graham C[1], Wu N[1], Eckhardt AE[1], Meguid N[1], Millington DS[2], Bali D[2], Pamula VK[1]. [1] Advanced Liquid Logic Inc., Research Triangle Park, NC; [2] Division of Biochemical Genetics, Duke University Medical Center, Durham, NC.

Biotinidase deficiency and galactosemia are among the most common enzymatic assays performed in newborn screening (NBS) programs. The introduction of screening for lysosomal storage diseases (LSD) expanded the menu of enzymatic assays. Current technologies include manual or automated microplate fluorometry, flow-through fluorometry, or mass spectrometry.

We developed a novel technology – Digital Microfluidics – which harnesses the electrowetting effect to precisely manipulate discrete droplets within a sealed disposable cartridge in a programmable fashion to automatically perform fluorescence enzymatic assays. A cartridge is inserted into an analyzer with a built-in fluorometer, combining the automation of a fluid handling robot (cartridge) with the detection setup of a microplate reader (analyzer). Each cartridge has 48 reservoirs for samples, quality control, and calibrants and 10 reagent reservoirs.

Due to the intrinsic flexibility in cartridge design, assays requiring different reaction conditions can be programmed on the same cartridge. Thus, enzymatic assays for LSDs, biotinidase and galactose-1-phosphate uridyl transferase (galactosemia) screening can be performed on the same disposable cartridge and analyzer. Assays use only 100 nL of the same dried blood spot (DBS) extract (100 µL) from a single punch, thus generating a novel multipurpose, high throughput NBS platform.

We developed a multiplex assay for Pompe, Fabry, Hunter, Gaucher, and Hurler LSDs on a single disposable cartridge using 4-methylumbelliferyl fluorescence based enzymes. One droplet of DBS extract from 600 presumed normal and affected (12 Pompe, 7 Fabry, 10 Hunter, 10 Gaucher, and 10 Hurler) DBS samples was mixed with one droplet of reagent and incubated for 1 hour at 37°C. The reaction was stopped with sodium bicarbonate, and fluorescence values were measured at 370 nm excitation and 460 nm emission.

The protocol for screening of biotinidase and galactosemia is similar to that for LSDs and utilizes the same digital microfluidic cartridge. One droplet of DBS extract from 10 biotinidase proficiency quality control samples (7 normal and 3 deficient samples) was mixed with one droplet of reagent (4-methylumbelliferyl biotin) and incubated for one hour at 37°C. The reaction was stopped with sodium bicarbonate; fluorescence was detected at 360 nm excitation/460 nm emission. Galactosemia detection was optimized with a fluorescence microtiter plate assay and is currently being translated onto a digital microfluidic cartridge. One droplet of DBS extract from 5 proficiency samples (including 1 deficient sample) was combined with reagent (galactose 1-phosphate) and incubated for three hours at 37°C. Fluorescence was measured as above with biotinidase samples.

We demonstrated screening for LSDs (Pompe, Fabry, Hunter, Gaucher, and Hurler) in a 5-plex enzymatic assay. This system is under pilot in the Missouri State Public Health Laboratory. We demonstrated proof of feasibility of performing fluorometric enzymatic assays for detection of galactosemia and biotinidase deficiency by correctly identifying deficient and normal samples. We are currently optimizing conditions for validation using a larger set of newborn screening samples. A single, easy to use, inexpensive, and automated platform allows for consolidation of several assay modalities onto a single instrument for a variety of newborn screens.

A novel, low-cost point-of-care testing platform based on direct measurement of diffusion and interaction in the sample matrix.

Noble M[1], Rippeth J[1], Brandon-Jones S[2], Edington DWN[2]. [1]Exacsys Limited, Cambridge, UK; [2]PA Consulting Group Limited, Cambridge, UK.

A novel point of care testing platform is described, and feasibility for a number of promising applications is shown. The underpinning technology involves the introduction of an indicator species to a liquid sample and subsequent measurement of the flux of this indicator through the sample.

Prior knowledge of the expected flux enables measurement of clinically-relevant parameters related to diffusivity in the sample matrix (e.g., hematocrit). Alternatively, changes to the expected flux due to binding or sequestration of the indicator can be used to measure interactions in the sample (e.g., plasma protein binding for drug dose optimisation). Measurement of diffusivity via indicator flux can also be used to reduce inaccuracy in existing POCT assays where diffusivity contributes to analytical error (e.g., blood glucose monitoring for glycemic control).

