

Poster 1

Two-Dimensional reverse-phase protein microarrays: A new approach to comprehensive, disease specific, protein profiling of patient samples. Barder T. Eprogen, Darien, Ill.

DNA arrays are useful to detect the presence, translocation and, amplification of thousands of genes. Studying complex nonlinear interactions of cellular proteins and post-translational modification as it directly relates to various tumor cell phenotypes has been difficult to do at the scale needed for complete understanding of a disease. Here we present a new approach to assaying large numbers of serum samples to establish protein profiles that directly identify proteins specifically related to the disease expression or phenotype. Melanoma Cells were extracted for the lymph nodes of patients, expanded and isolated. The cells were lysed and the proteins separated using 2D liquid chromatography into ~1000 unique pI/hydrophobicity fractions, each fraction containing a small subset of the proteins expressed in the tumors. These fractions were then spotted onto microarray slides and assayed using the sera from a cohort of 120 patients comprised of normal, advanced and early stage melanoma and non-melanoma cancer patients. The autoantibody signature profiles were analyzed using M-statistics and protein fractions identified that classified the patients into their respective classes. These fractions were then analyzed by MS to ID the proteins present. This technique was able to clearly establish unique sets of proteins which readily classify early stage and normal controls patients from late stage melanoma patients and a smaller set of proteins that classify early stage melanoma and advanced disease. This general methodology using defined cohorts of patient sera for autoantibody response provides for a utilitarian “Bedside-to-Bench” approach to patient stratification through identification of those biologically relevant proteins in patients that actually are expressed as biomarkers highly specific to the disease expression. It can serve as a new tool to help discover what we don’t know about a disease phenotype through use of clinical samples.



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Poster 2

The sensitivity of the Partec CyScope® in the diagnosis of malaria parasites. A cost effective and time saving alternative for poor endemic areas? Nkrumah B^{1,2}, Agyekum A^{1,2}, Acquah SEK^{1,2}, May J³, Tannich E³, Brattig N³, Hüniger H², Adu-Sarkodie Y¹. ¹Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. ²Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana. ³ernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany.

Introduction: Malaria remains the single largest cause of death in Africa. Rapid and accurate diagnosis is therefore the cornerstone of good malaria control. Initiation of malaria treatment largely depends on good laboratory-confirmed diagnosis but in many endemic countries, clinical diagnosis is the only method used to decide on treatments as laboratory techniques to confirm the clinical suspicion are considered to be too labour intensive or are not sensitive enough⁽¹⁾.

Aim: To evaluate the Partec CyScope® for the detection of Plasmodia in human blood from patients in an endemic area.

Materials and Methods: 263 samples from patients attending the Under Five Clinic and Out Patient Department of the Agogo Presbyterian Hospital in the Asante Akim North District, Ghana were examined with the four different tests independently and blinded. The test methods employed in the study were: (i) Giemsa-stained blood smears (ii) Partec CyScope® (iii) Binax Now® RDT and (iii) PCR⁽²⁾.

Results: Out of 263 individuals analyzed, 107 (40.7%), 111 (42.2%), 114(43.3%) and 181 (68.8%) were positive for the presence of Plasmodia by Giemsa stain, Partec CyScope®, BN RDT and PCR respectively. There was a high agreement between the results of Giemsa stain and Partec Cyscope® (k=0.97). Compared to Giemsa stain the sensitivity of Partec CyScope® test was 100% and the specificity was 97.4% at 95% CI. The Positive and Negative Predictive Values were 96.4% and 100% at 95% CI.

Discussion/Conclusion: The test performance of Partec CyScope® was very similar compared to Giemsa-stained blood smears with slightly more false positives. This might be due to the fact that this method uses unspecific fluorescent stain (DAPI) which detects any intracellular DNA that might be present in erythrocytes. Thus artifacts might have been misinterpreted as plasmodial DNA. However, the CyScope® had other added advantages; it has a standby battery and can be used on the field, it was faster, easier to use, less expensive in terms of cost per test and test equipment than the Giemsa stain. PCR has much higher analytical sensitivity (0.01-0.02 parasites/μl)⁽²⁾ than the other methods and therefore detected more positives. The CyScope® may therefore be used as an alternative method for Giemsa thick film.

References:

1. Jonkman A, Chibwe RA, Khoromana CO et al. 1995. Cost-saving through microscopy based versus presumptive diagnosis of malaria in adult outpatients in Malawi. *Bulletin of the WHO* 73, 223-227
2. Mangold KA, Manson RU, Koay ESC, Stephens L, Regner MA, Thomson Jr RB, Lance R, Peterson LR, Kaul KL. 2005. Real-Time PCR for Detection and Identification of Plasmodium spp. *Journal of Clinical Microbiology*; 43(5): 2435–2440.



Poster 3

Enzymatic lateral flow assay technologies. Song X. Corporate Research & Engineering, Kimberly Clark Worldwide, Roswell, GA.

Enzymatic lateral flow assay technologies developed in Kimberly Clark integrate enzyme reactions and magnetic particle separation technology into a user-friendly and low cost lateral flow assay format for detection of any species involved in an enzyme reaction, including an enzyme, an enzyme inhibitor, and/or a co-factor. The assay technology uses a magnetic particle-based enzyme substrate conjugate that consist of an enzyme substrate (e.g., a peptide or a protein or a nucleic acid oligomer) tagged to a magnetic particle through a linker on one end. A probe (e.g., biotin) is tagged to the other end of the substrate. An enzyme cleaves the substrate to release the probes from the magnetic particle that are subsequently detected by a standard lateral flow device while the non-released probes still attached to the magnetic particles are removed by a magnet. The enzyme reaction, the removal of the magnetic particles and the detection of the released probes can easily integrated into one single lateral flow device, making the assay technologies particularly suitable for point-of-care and over-the-counter test market. The assay technology can be easily adapted for different enzyme reactions by standardizing magnetic particle and probe used, so that only one type of lateral flow device for detecting the standard probe is needed for all the enzyme reactions. Only modification needed is to change the substrate for different enzymes. Furthermore, we have also developed a lateral flow assay technology to detect enzyme activity and the amount of the same enzyme simultaneously by coupling the enzyme detection technology with traditional lateral flow immunoassays on the same device.



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Poster 4

Serum Cathepsins K, L and Cystatin C: Can be used as biomarkers for the severity of Coronary Artery Ectasia? Haliassos A¹, Zografos TA², Korovesis S², Giazitzoglou E², Voridis E², Katritsis G².¹Diamedica S.A., Athens, Greece and ²Department of Cardiology, Athens Euroclinic, Athens, Greece.

Coronary artery ectasia (CAE) is an inappropriate dilatation of the coronary vasculature, which is characterized by marked degradation of the musculoelastic elements of the tunica media and disruption of the internal and external elastic lamina. Proteolytic enzymes, such as matrix metalloproteinases (MMPs) and cysteine proteases can degrade the extracellular matrix and have been implicated in the process of atherosclerosis and arterial aneurysm formation. Although there are reports of increased MMPs in patients with CAE, the role of cathepsin cysteine proteases in CAE has not been fully investigated. We investigated the levels of serum cathepsins K and L and their major endogenous inhibitor, cystatin C, in patients with CAE compared to controls.

In our study thirty four patients with angiographically proven CAE were matched to thirty five controls for age, sex, BMI and coronary artery disease (CAD) severity. Cathepsin K and L concentrations were determined using enzyme immunoassays (ELISA) and cystatin C using a particle-enhanced turbidimetric assay on the Abbott Arcitect ci8200 analyzer.

We observed a significant correlation between serum cathepsin L and the number of diseased vessels in our patients ($r_s=0.436$, $p<0.01$), although there was not statistically significant differences in serum cathepsin K, L or cystatin C levels between CAE patients and controls.

We conclude that serum cathepsin L concentration is correlated with the severity of CAD and further studies are needed to validate its use as a potential biomarker for CAD severity. However, cathepsin K and L or cystatin C levels are not useful for the detection of patients with CAE.

Poster 5

A liquid stable enzymatic lithium assay. Bezverkov R, Dou C, Data A, and Yuan C. Diazyme Laboratories, Poway, CA.

Background: Lithium carbonate is used as a therapeutic agent for the treatment of bipolar (manic depression) disorder as well as for mood stabilization. Lithium has a narrow therapeutic range (0.4 – 1.4 mM) and, as a result, regular monitoring of the patient's clinical state and serum lithium levels are required. Current assay methods for lithium serum concentration include flame atomic emission spectrometry, ion-selective electrode (ISE) measurement, and porphyrin dye spectrometry. Recently, we have developed a liquid stable, fully automated enzymatic method which has significant improvements over the previous lyophilized powder version in both user friendliness and assay performance.