Here we demonstrate feasibility of the technology when implemented via minor modifications to a ubiquitous low cost platform (self-monitoring of blood glucose - SMBG), and illustrate three potential POCT applications:

- Measurement of hematocrit
- Measurement of plasma protein binding of a drug
- Improvement of the accuracy of an SMBG product via on-strip calibration

Commercially available electrochemical SMBG test strips were purchased and the cover was replaced with a polymer film prepared with a controlled release coating. This coating was formulated to release an indicator through dissolution in the sample. Chronoamperometric measurements were made using a bespoke potentiostat. Venous blood obtained from healthy volunteers was manipulated to create a range of hematocrit and glucose levels.

Firstly, we used glucose as an indicator to exploit the detection chemistry present on the SMBG strips. During the first few seconds of measurement after blood sample introduction, a conventional Cottrell decay of current with time is observed. Subsequently, the current begins to increase due to the arrival of the indicator after diffusing through the blood. The level of this increase was found to be strongly correlated to hematocrit, allowing direct measurement of hematocrit in the range 0-65%.

To measure plasma protein binding, we used acetaminophen as an indicator in the controlled release coating. Measurements of flux at the test strip electrodes were made by direct oxidation of acetaminophen with a baseline determined using a PBS control. A reduction of 25% in flux measured in blood plasma was found, matching the expected level of plasma protein binding.

Finally, we used glucose as an indicator to investigate the potential of the technology to enable "on-strip calibration" in SMBG. The SMBG assay is diffusion-limited, so changes in glucose diffusivity in the sample are a major source of inaccuracy. We found the indicator flux to be strongly correlated to analytical error compared to a reference instrument (YSI 2300 STAT Plus), and that this information could be used to reduce the mean absolute relative error of a conventional test strip from 22.2% to 4.7%.

Development of a new point-of-care system for CD4 counts, %CD4, and hemoglobin determinations.

Bornheimer S[1], Bui N[1], Le D[1], Wai H[1], Tran A,[1] Wu A[1], Lee S[1], Goldberg E[1], Bouic P[2], Huang W[1], Lowe L[1], Sugarman J[1], Crow M[1], Kumar V[1], Tomas C[1], Zhu J[1], Yu J,[2] Clark A[2], Broszeit E[2], Chen R[1], Bush-Donovan C[1]. [1]BD Biosciences, San Jose, CA; [2]University of Stellenbosch, Cape Town, South Africa.

Background:

This abstract describes the preliminary performance of the BD FACSPresto™ system*. This point-of-care system produces absolute $CD4^+$ cell counts, %CD4, and hemoglobin (Hb) determination for staging and monitoring HIV patients. This compact BD FACSPresto system is fully integrated with a fluorescence digital imaging camera and a visible/IR spectrophotometer. It provides a user-friendly touchscreen, pictorial prompts, automated data analysis, and report printing. The system also features a unitized reagent cartridge containing dry-formulated antibody-fluorophore reagents and in-device instrument and reagent QC. To perform a test, the operator does not need special laboratory training. The system accepts fingerstick or venipuncture blood specimens.

Materials and Methods:

The unitized reagent cartridge includes dry reagents required for counting $CD4^+$ lymphocytes and total lymphocytes: CD4 PE-Cy™5 and CD45RA APC for enumeration of $CD4^+$ cells and $\%CD4^+$ lymphocytes. The embedded spectrophotometer requires no reagents to directly measure Hb content in whole blood specimens. The test protocol includes first collecting a whole blood sample by fingerstick or venipuncture and applying it to sample port of the cartridge. The cartridge is subsequently incubated for 18 minutes before insertion into the instrument to generate CD4, %CD4, and Hb results.
(a)To evaluate the accuracy of CD4 and %CD4 assays analyzed on the BD FACSPresto system, results from fingerstick samples were compared to those from venipuncture samples tested with BD CD4 Tritest™ reagent (CD3/CD4/CD45 plus BD Trucount™ beads) and analyzed on the BD FACSCalibur™ system. A total of 69 HIV^+ fingerstick and venipuncture samples were tested (with patient consent). (b)To evaluate the accuracy of the Hb assay analyzed on the BD FACSPresto system, results were compared to those on the Sysmex® system. A total of 211 HIV^+ venipuncture samples were tested. (c)To assess the system precision, commercially available CD4 controls and Hb controls were used. A replicate of 2 for each level was tested daily for a total of 9 days.

Results:

CD4 and %CD4 Accuracy : The Deming regression analysis of CD4 absolute counts comparing the BD FACSPresto to the BD FACSCalibur system showed a correlation coefficient of 0.98 and a slope of 0.92. For %CD4, the analysis showed a correlation coefficient of 0.97 and a slope of 0.98.
Hb Accuracy: The Deming regression analysis of venipuncture Hb results comparing the BD FACSPresto to the Sysmex system showed a correlation coefficient of 0.96 and a slope of 0.92.
Precision: The 9-day study showed %CVs for CD4 counts were below 7%. The %CVs for %CD4 were below 5%, and for Hb were below 3%.

Conclusions:

The BD FACSPresto system, designed as an easy-to-use point-of-care system, provides accurate and precise CD4 counts, %CD4, and hemoglobin results for HIV patient staging and monitoring

* Currently under development, not available for sale or use.

Reader-free, CMOS-based POC device for sensitive quantification of cTnI.

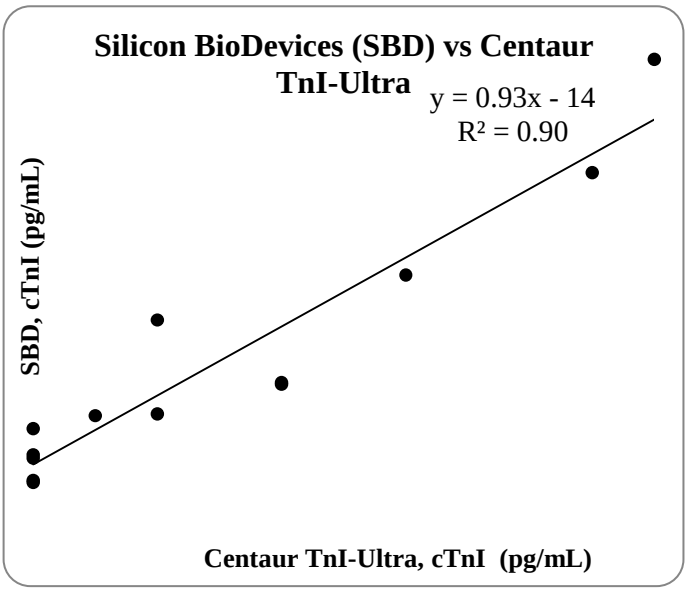
Florescu O[1], Martin T[1], Cromer R[1], Yamasaki D[1], Wang K[1,2], Christenson R[1,3]. [1]Silicon BioDevices, Inc., Berkeley, CA; [2]Tufts University, Medford, MA; [3]University of Maryland School of Medicine, Baltimore, MD.

We present a disposable, reader-free point-of-care (POC) device designed to meet or exceed laboratory guideline specifications for the precise and rapid turnaround measurement of cardiac troponin I (cTnI).

The self-contained device yields digital results in one step from a single drop of whole blood in less than 15 minutes. Its core consists of a complementary metal oxide semiconductor (CMOS) integrated circuit (IC) that performs a magnetic bead-based sandwich immunoassay from as little as 2 µL of neat plasma. For whole blood measurements, the IC is mated to a passive blood separator that delivers plasma from a finger stick or collection tube. Wireless transmission can be embedded on-chip, enabling automatic, compliant reporting to hospital EMR or mobile apparatuses. Since this fully integrated device requires only one CMOS IC, a battery and a display, it is inexpensive to manufacture and can be packaged in any physical form, such as a thumb-drive or smart-phone.

Feasibility studies used normal human plasma samples spiked with recombinant cTnI for calibration. The analytical sensitivity was determined by running 22 replicates of normal plasma and calculating the mean signal + 2 SD. The LoD was calculated as 12 pg/mL. Regression analysis of the system versus Centaur TnI-Ultra for clinical plasma samples with cTnI yielded correlation factors R^2=0.95 (N=22) for values up to 5 ng/mL and R^2=0.90 (N=13) for values below 120 pg/mL (see graph).

Silicon Biodevices' unique platform technology has the potential to achieve high sensitive performance characteristics with rapid turnaround time and extend clinical lab-quality testing by caregivers and patients alike to any setting – including the home.