Methods and Results: The liquid stable enzymatic lithium assay is based on an enzyme whose activity is lithium concentration dependent. The enzyme, a phosphatase, converts its substrate, adenosine diphosphate, to hypoxanthine through coupled enzymatic reactions and generates hydrogen peroxide (H_2O_2), which is quantified by a conventional Trinder reaction. The assay consists of two liquid stable reagents, takes less than 10 min and uses only 5 μ l of a serum sample. The reagent on-board stability was determined, based on NCCLS guidance, on two different instruments, the Hitachi 917 and the Beckman CX-7 analyzers. It was found that the reagent on-board stability on both Hitachi 917 and Beckman CX-7 is 8 weeks. The calibration frequency is at least two weeks for both of the instruments. The reagent shelf-life is 15 months when stored at 4°C. The assay had within-run imprecision of CV = 4.3% at 1.0 mM lithium and CV = 1.2% at 2.5 mM lithium. The total imprecision values were CV = 4.8% at 1.0 mM lithium and CV = 1.3% at 2.5 mM lithium. The reportable range of the assay was found to be from 0.19 - 3.10 mM lithium on various instruments. The assay was not interfered with significantly (< 10%) by other ions including Na^+ (200 mM), K^+ (20 mM), Ca^{++} (4.0 mM), and NH_4^+ (1.0 mM). The assay was also not interfered by other phosphatases such as alkaline phosphatase (1000 U/L) and acid phosphatase (1000 U/L). The assay results determined by the liquid stable enzymatic lithium assay correlated well with both the results obtained by the ISE method and by the porphyrin dye method (Thermo Trace) with R^2 values of 0.992 and 0.990, respectively, when tested on the Hitachi 717 chemistry analyzer.

Conclusion: The liquid stable enzymatic lithium assay is a significantly improved method for the determination of serum levels of lithium. It is a much more user friendly assay and is suitable for applications on most clinical chemistry analyzers.



Poster 6

Integration of nucleic acid, protein and small molecule detection on a rapid, point-of-care multi-parameter platform. Blair ED¹, Salmon JC², Bachmann TT³ and Pritchard DJ⁴. ¹ITI-Scottish Enterprise, Glasgow Scotland; ²Lab901, Edinburgh, Scotland; ³Division of Pathway Medicine, Edinburgh, Scotland and ⁴Axis-Shield Diagnostics, Dundee, Scotland.

We have developed a prototype instrument and cartridge combination to demonstrate our biosensor platform “sample to answer” concept, using hepatitis C infection as the demonstrator application.

A novel process allows 2mL of plasma to be isolated from up to 4mL of fresh whole blood in 2 minutes, with red cell removal efficiency of 99.99% and white cell capture of greater than 97%.

The two-part cartridge, consisting of dry-reagent storage and waste-handling components together with integrated microfluidic chips, processes plasma for detection of three analyte classes. Nucleic acid (RNA) is detected by reverse transcription and real-time qPCR in approximately 13 minutes, detection of proteins by both ELISA-type and enzyme-activity assays is executed in less than 10 minutes, and a homogenous assay for small molecule detection has also been demonstrated. The microfluidics chips are prepared by a co-extrusion lamination process, giving a seven layer format that allows for accurate liquid handling and parallel control/ calibrator channels. One chip design can run two assays (qPCR and enzyme) simultaneously, thus demonstrating the multi-parameter capability, while also accommodating multiplex assay formats. The assay timescales are shortened by a novel rapid-mixing chip design feature.

The prototype instrument has been developed on a modular basis to maximise development flexibility. It consists of four processing ‘slices’, comprising a motion controller, a fluid and bead manipulator, an optical detection unit and a qPCR unit. Each of these ‘slices’ is able to operate in unison with the others, as appropriate, to execute the requisite assays on the microfluidic chips. The detection capabilities are consistent with the analytical performance dictated by a detailed functional requirements gathering exercise. All features, including a novel means of connecting the cartridge to the instrument, are capable of miniaturisation during further development. A draft control script provides a simple user interface that facilitates revisions and adaptations.



Figure 1. The EBB instrument (left), user interface (centre) and the multi-parameter EBB cartridge.

Poster 7

A novel, rapid, automated enzymatic assay for detection of myeloperoxidase in human plasma.

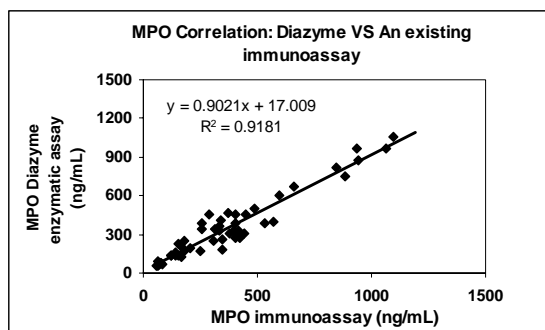
Gong XM¹, Dou C², and Yuan C².¹General Atomics, San Diego, CA and ² Diazyme Laboratory, Poway, CA.

Background: Myeloperoxidase (MPO) is a hemoprotein present in leukocytes, catalyzing the peroxidation of halide ions to produce strong reactive oxidant species against a broad range of invading parasites and pathogens. MPO plays therefore an important role in human innate host-defense mechanism. However, MPO-derived reactive oxidants cause host tissue injury through lipid and protein peroxidations leading to cardiovascular inflammation. Elevated MPO in plasma is a sensitive clinical indicator of cardiovascular and other chronic inflammatory diseases. Recently, an MPO immunoassay was developed for uses on specific immunoassay instruments. In this study, we report a novel enzymatic MPO assay that can be run on general clinical chemistry analyzers.

Methods and Results: Diazyme's enzymatic MPO test is a two-step assay. The first step measures the total peroxidase activity in plasma (rate A) followed by the second step measuring the non-MPO peroxidase activity (rate B) after adding a MPO specific inhibitor. The MPO specific activity, obtained by subtraction of the rates, is proportional to the MPO mass in samples. The kit consists of three liquid stable reagents, and can be run on most clinical chemistry analyzers with the first result available within 10 min. The assay is linear from 50 to 1300 ng/mL (9.5 to 247.0 mU/mL). Using NCCLS guidelines, the limits of blank, detection and quantitation are 19, 36.6, and 56 ng/mL, respectively. Intra- and inter-assay imprecision is CV < 6%. Other features of the Diazyme MPO assay include no significant interference from eosinophil peroxidase, ascorbic acid, bilirubin, or triglycerides. The results from Diazyme's enzymatic MPO assay correlate well with those obtained from immunoassay (Figure 1).

Conclusion: The enzymatic MPO assay reported here represents the first such assay designed for clinical chemistry analyzers. The assay reagents are liquid stable; the assay has a wide dynamic range and good correlation with an existing immunoassay.

Fig. 1. Correlation of Diazyme enzymatic MPO assay with an existing immunoassay. A total of 44 human plasma samples and 6 spiked plasma samples were used for the comparison. Diazyme MPO assay was performed on Hitachi 917. The specific activity of human MPO is 0.19 mU/ng. The conversion factor between the MPO mass unit (ng/mL) and activity (mU/mL) is: 1 ng/mL MPO = 0.19 mU/mL.



Poster 8

Withdrawn



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Poster 9

An integrated plastic chip for PCR detection of influenza A. Cao Q¹, Mahalanabis M², Klapperich C^{1,2}. Departments of Mechanical Engineering¹ and Biomedical Engineering², Boston University, Boston, MA.

We have developed a simplified, low cost, and disposable integrated microfluidic device with the ability to extract and purify RNA, synthesize cDNA from RNA and amplify the cDNA in the presence of a fluorescent tag. The device was fabricated with a stable medical grade cyclic olefin polymer substrate, Zeonex 690R (Zeon Chemicals, Louisville, KY). Micro-channels were made by hot embossing with an epoxy master mold, and chips were sealed with heat sealing on a hot press. To simplify the thermal control, continuous flow PCR was used and only two thin film heaters were needed to obtain the desired temperature distribution in the fluid. A high efficiency passive mixer was designed to mix the purified RNA eluted from the on-chip solid phase extraction column (SPE) with the reverse transcription polymerase chain reaction (RT-PCR) reagents. A simple thermally controlled paraffin valve was used to avoid contaminating the RT and PCR channels with wash buffer from the SPE. The integrated chip was demonstrated using a lab strain of influenza A (**VR-1469** Manassas, VA) harvested from MDCK cell culture (**CCL-34**, Manassas, VA). Virus in cell culture medium was mixed with GuSCN (guanidium thiocyanate) containing lysis buffer in a 1:3 ratio and pumped through the SPE channel, where the RNA bonded to the silica particles in the monolithic column. After washing with ethanol, 30 μ L of RNase free water was flowed through the channel for 3 min to elute the extracted RNA. At the same time, the paraffin valve was opened, so that the extracted RNA flowed into the mixer and mixed with the RT-PCR reagents. After mixing, they flowed into the RT channel, whose temperature was set at 50C. Reverse transcription proceeded for 30 minutes. The reagents were then pumped through the continuous flow PCR channel, and the cDNA was amplified. We intend to pursue this relatively simple combination sample preparation/amplification device for global health applications.

Poster 10

Point-of-care diagnosis of human immunodeficiency virus (HIV-1) viral loads using nano-based reagents and isothermal amplification. Leautaud V¹, Javier DJ¹, Sutton RE², Molyneux E³, Richards-Kortum R¹. ¹Rice University, Houston, TX, ²Yale University Medical School, New Haven, CT, ³Queen Elizabeth Hospital, Blantyre, Malawi.

Objective: As access to HIV-1 antiretroviral therapy becomes more widely available, sensitive assays to monitor plasma viral loads at the point of care (POC) are critically needed. Viral load determination is necessary to determine when to initiate therapy, to monitor compliance, and most importantly, as an early indicator of therapeutic failure. Moreover, one of the major challenges to universal access to therapy is the lack of adequate diagnosis and treatment of pediatric HIV-1 disease. Thus, our goal is to develop an integrated diagnostic test for HIV-1 viral load with high sensitivity, specificity and reproducibility for use in low resource settings.

Results and Methods: In developing diagnostic reagents, gold nanoparticles offer a cheap, versatile, and light/ heat resistant alternative for detection of viral nucleic acids. Thus the goal of our work is to use gold nanoparticles in the development of inexpensive point-of-care diagnostic reagents for detection of HIV-1 in biological samples. Our overall strategy in the development of these reagents can be summarized as: 1) isolation and purification of viral nucleic acids using magnetic microbeads; 2) signal amplification through nucleic acid amplification; 3) optical detection of viral nucleic acids with gold nanoparticles.

We have explored both gene-specific methods and Boom chemistry reagents for the isolation of viral RNA. Using commercially available streptavidin-coated magnetic beads and a synthetic biotinylated oligonucleotide complementary to the mRNA of interest, we have successfully recovered a targeted mRNA from a pool of total cellular RNA. To improve the efficiency of recovery, we have also used commercially available reagents such as the Silane Dynabeads for viral nucleic acids to successfully recover an *in vitro-transcribed* HIV-1 gag mRNA, adding a sample prep cost of \$3.00/rx.

Key to our strategy is the isothermal amplification of HIV *gag* mRNA, aimed at increasing diagnostic sensitivity, while circumventing the cost of electricity requirements of standard thermal cycling amplification. The HIV-1 *gag* target sequence is highly conserved among all group M clades, conferring a broad spectrum of detection to our assay. We have established appropriate conditions for nucleic-acid sequence-based amplification (NASBA) of viral RNAs, an approach successfully used in the detection of HIV-1 and other bacterial and viral pathogens. Using this approach we are able to detect amplification products with a lower detection limit of 10² *gag* mRNA copies per reaction.

Finally, previous research in our laboratory has focused in the functionalization of gold nanoparticles with protein and nucleic acids. Based on their distance-dependent optical properties, gold nanoparticles have been used as a colorimetric detection method, an approach successfully implemented in our laboratory for detection of attomolar (10⁻¹⁸) concentrations of mRNA from the diarrheal parasite *Cryptosporidium parvum*. We intend to use this approach for the detection of the recovered and amplified nucleic acid targets.

Conclusions: We expect that the high sensitivity of both isothermal amplification and of oligo-Au aggregation assays will make the fully integrated diagnostic platform more robust and better suited to overcome the technical limitations in a minimal resource facility.



Poster 11

Point-of-care instrument platform based on magnetic beads and silver nanoparticle metalloimmunoassay. Wilson PK¹, Szymanski, M², Porter R¹. ¹Argento Diagnostics Ltd., Twickenham, Middlesex, UK and ²Knowledge and Innovation Centre, National Physical Laboratory, Teddington, Middlesex, UK.

The Argento system is a hand-held instrument platform, based on a novel rapid metalloimmunoassay utilising silver nanoparticles and magnetic beads. The small size of the instrument and use of non-hazardous reagents, combined with a total test time of 15 minutes or less make it ideally suited to point-of-care testing. The incorporation of data logging and mobile phone Bluetooth and Wi-Fi technology enables easy transfer of data to centralised systems. The system has won a number of prizes and awards including reaching the final three products in the 2009 UK Government Technology Strategy Board Innovation Awards in the “Next Big Thing” category.

The assay system is based on conjugating antibodies to silver nanoparticles and either a second antibody or an antigen (depending on the assay format) to magnetic beads. These reagents are then introduced into the sample and allowed to bind to their targets. The magnetic beads and any silver nanoparticles bound to them are then transferred to an assay area, where the silver is reacted with ammonium thiocyanate to create a negatively charged particle. This is migrated to an electrode under a positive potential and dissolved at an electrode. The resultant silver ions are then measured by anodic stripping voltametry. The instrument software calculates the amount of target present, records the data and presents it to the user in an appropriate format, which can be customised for each application.

At present this technology is being developed for targets related to elite sport, cardiac markers, and viruses, but could in principle be applied to any analyte for which antibodies are available. Assays demonstrated to date indicate that the sensitivity of the Argento platform can be significantly greater than ELISA using the same reagents. Since the readout is electrochemical, measurement can be carried out in any sample matrix. Initial assays are to be based on saliva primarily for ease of sample collection.

Poster 12

Multiplexed, high performance finger-prick test for POC diagnostics using optomagnetic technology. Dittmer WU¹, Orsel JG, Neijzen JHM¹, Ovsyanko M¹, Beerling BJM¹, van Boekel MAM¹, de Theije FK¹, Nieuwenhuis JH¹, Immink AHJ¹, Dekkers DWC², Hefti MH², Vissers JLM², Martens MFWC². ¹Philips Corporate Technologies, Eindhoven, The Netherlands and ²Future Diagnostics BV, Wijchen, The Netherlands.

We present an integrated testing device that is able to perform multiple assays in parallel from a single finger-prick sample based on a recently developed optomagnetic technology known as Magnotech. Multiplexing will become increasingly important for POC diagnostics, as the specificity of the test outcome can be significantly enhanced by increasing the number of markers analyzed. A key feature of our technology is the high degree of independence between the assays which has been achieved by using microfluidics and multiple distinct magnetic actuation zones to separate the assays. As a result tailored reagents and assay protocols, employing magnetic actuation, optimal for each analyte can be used to achieve high analytical performance for panel applications.

In the first step of the heterogeneous assay, analyte molecules are captured on 500 nm magnetic particles functionalized with tracer antibodies. Thereafter the particles are attracted to the detection surface, to which particles bind depending on the concentration of analyte in solution. The motion of the magnetic particles (and the related binding and free-bound separation reactions) is precisely and swiftly controlled by the electromagnetic coils positioned above and below the disposable. Particles specifically bound to the sensor surface are sensitively detected using the optical technique of frustrated total internal reflection (f-TIR).

A compact, injection molded plastic disposable containing four chambers was designed with fast and efficient on-board microfluidic separation of plasma from whole blood and an on-board dry magnetic particle and reagent formulation was investigated for extended stability. Assays for cardiac markers (including cardiac troponin, NT-proBNP) and parathyroid hormone were developed that demonstrated sensitivities in the picomolar range for a total assay time of approximately 5 minutes.

The combination of magnetic particles and their fine actuation with electromagnets permits the rapid and sensitive detection of proteins. Because of the high analytical performance and ease-of-use of the test, it is well suited for demanding point-of-care medical diagnostic applications.



Poster 13

A PDMS sample pretreatment microdevice to enable downstream electrokinetic manipulations in bovine serum. Abram T and Clague D. California Polytechnic State University San Luis Obispo, CA.

The notion of sample preconditioning, or pretreatment, as a micro-unit operation in a Lab on a Chip (LOC) system has yet to be realized in commercial practice. As is well known, Biomarker detection in complex, biological samples, such as blood requires a series of pretreatment steps to enable detection of specific markers. On chip, such a process usually relies on “off-chip” sample pretreatment prior to “on-chip” analyte manipulations and detection. Presented in this paper is a PDMS, pretreatment chip based on the design of Oddy *et al.*¹ with a view to enable a self-contained LOC platform. The chip was designed to directly manipulate the suspended species while adjusting fluid properties using buffer volumes less than 1 ml. Using previous literature related to capillary electrophoresis, a bench-scale pretreatment protocol was developed to tune specific fluidic parameters to an optimal range, namely pH, conductivity, and viscosity. A PDMS device was fabricated and used to combine a raw, bovine serum sample with specific buffer solutions. Off-chip electrodes were used to induce DC-electrokinetic micro-mixing of the target analyte in the mixing chamber, where a homogeneous analyte distribution was achieved in less than one second using an 800V DC pulse wave. Additionally, the desired solution viscosity and pH were achieved using less than 1 ml of buffer solution. Adjustment of sample conductivity, which is driven by sample fluid volume, remains an open area of research.

Keywords: Pretreatment, Microfluidic, Micro-mixing, Diagnostic, DC-Electrokinetic, PDMS

References:

1. M.H. Oddy, J. G. Santiago, and J.C. Mikkelsen *Analytical Chemistry*, 73, 5822-5832 (2001).



Poster 14

Single Cell Impedance Sensing on Chip. Hernandez S, Fadriquel J, Szlavik R, Clague D.
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Obispo, CA.

Common methods for disease quantification, e.g., needle biopsy for cancer, are invasive and use tissue, surrounding body fluid and symptom level quantification. The common quantifications being stages 0, I, II, III and stage IV cancer, or the TNM measures, or a combination of the two quantifications. Cell-lines have unique complex impedance spectra [Jones] and Cancerous cells are known to exhibit tell-tail changes in impedance spectra.[gascoyne] Flow through Impedance Sensors. exist today; however, these sensors lack desired precision in spectra owing to variations in cell position, cell concentration and time of analysis. To address these concerns, a Single Cell, PDMS, impedance micro-chip was fabricated and demonstrated using polystyrene beads and yeast cells. The single cell capture device is presented and discussed. Results for single cell and small numbers of cells in the detection chamber are shown, and the m-fluidic cell isolation protocol is elucidated. An overview of the underlying electronics and Lab View interface is also presented. Resultant impedance spectra for an empty chamber, a polystyrene bead and a small group of yeast cells are shown. Ongoing work, conclusions and future directions are discussed.