Expanded assay platforms utilizing yeast-scFv affinity reagents.

Spadafora LJ[1], Grewal YS[2], Shiddiky MJA[2], Weigel KM[3], Trau M[2], Cangelosi GA[1,3].
[1]Interdisciplinary Program in Pathobiology, Department of Global Health, University of Washington, Seattle WA; [2]Australian Institute of Bioengineering and Nanotechnology, University of Queensland, St. Lucia, QLD, Australia; [3]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle WA.

Molecular probes, typically monoclonal antibodies (mAbs), are critical for biomarker discovery, diagnostic testing, and basic biomedical research, but their expensive and months-long production process has created a significant bottleneck for such endeavors. The Accelerated Molecular Probe Pipeline (AMPP) is designed to reduce discovery and production time of molecular probes to 2-3 weeks, utilizing a library of antibody-like single chain fragment variable (scFv) molecules displayed on whole yeast cells.

Yeast probes specific to target antigens and with comparable affinities to traditional mAbs have been successfully selected from our diverse scFv library through two rounds of magnetic cell separation (MACS) and 1-2 rounds of fluorescently-activated cell sorting (FACS). However, selected scFvs usually lose activity when culled into soluble reagents and thus have not replaced traditional mAbs as first-line molecular probes. To overcome this limitation, we have developed strategies that maintain scFv in the environments in which they were selected to function, namely tethered by Aga1-Aga2 linkages to yeast cell walls. Whole-cell or fragmented yeast-scFv affinity reagents are produced, stabilized, and utilized as assay reagents in this environment.

Here we present two new yeast-scFv-based assay formats: A whole yeast-scFv sandwich ELISA and a cell-free, label-free electrochemical assay utilizing yeast-scFv fragments. The yeast-scFv used in these assays were selected against candidate cyst antigens of diarrheal pathogen *Entamoeba histolytica*, as part of a model biomarker validation project within AMPP.

Development of a broad-specificity pathogen capture system.

Clarizia L-J, Dryga SA, Adams EW, Ung T, Norvell M, Dykes CW, Esch V. nanoMR Inc., Albuquerque, NM.

Background:

Current pathogen ID systems, including blood culture for detection of bloodstream infections (BSIs), are too slow to significantly impact therapy. New molecular methods lack the sensitivity to detect pathogens in blood at clinically relevant concentrations (~1 CFU/mL), and must be used post-positive blood culture. Our goal was to develop a system to isolate prevalent pathogens present at 1 CFU/mL in blood, and other matrices, and provide purified pathogen DNA for subsequent analysis, in an hour or less.

Methods:

IgG from goats immunized with antigens representing the diversity of major pathogens causing bloodstream infections were purified from sera by affinity chromatography. IgG was conjugated to polystyrene superparamagnetic beads prepared via emulsion polymerization. Capture system components were optimized for rapid, efficient pathogen capture and removal of sample matrix in both contrived and clinical samples by agar plating, or molecular ID following DNA extraction.

Results:

1. Blood samples spiked with individual pathogens in the range 1-10,000 CFU/mL were mixed with magnetic beads and buffer to a final volume of 0.5 mL. After 30 minutes incubation, bead/pathogen fractions were captured in a magnetic trap, then washed and plated to measure pathogen recovery. Capture efficiencies (CE = [CFU captured/input CFU]*100) >50 % were reproducibly achieved for the 19 pathogens tested, including the most prevalent bacteria and Candida species found in BSIs. CE remained constant across the range of concentrations tested (e.g., a line fit of captured vs. input CFU gave correlation coefficients of 0.999, 0.9995, and 1.0 for *E. faecium*, *S. aureus*, and *E. coli*, respectively).
2. There were no statistically significant differences in CE of *S. aureus* spiked at 100 CFU/mL into triplicate samples of human blood, human urine, and ground beef extract.
3. A study of 674 blood samples from patients with suspected BSIs, where the recovered fractions were analyzed by plating, followed by conventional ID, showed superior sensitivity and specificity, 83.33% and 99.84% respectively, for the nanoMR system compared to 77.22% and 97.65% for blood culture. The use of newer IgG preparations, with wider coverage of pathogen diversity is expected to provide >95% sensitivity.
4. Analysis of pathogen DNA extracted from bead/pathogen fractions using the Zymo D6007 kit and analyzed by qPCR using species-specific primers with the Roche LightCycler®2 gave limit of detection values close to 1 CFU/mL for all species tested (range 0.1–4.0 CFU/mL), similar to LODs measured by plating, in around three hours. Initial results on a small set of clinical samples analyzed using a commercial multiplexed PCR system (three hour assay time) have successfully detected bloodstream pathogens in around four hours.

Conclusion:

This immunomagnetic pathogen capture system has the potential to revolutionize diagnosis of bloodstream infections by providing actionable information to physicians in a clinically meaningful time frame.

High definition immunoassays (HDIA) with spatially resolved detection.

Ruan Q, Skinner JP, Gayda S, Tetin SY. Abbott Diagnostics, Diagnostics Research Division, Abbott Park, IL

Non-specific binding greatly affects assay sensitivity. In conventional immunoassay, total signals are collected from the entire reaction vessel, thus non-specific signals from solution and vessel surfaces are included, resulting in a rise in background signals. Here we introduce a new detection concept: fluorescence imaging based high definition immunoassays (HDIA). Fluorescence images of analyte bound microparticles were examined pixel-by-pixel, irrelevant signals were filtered out using imaging process algorithms; only relevant signals is included to determine analyte concentration. HDIA can be performed in both sandwich and competitive format. With the HDIA approach, highly sensitive (sub-picomolar) immunoassays can be performed in 15 minutes with minimal washing steps. Using troponin and NGAL (neutrophil gelatinase-associated lipocalin) as model analyte systems, we developed immunoassays with both high sensitivity and wide dynamic range. HDIA can also be performed in homogeneous mode because background and unwanted signals are filtered out with imaging analysis. Furthermore, this approach is insensitive to microparticle number variation under certain conditions during the reaction and the wash, which eliminates one main source of error from microparticle handling in immunoassays.

HDIA detection requires only a light source, simple optics and a digital camera. It can be readily adapted for quick assay prototyping and high throughput screening on any conventional fluorescence imaging system.

The use of adaptive focused acoustics to enhance HTS immunodiagnostic binding assays.

Green DJ[1], Rudd EA[2], Laugharn JA[2]. [1]Biotechnology Consulting Associates, Fitzwilliam, NH; [2]Covaris Inc., Woburn, MA.

Objective: To demonstrate the use of low-power Adaptive Focused Acoustics (AFA™) energy to provide rapid binding of antibody to antigen in a controlled, reproducible, isothermal process.

Methods and Materials: A microtiter plate sandwich ELISA that measures HIV-1 p24 levels in tissue culture samples (ABL, Inc., Bethesda, MD) was employed in this study. The effect of AFA on the first binding step in this assay – the binding of HIV p24 to immobilized capture antibody – was studied. Dose response curves of p24 binding were determined at different levels of precisely controlled acoustic energy and compared to the passive binding stipulated in the kit protocol. Other assay steps were performed according to the kit procedures. AFA treatments were performed in HTS parallel processing AFA instruments (Covaris, Inc., Woburn, MA) in which enhancement of immunoassay binding kinetics has not been previously demonstrated.

Results: AFA administered at 20°C to a 96-well microtiter plate in a whole plate sweeping modality provided a two-fold increase in the rate of binding of HIV-1 p24 to capture antibody compared to conventional binding at 37°C for one hour. Increasing the rate at which repetitive AFA treatments were applied at 20°C to one eight-well column resulted in a more than four-fold increase in antigen binding kinetics compared to the kit protocol performed at 37°C. Heat was not applied in any of the AFA assays, as it was in the kit protocol. Low inter and intra-assay CV's were consistently observed in these kinetic studies. HIV-1 p24 calibration curves of the AFA-enhanced assay with the antigen/antibody binding step shortened to 20 minutes also consistently exhibited low inter and intra-assay CV's. Low standard deviations of both positive and negative samples resulted in superior Z' factors [*1*] indicating good potential for use of AFA-enhanced immunoassays in HTS applications.

Conclusions: AFA provides rapid, controlled, precise, reproducible, and isothermal enhancement of antigen binding in commercially available, off-the-shelf microtiter plate assays. This low power capability, together with the known compatibility of AFA instrumentation for high power DNA shearing widely employed in next generation DNA sequencing platforms [*2*], is another demonstration of tunable AFA energy for enabling multiple applications to life sciences, such as *in vitro* clinical diagnostics.