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Poster 15

Use of a plasma separator membrane for sensitive detection of Troponin in whole blood samples. Dubitsky A, Fomovska G. Pall Life Sciences, Port Washington, NY

Vivid™ Plasma Separation Membrane extracts plasma from small volumes of whole blood. The membrane has an asymmetric pore structure which traps cells inside the membrane, allowing plasma to flow through to a receiving material or capillary. A pre-treatment process is described that enables the use of Vivid membrane for microfluidic POC rapid detection of cardiac Troponin I.

When cardiac Troponin I is present in blood, it is primarily complexed to Troponin T and Troponin C. Troponin I is most commonly used as the target molecule for ELISA detection, but is poorly soluble in its isolated form. For this reason, we used radiolabeled Troponin ITC complex and recombinant Troponin IC complex as markers of cardiac Troponin behavior in whole blood and plasma.

The radiolabeled marker allowed tracking of molecules that are free in plasma or associated with cells. It also allowed determinations of adsorption by the membrane and transmission of the complexes to a receiving membrane (the downstream plasma fraction ready for analysis). Radiotracer studies were confirmed using unlabeled Troponin ITC complex and a commercial ELISA test for Troponin I.

Influent levels of Troponin complex were varied between 2ng/ml and 20ng/ml. Volumes of influent blood were between 30 – 50ul, applied to 25mm discs of Vivid membrane. Results show that up to 20% of Troponin complex spiked into whole blood may associate with the cellular fraction and would be unavailable for ELISA detection. Pre-treating Vivid PSM membrane with a combination of casein and surfactant enhances the plasma separation process and allows transmission of 90 - 100% of the available Troponin complex without interfering with downstream ELISA detection.

Vivid PSM membranes have been designed to provide reproducible, high efficiency yields of plasma from whole blood. Blood volumes of up to 50ul/cm² of membrane can be accommodated and yields for plasma will vary from 60 – 90% of available plasma, depending on the grade of the membrane. This study has shown that Vivid GF PSM membrane, after pretreatment, is useful for small volume Troponin I assays. It is likely that this pretreatment will also improve detection of other cardiac markers as well as other non-abundant or relatively insoluble plasma components.

Poster 16

A novel and sensitive rapid diagnostic test for Chagas disease. Barfield CA¹, Barney RS¹, Crudder CH¹, Wilmoth JL¹, Stevens DS¹, Yanovsky J². ¹ PATH, Seattle, WA, ² Laboratorio Lemos, Buenos Aires, Argentina.

Chagas disease, caused by infection with the parasite *Trypanosoma cruzi*, is one of the most significant neglected diseases in the developing world. Found throughout Latin America, it is especially prevalent in rural areas where poor housing conditions encourage disease transmission from insect vectors. Although an estimated 12 million people are infected with *T. cruzi* and more than 90 million are at risk of being infected every year according to the World Health Organization, there is currently no easy and inexpensive way to diagnose Chagas disease.

Improved and more affordable diagnostic tests for Chagas disease are urgently needed. Diagnosis of Chagas disease is challenging because the recommended practice requires the use of two tests, and no combination of tests commonly employed are appropriate for use at the point of care (POC). In particular, POC tests with high sensitivity are needed to accompany screening programs. Several ELISA tests for the diagnosis of Chagas disease have more than 99% sensitivity and specificity, but currently available POC tests have sensitivities of 95% at most. This is a crucial limitation, since the most important aspect of a screening campaign is to identify as many putative infected individuals as possible.

A new, highly sensitive rapid test for Chagas disease is under development at PATH, in collaboration with Laboratorio Lemos of Argentina, which utilizes Lemos' novel, multi-epitope, recombinant antigen for detection of antibodies to *T. cruzi*. The PATH-Lemos rapid test utilizes an immunochromatographic strip format, which offers many well understood advantages for testing in resource-constrained settings. The PATH-Lemos rapid test includes a control line reagent, test line reagent (recombinant *T. cruzi* antigen), and dried detector reagent. The test is performed by adding running buffer and 10 μ L of sample (serum or plasma) to a test tube into which a PATH-Lemos rapid test is then placed. As the running buffer, rehydrated detector reagent, and sample mixture migrate up the strip across the nitrocellulose membrane, the detector reagent binds to human immunoglobulins in the sample. If the sample contains antibodies to *T. cruzi*, the complex binds to the antigens in the test line, producing a red line and indicating a positive result. In the absence of antibodies to *T. cruzi*, no red line will form in the test line area, indicating a negative result.

To evaluate the performance of the PATH-Lemos rapid test, a total of 375 previously collected serum samples from Argentina (185 negative and 190 positive samples) were blinded using a randomization key. The samples were then independently tested using three separate assays: 1) the PATH-Lemos rapid test, 2) a commercially available rapid test (Chagas STAT-PAK[®], Chembio), and 3) an FDA-approved ELISA test (the Ortho *T. cruzi* ELISA, Johnson & Johnson). Compared to the ELISA test, the PATH-Lemos rapid test demonstrated a sensitivity of 99.5% and a specificity of 96.8%, while the Chagas STAT-PAK[®] demonstrated a specificity of 99.5% but a sensitivity of only 95.3%. These results indicate that the PATH-Lemos rapid test has great potential as a highly sensitive and appropriate diagnostic test for Chagas disease.

Poster 17

Highly multiplexed point-of-care diagnostic system for infectious disease panel assays.

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Point-of-care (POC) infectious disease testing has been significantly improved with the emergence of high quality rapid tests based on immunochromatographic strip, or lateral flow technology. Unfortunately, lateral flow tests are typically configured for only one or two target pathogens. In parts of the world with high infectious disease burden, health care providers are confronted with diagnosing disease that could originate from any number of etiologic agents, including HIV, malaria, hepatitis viruses, syphilis, etc. While rapid tests exist for all of these diseases, the cost, training, and quality control burden of managing multiple rapid tests in high disease prevalence clinical settings is operationally prohibitive. Further, some diseases, most notably tuberculosis, invoke a complex immune response that has prevented development of single marker rapid tests. Immunodiagnosis of tuberculosis, particularly in the context of HIV coinfection, will most likely be based on measurement of panels of pathogen markers.

mBio Diagnostics has developed a low-cost, multiplexed POC platform for infectious disease panel immunoassays. Based on a novel combination of planar waveguide technology and low density microarrays, the system delivers serodiagnosis for dozens of disease markers in parallel. It uses a 5 microliter sample in a simple, disposable cartridge with workflow similar to current rapid tests. Cartridges are read on a low-cost, low-power fluorescence imager amenable to implementation in low resource settings.

Clinical sample data are presented for an HIV / opportunistic infection antibody detection panel consisting of multiple antigens from HIV, hepatitis C virus (HCV), and *T. pallidum*. The panel also includes in-array sample and procedural quality controls. Data are presented for 170 clinical serum samples collected from a cohort of high-prevalence HIV/HCV/syphilis individuals in San Diego, CA. Based on preliminary positive/negative call algorithms, current analysis shows HIV specificity and sensitivity of 100% and 98.2% respectively, and 97.9% and 100% specificity and sensitivity for HCV serology. *T. pallidum* results show 100% correlation with confirmed RPR+/TPPA+ samples. The device has also been tested against commercial HIV seroconversion panels and data are presented showing excellent correlation with laboratory-based enzyme immunoassay results. Finally, as a complement to the qualitative serology results, the quantitative assay capability of the system is also demonstrated. Specifically, clinically relevant standard curve data are presented for a cartridge-based hepatitis B surface antigen capture assay.

Poster 18

Affordable point-of-care viral RNA specimen processing for low-resource settings. Stevens DS¹, Beddoe AG¹, Hubbard L¹, Crudder C¹, Gerlach J¹, Santos TDL¹, LaBarre PD¹, Weigl BH¹, Domingo GJ¹. ¹PATH, Seattle, WA.

A major challenge in developing diagnostic tests for RNA biomarkers for infectious diseases such as HIV, pandemic influenza, and dengue fever is employing specimen handling methods that protect the integrity of these labile molecules. Commercially available products that address this problem are expensive, technically demanding, and/or require some form of refrigeration, requirements which cannot easily be met in low-resource or remote settings. A low-cost, easy-to-use platform for extraction and stabilization of viral RNA at the point of specimen collection would introduce flexibility into an otherwise very rigid and costly centralized testing system. One particular need for this technology is antiretroviral therapy monitoring through HIV viral load testing.