1. Zhang et al. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen 1999; 4(2): 67-73.
2. Quail et al. A large genome center's improvements to the Illumina sequencing system. Nature Methods 2008; 5(12): 1005-10.

A next-gen sequencing assay for the simultaneous detection of bladder cancer-associated protein and DNA markers.

Fernandez CA, Millholland JM, Anderson LD, Campo MF, Shuber AP. Predictive Biosciences, Lexington, MA.

Protein and DNA biomarkers have traditionally been treated as separate entities such that diagnostic tests multiplex either only protein or only genomic markers. In both cases, as sensitivity is increased, specificity decreases and vice versa. We have recently described a unique non-invasive assay that combines protein and genomic markers to triage a population of patients undergoing evaluation for bladder cancer. The high specificity of DNA markers combined with the high sensitivity achieved by quantitative protein marker cutoffs maximizes specificity while simultaneously maintaining high sensitivity.

To eliminate the need to perform separate analytical procedures for protein and DNA, and to decrease assay complexity and cost while increasing assay throughput, we have now developed a method for simultaneous detection of protein and DNA biomarkers on a next-generation sequencing (NGS) platform. The increased analytical sensitivity of the NGS approach allows for detection of rare genetic events in a dilute medium such as urine resulting in increased clinical sensitivity. We have recently described an ultra-deep amplicon sequencing method for detection of FGFR3 mutations in urine that closely matches the frequency of mutations found in bladder cancer tissue. While FGFR3 mutations are present in a large fraction of non-invasive tumors, these mutations are rarely detected in invasive bladder tumors. To complement our existing FGFR3 NGS assay, we have now also developed a multiplex NGS assay to simultaneously detect 133 bladder cancer-associated mutations in tumor protein (TP)53. Mutations in TP53 are commonly found in advanced bladder cancer, and show little overlap with FGFR3 mutations.

In order to simultaneously detect the matrix metalloprteinase-2 (MMP-2) protein marker, we have developed an aptamer-based assay such that all analytes may be detected in one sequencing reaction. DNA aptamers are single stranded DNA oligonucleotides that can bind specifically to their target molecules, including proteins. Once bound to the protein, these aptamers can then be used as templates for PCR and then quantitated on the NGS platform. Here, we demonstrate linear detection of MMP-2 protein via sequencing and the simultaneous multiplex detection of genomic and proteomic markers in an NGS platform.

Our unique multi-analyte approach of combining proteomic and genomic markers in a single assay improves clinical performance using a minimal number of markers. The adoption of the NGS platform further increases performance by increasing analytical sensitivity for rare genomic events in a bodily fluid and the ability to identify a large number of potential mutations. Lastly, to decrease assay complexity and increase throughput we now demonstrate the ability to simultaneously analyze protein and DNA markers in a single NGS platform.

Molecular Pap smear for cervical cancer screening: A combination of DNA methylation with oncogenic HPV typing.

Liou Y-L[1,5], Liao C-I[3], Zhang Y[4], Qin C[1], Zhang T[1], Mo M-H[5], Chen CH[5], Chang C-F[5], Kan Y-Y[2*], Zhou H[1*]. [1]Institute of Clinical Pharmacology, Central South University Xiangya School of Medicine, Changsha, Hunan, China; [2]Yuan's General Hospital, Kaohsiung, Taiwan; [3]Department of Obstetrics and Gynecology, Kaohsiung Veterans General Hospital, Kaohsiung, [4]Taiwan; Xianya Hospital, Changsha, China; [5]iStat healthcare Consulting Co. Ltd., Taipei, Taiwan.

Background: Oncogenic human papilloma virus (HPV) DNA testing is an appealing method since its etiological role of cervical cancer is well established. However, the poor specificity of HPV testing lessens its value in cervical cancer screening. Recent findings suggest that DNA methylation biomarkers, alone or in combination with current cytology or virus-based methods for cervical cancer screening, might possess clinical potential. Here, the molecular Pap smear—HPV subtyping16 & 18 combined with specific methylation DNA— is proposed as a potential tool for the detection of cervical cancer.