We have developed a low-cost technology to extract HIV viral RNA at the point of care and stabilize it, removing the need for cold chain preservation of the specimen. A modular design has been developed in order to confer versatility to the product for applicability to other targets (such as avian flu), specimens (such as nasal swabs), and downstream applications (such as PCR or transcription-mediated amplification). To ease scalability and realize potential economies of scale, the platform components are being built around low-cost, off-the-shelf laboratory disposables. The final product is designed for production by local or regional diagnostic enterprises. The performance of the platform as shown on clinical specimens matches the performance of current gold-standard viral RNA extraction technologies. Additionally, we show the quantitative stabilization of RNA from clinical specimens at 45°C over a period of one week, removing the need for cold chain specimen handling.



April 22 & 23, 2010 San José, Calif.

Poster 19

Simple, inexpensive pro-viral DNA preparation at the point-of-care. Stevens DS¹, Barfield CA¹, Emery S², Overbaugh J², and Gerlach J¹. ¹PATH, Seattle, WA, ²Fred Hutchinson Cancer Research Center, Seattle, WA.

We are developing a new type of sample collection card that will capture and purify pro-viral DNA at the point of sample collection. The card is designed to be compatible with a broad range of nucleic acid amplification and detection platforms. Further, the design seeks to maximize ease of use for collecting and preparing specimens as well as setting up PCR (or other nucleic acid amplification) reactions with prepared specimens. The entire process, from sample collection to PCR-ready sample, takes less than five minutes to complete and requires virtually no technical expertise or expensive equipment.

Our extraction process selectively captures peripheral blood mononuclear cells on a membrane. The membrane is integrated into the collection card, which utilizes an absorbent pad to drive fluid flow and contain flow-through waste for easy disposal. Whole blood is deposited on the membrane, and wash buffer is applied to rinse away red blood cells and their associated PCR inhibitors. The membrane matrix can be placed directly in the reaction tube for testing. We are designing the collection card to be compatible with standard reaction tubes available from a variety of suppliers, paying particular attention to ensuring that the presence of the membrane in the tube will not disrupt the light path used for fluorescent or other optical amplicon detection methods. The method is suitable for preparing specimens to perform nucleic acid tests at the point of specimen collection or for drying specimens and transporting them to a laboratory for testing (much like dried blood spots [DBS]).

This is a particularly attractive alternative to conventional DBS methodologies for pediatric HIV diagnosis in low-resource settings. To investigate this application, we have evaluated our system using ACH2 cells spiked into whole blood. ACH2 cells are a highly characterized T-cell line with a single copy per cell of the full HIV-1 genome. These properties make ACH2 cells ideal for exploring the cellular capture efficiency of our extraction protocol. A well-established generic HIV-1 quantitative PCR was used to determine the limit of detection (LOD) of ACH2 cells that were isolated using our extraction system. For comparison, whole blood dilutions of ACH2 cells were prepared and extracted using both Qiagen DBS and Qiagen whole blood standard protocols. Our system (LOD 10 c/mL) outperformed the Qiagen DBS extraction (LOD 100 c/mL), and it had equivalent performance to the Qiagen whole blood extraction (LOD 10 c/mL).



Poster 20

Fluorescence imaging system for point-of-care CD4 cell counting in low resource settings.

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mBio has developed a point-of-care diagnostic system that combines a disposable plastic cartridge with an extremely low cost fluorescence imaging instrument. A principal application for this system has been measuring helper T cell concentrations in HIV positive patients in low resource settings. Helper T cells, or CD3+/4+ cells, decrease in number as an HIV infection progresses. Quantitative cell counts over the range of about 200 to 1500 cells/uL are a critical indicator for initiating and optimizing anti-retroviral treatment and preventing viral drug resistance. In developed countries, flow cytometry is the standard method of measuring cell counts, but the costs of flow cytometry are not practical for low resource settings. In contrast, the mBio imaging system is a low cost, rapid, and fieldable system for CD3+/4+ counts.

A sample processing procedure to lyse red blood cells and stain relevant lymphocytes was developed, and specifically does not involve centrifugation, consistent with the low resource application. Prepared sample is placed in a disposable cartridge that is inserted into the imaging instrument. The cartridge manages fluid flow via a passive wick design, and incorporates robust light coupling into a planar polymer waveguide for broad illumination across the image area. The imager uses consumer optoelectronic components for low manufacturing cost and limited power consumption, resulting in a compact unit powered by a battery operated laptop and USB port connection. The imager records a brightfield image and fluorescent images for two different fluorescence channels. Captured images are co-registered to resolve debris, unlabelled cells, and cells with either or both stains. The total time from specimen to counts is approximately 25 minutes, and 8 to 16 cartridges are routinely run in parallel.

Helper T cell concentrations from 0 to about 1600 cells/uL have been measured and compared to flow cytometry results. Reproducibility is primarily limited by statistical noise, and sample preparation parameters such as incubation times and lysis buffer concentration have been characterized. Histograms of frequency vs. signal strength have been combined with different fluors and stains to optimize exposure time and other imaging factors, distinguish T cells from monocytes and other image features, and develop image analysis algorithms. Isotype controls and other negative samples have also been used to characterize non-specific binding. Continued development and packaging will lead to expanded clinical testing, field trials, and practical cell counting in low resource regions.



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Poster 21

Rapid sample preparation, DNA amplification and detection of Herpes Simplex Virus suitable for point of care applications. Ferguson TM¹, Erwin B¹, Perez M², Hickerson A¹, Roskos K¹, Doebler R³, Irvine B³, Sterling J¹, Voelkerding K^{2,4}, Niemz A¹. ¹Keck Graduate Institute, Claremont, CA, ²ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, ³Claremont BioSolutions, Upland, CA, and ⁴University of Utah, Department of Pathology, Salt Lake City, UT.

Nucleic acid amplification technologies enable sensitive and specific pathogen diagnosis and surveillance in developed and developing countries. Point-of-care testing in low resource settings requires rapid, simple to use, and inexpensive nucleic acid based diagnostic assays and related devices that address the entire process from sample in to answer out. We are developing such a platform technology applicable to a variety of pathogens, and as proof of concept are addressing the rapid diagnosis of Herpes Simplex Virus (HSV) from DNA extracts of clinical swab samples. HSV can lead to life-threatening infections in newborns and immuno-compromised individuals; therefore a need exists for rapid HSV diagnosis in STD clinics and maternity wards.

Isothermal technologies require a single reaction temperature for nucleic acid amplification, and can be implemented through simple instrumentation suited for point-of-care molecular diagnostics. Our system uses assays related to the Exponential Amplification Reaction (EXPAR) to (a) generate specific trigger oligonucleotides from HSV target genomic DNA, (b) exponentially amplify the trigger oligonucleotides, and (c) convert trigger into reporter oligonucleotides, all within one homogeneous reaction. To facilitate visual detection with minimal equipment needs, we have coupled this reaction with nucleic acid lateral flow (NALF). Using EXPAR coupled with NALF, we can detect 1 aM (18 copies in a 30 µl reaction volume) of a plasmid vector with HSV-1 derived insert, and based on a small preliminary clinical study we can distinguish HSV-1 positive from HSV negative DNA isolates of clinical swab samples. We are further implementing EXPAR amplification and NALF detection in a disposable pouch-based cartridge prototype coupled to a miniaturized heater, with proof of principle established.

To facilitate sample preparation, we have coupled this reaction upstream with a miniaturized, inexpensive, battery operated and disposable bead-based device for mechanical pathogen lysis and solid phase DNA extraction. We demonstrated effective sample preparation coupled to PCR-based HSV detection using swabs of HSV infected human epithelial cells as surrogate for herpetic lesions. We also demonstrated effective sample preparation coupled to EXPAR amplification and lateral flow based detection of HSV derived sequences, using *E. coli* cells that express the above mentioned plasmid vector with HSV insert. Building on these proof of principle results, our ultimate goal is to integrate sample preparation, EXPAR amplification and NALF detection into a single, closed unit, disposable cartridge coupled to a handheld device to enable sensitive and specific detection of HSV from swab samples of herpetic lesions in 30 minutes sample in to answer out. We further are expanding our efforts to other pathogens relevant to point- of-care diagnostics in low resource settings.



Poster 22

Improved enzymatic cycling method for determination of ethanol concentration in

Biological sample. Wang T, Tang Y, He P. Yujiang Medical College for Nationalities, Baiso, China.

Introduction: Gas chromatography is considered to be the reference method for ethanol determination. However, enzymatic ethanol assays have been selected as routine screening method. The most frequently used enzymatic assay utilizes the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase with concurrent reduction of nicotinamide adenine dinucleotide (NAD) to NADH while monitoring the increase in absorbance at 340 nm. Previously, several authors reported that increased concentrations of lactate and lactate dehydrogenase (LDH) can cause false-positive results in haemolytic sample, because of the NADH produced by LDH. The another disadvantage of the NAD-ADH system is insensitive in low alcohol sample. In this paper, we present an enzymatic cycling method to overcome the weakness of the NAD-ADH system, which includes two steps, in first step, ethanol is converted to acetaldehyde by ADH, and the potential lactate is depleted. In second step, acetaldehyde formed is subsequently oxidized to acetic acid by aldehyde dehydrogenase, while thio nicotinamide adenine dinucleotide(thio-NAD) is reduced to reduced thio nicotinamide adenine dinucleotide(thio-NADH), in the presence of NADH, acetic acid is reversely catalyzed to acetaldehyde,. Thio NADH signal is amplified during the cycling reaction and can be monitored at 405nm.

Methods: The method involves use of thio-NAD(+), NADH , alcohol dehydrogenase(EC 1.1.1.1) and aldehyde dehydrogenase (EC. 1.2.1.5) , and measurement of the increase in absorbance at 405 nm of thio-NADH at 37 degrees C.

Results: The calibration curve for ethanol was linear ($r=0.99$) between 0.5 and 120 mmol/l. Analytical recoveries of exogenous ethanol added to serum and urine were 100-105% and 98-103%, respectively. Within-run and between-run coefficient of variation (CV) were 2.8%(1 mmol/l), 1.6%(30 mmol/l) and 0.8%(80mmol/l) in blood sample, and 3.4%(1 mmol/l), 2.5%(30 mmol/l) and 1.2%(80mmol/l) in urine sample, respectively. There were no significant difference($p>0.05$) in the results of the congenerous samples with or without lactate and LDH added(lactate 3.0 mmol/l, LDH 400 U/l).

Conclusions: An improved enzymatic cycling assay for ethanol determination was successfully developed, which was more sensitive and able to eliminate the interference of lactate and LDH. This method is especially suit for the low ethanol sample and haemolytic sample.

Poster 23

Use of AssayCheX™ to increase confidence in xMAP® assay results. Vahedi A, Nghima-Tahiri R, Cheung Y, Millar L, Vijayakumar S, Sarma S, Wang, B. Nolan N, Phelps BH, Beggs MJ. Aviiir, Inc., Palo Alto, CA.

The multiplexing capabilities of Luminex's xMAP technology offers many benefits, yet running multiple immunoassays simultaneously in one well of an assay plate also increases the complexity and uncertainty of monitoring assay quality in each well. Radix® BioSolutions offers the AssayCheX® set of internal control microparticles to monitor the quality of xMAP assay results. We evaluated the effectiveness of using AssayCheX as internal control microparticles during a large clinical sample study with 49 serum protein xMAP assays multiplexed across three assay panels.

The multiplexed immunoassay panels consisted of custom 12-plex and 16-plex assays developed with R&D Systems, and the Human Cytokine 21-plex assays marketed by Bio-Rad. An initial study demonstrated that the use of AssayCheX control microparticles allowed for detection of reagent pipetting errors. We also saw that there was little or no interference of the AssayCheX with the specific immunoassays in the panels. Multiple plates of each panel were run to determine the expected ranges of AssayCheX signals and to define acceptance ranges. These acceptance ranges were used to assess the quality of data generated by testing over 1400 clinical samples in duplicate across each panel. Forty-five plates, each containing 32 serum samples in duplicate were run with each of the three multiplex panels, generating over 4300 data points for each of the AssayCheX microparticles per immunoassay. In order to implement an effective assay QC process, each AssayCheX microparticle required a different approach when defining and applying its acceptance ranges to avoid false rejections. Because each of the four AssayCheX microparticles provided a different insight into what may have happened with the multiplexed assays in each well, we were able to identify assay outliers for clinical samples exhibiting a large %CV between duplicates. By excluding just the duplicate well where the AssayCheX signals were outside of their acceptance ranges, we were able to salvage the remaining replicate, thereby allowing us to obtain valid values for several samples that would have been rejected in our study. This was especially important when the sample volume was limiting and prevented any opportunity to retest.

AssayCheX can also provide information to help discern the cause for signal shifts observed in assay results. For example, drift of the assay signal across individual plates is sometimes observed. We were able to eliminate Bio-Plex® Reader malfunction as the cause for this variability by analyzing the signal from the AssayCheX instrument control bead that monitors instrument functionality.

In conclusion, with appropriately set ranges, the AssayCheX control microparticles provided internal controls for the xMAP assays by indicating which wells had an assay problem, thereby reducing the number of samples rejected due to outlying results and increasing confidence in the final data set. Using AssayCheX control microparticles can help with monitoring assay quality in each well and also provide troubleshooting information regarding signal trends.



Poster 24

Paper-based ELISA. Cheng C-M, Martinez AW, Gong J, Mace CR, Phillip ST, Carrilho E, Mirica KA, Whitesides GM. Department of Chemistry & Chemical Biology, Harvard University, Cambridge MA.

ELISA (enzyme-linked immunosorbent assays) technology is commonly used for detecting antibodies and antigens in both developing and developed nations; especially, developing nations are necessary of new diagnostics technologies designed specifically for use in resource-poor settings. In this abstract, we describe the development of paper-based ELISA (P-ELISA), which basically demonstrates ELISA with SU-8 patterned paper 96-microzone plates, a new and inexpensive platform for biochemical analysis. P-ELISA is basically the same as the conventional ELISA, but differs in using SU-8 patterned paper 96-microzone plates rather than plastic wells or tubes as the reaction medium. SU-8 patterned paper 96-microzone plates for ELISA can have the same layout as plastic 96-well plates, but each test zone requires only $\sim 3 \mu\text{L}$ of samples and reagents, and the results can be quantified colorimetrically with the proper choice of enzyme and substrate. We first developed SU-8 patterning technique to fabricate paper 96-microzone plates with the diameter of 3.5 mm, which was filled with $1 \mu\text{L}$ of solution for each test zone. Besides, we found that $>90\%$ of the proteins (e.g., TRITC-labeled rabbit IgG) added to the test zones remained adsorbed onto the surface of the paper after drying and washing the test zones under ambient conditions. To demonstrate an indirect P-ELISA, we used rabbit IgG as model antigen and to evaluate the flexibility of P-ELISA, we developed a three-step protocol that i) immobilized rabbit IgG and then added its primary antibody on a SU-8 patterned paper 96-microzone plate; ii) prevented non-specific binding of proteins in the test zones and iii) washed unbound proteins out of the test zones, by using TRITC-labeled rabbit IgG as an indicator to develop this protocol. In addition, we also developed the colorimetric indicator which used alkaline phosphatase as the enzyme and BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) as the substrate; they produced a color change from light yellow to dark purple, which had excellent contrast with the white background provided by paper. The result indicated that P-ELISA was capable of detecting 0.67 fmoles of rabbit IgG immobilized in single paper-based test zones, which is comparable with the bench-top ELISA. Furthermore, we then demonstrated that this P-ELISA can be used to detect HIV antigen in human serum, which is an obvious choice as a model immunoassay with direct relevance to human health. Several HIV-positive samples with various diluted concentrations and control samples were examined; results indicated that colorimetric signal intensities of both samples decrease upon dilutions. The positive result could be distinguished even for a ten-fold dilution of the human serum, suggesting that it is possible to develop quantitative paper-based ELISA for HIV-1 env antigen from human serum. The methods developed for P-ELISA, we believe, broaden the potential applications of paper-based diagnostic devices such as the detection and analysis of infectious diseases in developing nations.

Poster 25

Highly scalable barcoded magnetic beads for multiplexed assays. Ho WZ, Collins J, Chen G, Low P, Vera L. Hou, C. Applied Biocode, Santa Fe Springs, CA.

A novel Barcoded Magnetic Bead (BMB) technology for multiplexed bioassays will be presented. BMBs utilize digital technology instead of conventional analog methodology to offer unmatched decoding accuracy; precise fluorescence detection; and an unlimited number of barcodes for use in multiplex tests. Marrying BMB technology to biotechnology results in an extremely flexible platform suited for a wide variety of biological applications, such as clinical diagnostics, gene mutation analysis, drug resistance genotyping, and pharmaceutical drug discovery. The polymer-based BMB (100x30x6 μ m) is manufactured using a well-established and highly reproducible semiconductor lithographic process. All BMBs are created equal, with few bead-to-bead and lot-to-lot variations. BMBs are permanently encoded with paramagnetic material, which not only provides the digital pattern for accurate decoding (no classification ambiguity), but also enables easy washing, separation, and automation. The digital pattern is highly scalable, allowing 2^N (N= number of digits) unique codes. BMBs with N=5, 7, and 10 have been mass produced. These beads show a “high-contrast transmitted barcode” pattern when illuminated with a light source.

Three types of beads are available: (1) a standard BMB for physical adsorption, (2) a carboxyl BMB for covalent attachment, and (3) a streptavidin BMB for SA-biotin coupling. Carboxyl beads permit probes and specific primers to bind the bead surface covalently via NH₂-modified 5' termini. All bead types enable attachment of proteins, peptides, nucleic acids, and other ligands in a highly multiplexed format with high stability and low nonspecific binding. This simple and flexible immobilization chemistry enables rapid assay development for a variety of applications. Assays use one of two analytical methods for barcode identification and fluorescence detection: (1) an imaging-based system for low-to-medium multiplexed assays (up to 128-plex), and (2) a flow-based system for high multiplexed (up to 1,024-plex) assays. The image analyzer is robust and simple to use for a 96-well microplate format. The system can scan the barcode and detect the fluorescence of every single bead in a microwell in 40 seconds or process 96 samples in one hour. It has a detection sensitivity of 0.5fmole for GAPDH (DNA) and 1.0 pg/ml for IL-2 (protein). The system has been used for a variety of panel tests.