Method: Following the GCP guidance, subjects were recruited in Yuan's General Hospital in Taiwan and Xiangya Hospital in China. Inclusion criteria were female age ≥20 and sexual experience. The exclusion criteria included: women had history of cancer related to reproductive tract, had therapy for cervical lesions, had received HPV vaccination or at pregnancy. The results of Pap and DNA methylation biomarkers including $PAX1^m$, $SOX1^m$, and $NKX6.1^m$ were determined by using Q-PCR. HPV typing tests were determined by semi-nested PCR and reverse hybridization. Sensitivity, specificity, and accuracy for HPV and the DNA methylated level were analyzed.

Results: Two hospital-based study was conducted on 557 subjects including normal Pap, abnormal Pap (n=257). The diagnosed according to cytological and histological reports of both Hospitals. The results indicated that the methylation levels of three genes were significantly higher in CIN3 and worse (CIN3+) lesions if compared to the level of methylation in normal cervix and CIN1 or CIN2($P<0.0001$). Among the three methylation genes, $PAX1^m$ was significantly higher in patients with CIN3+ lesions than those with CIN1, CIN2, and normal cervix than other methylation genes. The p value of the three genes is less than 0.0005 in the study. Among the three hyper-methylated genes, the sensitivity and specificity of $PAX1^m$ along are >79% and >75% respectively in both study side. The sensitivity of $PAX1^m$ combining with HPV 16 & 18 is above 90% and the specificity is over 73%.

Conclusion: The current results indicated that the real time PCR-based testing for DNA methylation of $PAX1^m$ is promising for cervical cancer detection and screening in Chinese population. Combining with the HPV 16 & 18 typing provide greater sensitivity and specificity for the detection of cervical cancer as first screening.

Towards small molecule diagnostics using aptamers and backscattering interferometry (BSI).

Jackson GW[1], Olmsted IR[2], Kussrow A[2], Morris MJ[1], Bornhop DJ[2]. [1]Base Pair Biotechnologies, Houston, TX; [2]Vanderbilt University, Nashville, TN.

Nucleic acid aptamers are high affinity, high-selectivity ligands produced *in vitro* by a process commonly known as "SELEX". Due to the expiration of major intellectual property restricting their use, the aptamer class of affinity reagents is poised for considerable growth in a variety of diagnostic application areas. Among other advantages, the ability to cheaply synthesize a well-defined, chemical affinity reagent represents a major advantage of aptamers over antibodies.

In vitro characterization of a new affinity ligand is a logical first step in the development of any new assay. Unfortunately, when the aptamer target is a "small molecule", characterization of the true Kd can be challenging. Specifically, solid-phase ligand binding techniques such as surface plasmon resonance (SPR) or biolayer interferometry (Forte Bio, Pall Corp.) present the challenge that if the aptamer is immobilized, the small molecule (having low molecular weight) will not produce enough response for good signal-to-noise. On the other hand, immobilization of the small molecule creates problems with target perturbation and steric hindrance. Similarly, the traditional solution phase approaches have limitations. Calorimetry requires large sample amounts (often prohibitive) and fluorescence polarization requires fluorophore labeling which can perturb the target or the aptamer.

A relatively new technique, backscattering interferometry (BSI), is truly label free, takes place completely in the solution phase, and is sensitive enough to address the concerns outlined above. Here we present binding data on several aptamers and their intended small molecule targets as well as specificity (cross-reactivity) data on molecules of similar molecular weight. The data also indicate that a wide range of affinities can be characterized, and these measurements can clearly be used to refine aptamer selections. Finally, because reliable BSI measurements have been demonstrated in complex sample matrices such as serum, we are presently investigating the development of several assays relevant to the clinical diagnostic arena in matrices such as saliva and serum.

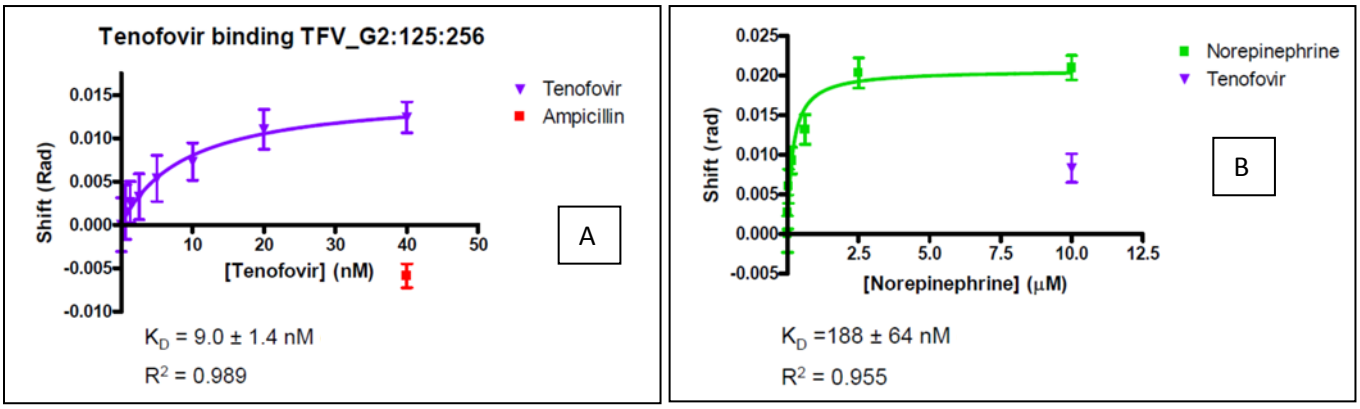


Figure 1. Examples of aptamer:small-molecule binding studies using backscattering interferometry (BSI). (A) A tenofovir specific aptamer is shown to have 9 nM binding and does not bind the small molecule ampicillin (B) An aptamer selected to norepinephrine has a Kd of 188 nM and shows some degree of cross-reactivity with the small molecule drug, tenofovir.

Accurate point-of-care hemoglobin A1C test using a low-cost bioelectronic single-measurement technology.

Ahrens M, Beal R, Gaustad A, Georganopoulou D, Hoo R, Palit R, Van Groll E, Bao P. Ohmx Corporation, Evanston, IL.

Background

Hemoglobin A1c (HbA1c) is a stable hemoglobin derivative that results from the non-enzymatic glycation of the N-terminal valine on the beta chains. It serves as a good biomarker for monitoring glycemic control since it correlates linearly with the average blood glucose level over the lifespan of red blood cells (2 to 3 months). Monitoring HbA1c is currently used to inform treatment decisions, to ensure compliance, and to help evaluate if patients are meeting their clinical goals. Since 2010, it is also the preferred method for diagnosing both Type II diabetes and pre-diabetes. Since HbA1c is measured as the percentage of total hemoglobin, most HbA1c tests are composed of two measurements; one to measure the glycated hemoglobin and the other to measure the total hemoglobin. Ohmx presents here the clinical validation for an innovative single measurement approach for quantifying HbA1c.*

Methods

Total hemoglobin capture antibody was immobilized on magnetic beads and used to equally bind all variants of hemoglobin in the sample. The captured HbA1c then binds to a specific probe antibody that is tagged with alkaline phosphatase (AP) forming an immune-sandwich complex. This target-antibody complex is then used to generate an in-situ surrogate target which is enzymatically enhanced producing an amplified substrate. The amplified substrate is further quantified using a bioelectronic measurement. We tested eight commercially available calibrators with HbA1c concentrations varying from 2.7% (6.0 mmol/mol) to 19.8% (192.9 mmol/mol) and established a standard curve directly correlating the electronic signal with the ratio of HbA1c. To verify the functionality of our HbA1c assay, we compared the results from 100 clinical whole blood samples with those values obtained by a Bio-Rad clinical immunoanalyzer Variant II Turbo.

Results

The total assay time of our HbA1c test is <5minutes with a linear response ranging from 2.7% (6.0 mmol/mol) to 19.8% (192.9 mmol/mol). The single-measurement based signal improves the assay's reproducibility, with intraday precision CV less than 3% and interday precision CV less than 5%. The Ohmx assay has been clinical validated with 100 clinical samples, and compared with data collected with Bio-Rad clinical immunoanalyzer showing high correlation ($R^2 = 0.94$).

Conclusions

There is excellent correlation between the data acquired by the single measurement Ohmx HbA1c test and the NGSP certified Bio-Rad assay. This technology is applicable for a variety of different analytes including ultrasensitive detection for proteins such as troponin I and thyroid stimulating hormone (TSH), also for small molecules such as flavin adenine dinucleotide (FAD), lipid panel and other targets.

*Assay currently under development and not for clinical use