Poster 26

Novel micro-fluidic actuation technologies for integrated clinical diagnostic devices. Den Toonder JM¹, Pelssers EGM¹, Wimberger-Friedl R². ¹Philips Applied Technologies and ²Philips Research, Eindhoven, The Netherlands.

Lab-on-Chip (LOC) approaches have enabled a number of new approaches for biological research which eventually will revolutionize medical diagnostics, in particular molecular diagnostics. A practical limitation for their application and commercialization often comes from the still not user friendly and/or robust interfaces which are required to reliably control the processes on the chip. By combining our micro-fluidics expertise with our broad technology competence base, we develop and offer concepts that overcome this limitation. One of our focal points is on fluid actuation concepts which do not require a mechanical or electrical interface, but rather make use of optical and magnetic fields. Such contactless technologies allow a low-cost and robust design and fabrication of the disposable cartridges and the instruments and, importantly, are very robust in practical use. Here we present three technologies for the contactless fluid actuation of very small volumes of sample liquid inside a cartridge:

1: *Polymer micro-actuators attached to the channel wall.* We have developed polymer based magnetic micro-actuators attached in a micro-fluidic channel which are actuated using external electromagnets. By the appropriate actuation fields this generates a flow with velocities of up to 1 mm/s as well as very effective local mixing (see figure a).

2: *Actuated magnetic beads dispersed in the liquid.* Instead of microstructures at the wall, dispersed superparamagnetic beads can be used to enhance mixing in an even laminar flow by creating chaotic advection (see figure b). This can be used for rapid micro-mixing of reagents, but we have also used it to substantially enhance the target capture efficiency and detection sensitivity in an on-chip immunoassay, and developed a rapid and fully automatic handheld drugs-of-abuse testing device on the basis of this principle.

3: *Droplet manipulation on a surface by optically activated electrowetting.* For even smaller volumes droplets forming micro-liter sized reactors in the disposable chip are used. Experiments have shown that the inclusion of a photo-sensitive layer at the surface of the device (see figure c) makes it possible to use a scanning laser beam to freely move the droplet to successive locations, where processes like cell lysis, and/or PCR can take place.



Poster 27

Specimen type comparison for circulating proGRP concentration in various lung diseases. Choi HJ^{1,*}, Kim HR^{2,*}, Shin MG¹, Park J³, Cho D¹, Kee SJ¹, Kim SH¹, Shin JH¹, Ryang D¹W, Suh SP¹.

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Background: Measuring circulating progastrin-releasing peptide (proGRP) levels has been shown to be helpful in distinguishing small cell lung cancer (SCLC) from other cancers. However, recent investigations have revealed that the stability of serum proGRP was worse than that of other cancer markers, likely due to the thrombin activity in serum. Therefore, it is recommended to use plasma to achieve more stable proGRP measurement. To date, most clinical data on proGRP have been based on concentrations in serum. This study evaluated the agreement between proGRP in fresh serum and plasma from healthy individuals and patients with various lung diseases including lung cancers.

Methods: From January 2009, pairs of serum and EDTA plasma were collected from 49 apparently healthy individuals (62.1±8.5 years, M:F = 27:22). At the same time, EDTA plasma of 178 patients with pulmonary diseases were prospectively collected at their first hospital visit (65.2±9.2 years, M:F = 137:41). ProGRP concentrations were measured using the Abbott ARCHITECT ProGRP. For healthy individuals, squamous cell carcinoma antigen (SCC) and neuron-specific enolase (NSE) concentrations in serum and plasma were also compared. The final diagnoses of the pulmonary patients were SCLC (N=37), non-SCLC (N=116) and benign lung diseases (N=23).

Results: ProGRP concentrations were higher in plasma than in serum by an average of 44.4%. The correlation coefficient between plasma and serum proGRP was lower than that of SCC. But no significant difference was found between proGRP and NSE in terms of the correlation coefficient of the 2 types of specimens. Plasma proGRP was higher in malignancy (336.4±925.4 pg/mL) than in benign conditions (40.1±11.5 pg/mL). The SCLC patients showed higher levels of proGRP (1256.3±1605.6 pg/mL) compared to the other types of lung cancer. Among the SCLC cases, the limited stage diseases had lower levels of plasma proGRP (33.0±759.6 pg/mL) than the extensive diseases (1940.1±1947.7 pg/mL).

Conclusion: Higher levels of proGRP in plasma than in serum agreed with previous studies, and that was thought to reflect the enhanced stability of plasma proGRP. The difference between proGRP values obtained in serum versus plasma was not significantly different from that of NSE which was not claimed by the manufacturer. Considering the different concentrations between benign diseases, non-SCLC and SCLC, plasma proGRP is thought to be capable of aiding in the initial diagnosis of a patient with SCLC.

Key words: ProGRP, serum, plasma, lung disease, SCLC.

* Hyun-Jung Choi and Hye-Ran Kim made equal contribution.



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Poster 28

Universal platform for rapid clinical diagnostics in unprocessed samples. Lowery TJ
Fritzemeier, ML, Neely, L, Rittershaus, C. T2 Biosystems, Cambridge, MA.

T2 Biosystem's universal NanoDx detection platform consists of a portable magnetic resonance (MR) device that analyzes nanoparticle-based assays on disposable cartridges. These assays work by using superparamagnetic nanoparticles decorated with selective binding agents that sensitize the particles to the desired target analyte. In the presence of the analyte, the particles undergo a transition in their clustering state, which can be detected non-optically by measuring a change in the MR signal from surrounding water molecules. This technology can be used to detect small molecules, proteins, nucleic acids, ions, and blood coagulation on a single sample.

To use the T2 Biosystems device, the user loads the biological specimen, such as whole blood or urine, onto the cartridge and inserts the cartridge into the instrument. After a short period of time the instrument provides a readout of a quantified result available at the point of care or reported through the laboratory information system. These devices are ideal for use in doctor's offices, emergency clinics, ambulances, and field settings.

The lack of sample preparation for both immunoassays and molecular assays is a particularly distinguishing feature for the NanoDx platform. We are currently developing a panel of whole-blood based immuno and nucleic acid tests that have a rapid turn-around-time with no sample prep and produce reference-lab equivalent sensitivity and precision. Unlike many other platforms that are working to automate sample preparation by use of microfluidics, T2 Bio avoids sample prep altogether by using reagents and a detector that are compatible with unprocessed whole blood and other samples.

In this presentation we provide an overview of the NanoDx platform and describe the underlying technology. We will present data for a menu of nanoparticle assays that detect nucleic acids, proteins, and small molecules with high sensitivity in serum, as well as unprocessed whole blood and urine.

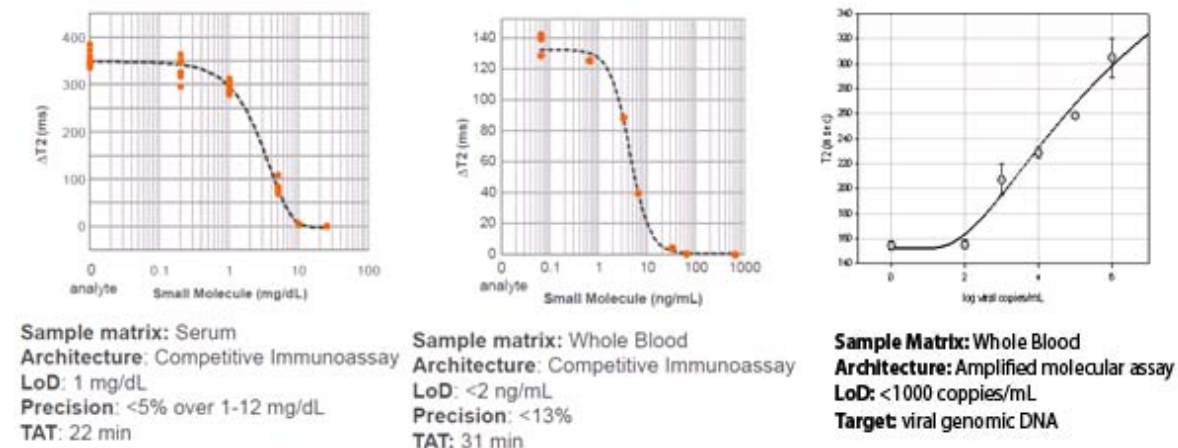


Figure 1. (a) NanoDx titration curve for a competitive assay in serum; (b) NanoDx titration curve for a competitive assay in whole blood; (c) NanoDx quantitative viral load assay performed in whole blood.

Poster 29

DNA-targeted gold aggregation assay for the detection of malaria. Cordray M¹, Orasionwu G², Amdahl M¹, Richards-Kortum R¹. ¹Rice University, Houston, TX, and ²Baylor College of Medicine, Houston, TX.

We present a new optically-based method for the detection of malaria based on the aggregation of gold nanoparticles coated with target specific oligonucleotide sequences. Malaria is one of the most serious of the diseases of poverty, causing around 1 million deaths annually. However, current diagnostic methods are often inadequate for use in the rural areas where malaria is most frequently endemic. We are investigating a gold-aggregation based assay with the potential for further development as a tool for use in these settings.

The assay requires two separate oligonucleotide sequences be designed to be complimentary to adjacent regions of a target sequence. We have designed these probe sequences for the four species of *Plasmodium* parasite that cause malaria in humans, as well as a pan-specific target, and targets for the currently identified markers of drug resistance in malaria. These probe oligonucleotides are bound onto the surface of 50nm gold nanoparticles. In the presence of target, the target DNA strand connects the two gold-bound probe sequences, building a network of closely linked gold particles which induces a red-shift in the scattering spectrum of the solution.

We have designed a cheap, easy to implement TIR system which allows for the illumination of the scattering spectra of these samples when placed on a glass slide, and allows for the easy optical detection of the result either with imaging or with portable spectroscopy. We have found that the aggregation induces a roughly 100nm red-shift in the peak scattering intensity, as well as peak broadening. We have found that the assay has a limit of detection of 40 fmoles of target. We have investigated varying the reaction conditions of the assay and have discovered that by increasing the incubation time or salt concentration of the assay, we can lower the limit of detection by an order of magnitude (to 400 amoles of target) as well as increase the contrast between positive and negative samples. We have further shown that these changed reaction conditions do not lower the specificity of the assay.

We find the aggregation assay to be promising for future development as a malaria diagnostic because of its relative ease of use, low limit of detection, and its potential for detecting different infecting strains of malaria and the markers of resistance. In future studies we will couple the aggregation assay with an isothermal amplification method to detect clinically relevant concentrations, and we will validate the system in cultured malaria parasites.

Poster 30

Evaluation of a fully automated NGAL particle-enhanced turbidimetric assay. Bangert K. BioPorto Diagnostics A/S, Gentofte, Denmark.

NGAL (neutrophil gelatinase-associated lipocalin) is a new, promising marker of acute kidney injury (AKI) in both urine and plasma. In AKI, NGAL levels in urine may increase from normal levels of up to about 20 ng/mL to levels above 25,000 ng/mL, while the range of expected levels in EDTA plasma is from about 50 to 5000 ng/mL. A fully automated, ready-to-use assay applicable to general clinical chemistry analyzers is needed in order to exploit NGAL's diagnostic potential fully. The purpose of this study was to evaluate an automated particle-enhanced turbidimetric assay for NGAL in urine and plasma samples.

The NGAL assay uses polystyrene particles coated with mouse monoclonal antibodies. The calibrator material is full-length recombinant human NGAL delivered in ready-to-use solutions at concentrations of 0, 150, 750, 1500, 3000 and 5000 ng/mL. The assay was run on a Hitachi 917 clinical chemical analyzer utilizing 3 μ L of sample, 150 μ L of buffer and 50 μ L of antibody-coated particle suspension, with a total assay time of 10 min. The within-run imprecision was estimated from 20 daily measurements of calibrator material at two concentrations. The linearity of the assay and the upper limit of the security range were determined by measurement of serial dilutions of calibrator material in steps of 10 ng/mL from 0 to 100, steps of 500 ng/mL from 0 to 5000 and steps of 8000 ng/mL up to 40,000 ng/mL. The measuring range was defined as the concentration range over which sample recovery deviated <15% from the expected value. Interference of hemoglobin (500 g/L), bilirubin (30 g/L) and emulsified lipid (5.0%) on the measurement of control material was determined. NGAL was measured with the turbidimetric assay and a commercially available ELISA kit (BioPorto) in urine samples from 130 ICU patients and EDTA plasma samples from 40 ICU patients.

The measuring range of the turbidimetric assay was specified to be 25 to 5000 ng/mL. Within-run imprecision was 2.0% and 1.2% at 200 and 500 ng/mL, respectively. The assay was linear in range from 20 to 5000 ng/mL with deviations below 5% of expected values. No effect of antigen excess was seen up to a 40,000 ng/mL. Potentially interfering substances had only marginal effects (<4%) on measurements. There was excellent agreement between the ELISA and turbidimetric measurements of both urine and EDTA plasma samples. The Deming regression fits (95% confidence intervals in brackets) were: urine, slope 0.97 (0.85 to 1.09), x-axis intercept -57 (-187 to 74), plasma, slope 1.00 (0.87 to 1.13), x-axis intercept -55 (-124 to 15). These data were not significantly different from identity between the results of the two assays.

The NGAL turbidimetric assay is a fast, precise and convenient test that works well with both urine and EDTA plasma samples over the clinically relevant concentration range. With this assay the promise of NGAL measurement for the diagnosis of AKI can be translated into widespread clinical use.



Poster 31

Attomolar detection of proteins in serum using single molecule enzyme-linked immunosorbent assays. Rissin DM¹, Kan CW¹, Campbell TG¹, Howes SC¹, Fournier DR¹, Song L¹, Piech T¹, Patel PP¹, Chang L¹, Rivnak AJ¹, Ferrell EP¹, Randall JD¹, Provuncher GK¹, Walt DR², Duffy DC¹. ¹Quanterix Corporation, Cambridge, MA, and ²Tufts University, Medford, MA.

We describe a method for detecting single immunocomplexes formed in the enzyme-linked immunosorbent assay (ELISA); we call this method digital ELISA. This method is based on isolating single immunocomplexes labeled with an enzyme in arrays of femtoliter wells, sealing the arrays in the presence of the enzyme substrate, and fluorescently imaging the array. Fluorescent product molecules of the enzyme-substrate reaction are confined in the femtoliter volume, giving rise to a local high concentration that can be easily detected using a standard fluorescent microscope. By using high density arrays of femtoliter wells, hundreds to thousands of single immunocomplexes can be detected simultaneously. Isolation of single immunocomplexes in this way gives rise to a dramatic increase in sensitivity to enzyme labels over bulk, ensemble detection methods. This method was used to detect yoctomole levels of β -galactosidase enzyme label. The enzyme sensitivity using these single molecule arrays—which we term SiMoA—is $\sim 10^5$ times greater than the same enzyme measured on a fluorescent plate reader and over 100 times greater than detection of alkaline phosphatase using chemiluminescence, the gold-standard of sensitive ELISA detection. Ultra-sensitive immunoassays for detecting clinically-relevant proteins have been developed using digital ELISAs that can detect sub-femtomolar concentrations of the proteins in serum. These assays have been used to detect proteins in clinical samples at concentrations that are well below the detection limits of current immunoanalyzers. With the aim of detecting very low concentrations of nucleic acids without recourse to PCR (or other target amplification), a single molecule assay for DNA was also developed. Attomolar limits of detection for direct detection of single molecules of DNA in a sandwich assay were achieved. This single molecule detection technology couples directly to the back-end of established clinical immunoassays providing a two to four log improvement over the detection limit of current clinical methods. The method overcomes the complexity associated with existing ultra-sensitive protein detection methods, bringing single molecule sensitivity to clinical immunodiagnostics that should lead to downstream benefits in patient care.

Poster 32

CaO-heated DNA amplification obviates electricity requirement at point-of-care. Hawkins K¹, Singleton J¹, Wilmoth J¹, Beddoe A¹, Gerlach J¹, LaBarre P¹. ¹PATH, Seattle, WA.

Many infectious diseases that affect global health are most accurately diagnosed through nucleic acid (NA) amplification and detection. Despite multiple attempts to develop small, portable, low-cost, instrument-free nucleic acid amplification tests (NAATs), none are commercially available. PATH has developed a technology that can result in a low-cost NAAT kit with the sensitivity of PCR, the simplicity of a strip test and sufficient stability for storage out of the cold chain for long periods of time. This kit will advance evidence-based medical practice at the point-of-care in global health settings.

We have developed proof of concept on individual components of the NAAT kit and are currently integrating these components. We plan to validate this kit as the first electricity-free, instrument-free, easy to use, low cost NAAT for point of care use in low resource settings.

The key components of this kit include: (1) reusable, electricity-free isothermal incubator; (100) rechargeable CaO heat modules; (1) LED keychain for fluorescent naked eye detection; (100) assay sub-kits.

Each assay sub-kit contains

- **Assay:** One set of highly accurate loop-mediated isothermal amplification (LAMP) assay reagents that can reliably detect pathogens in a whole blood sample without need for extraction, heating or centrifuge. The LAMP mastermix will be in dry stable format and packaging that obviates the need for cold-chain storage.
- **Packaging:** One pre-filled two-chamber reaction tube with locking cap that separately contains the fluorescent quencher and lyophilized LAMP mastermix to eliminate sources of sample contamination. A frangible seal allows post -amplification quencher addition without tube entry.
- **Controls:** negative and positive control tubes
- **Sample collection:** finger prick lance and blood collection capillary
- **Dilution:** pre-filled dispensers with the sample and control dilutions

Poster 33

Multiplex measurement of 39 cytokines in cerebrospinal fluid in central nervous system inflammatory disorders via Milliplex Map on Luminex analyzer. Mohammad A¹, Rocco LP¹, Svensson AM¹, Perrotta PL¹, Petersen JR², Gyure K¹ and Ducatman BS¹. ¹Department of Pathology, West Virginia University, Morgantown, WV and ²Department of Pathology, University of Texas Medical Branch, Galveston, TX.

Multiple sclerosis (MS) is the most common neurological disease in young adults with the risk of subsequent chronic functional impairment and disability after 10 to 15 years of disease duration. MS is not a single inflammatory demyelinating central nervous system (CNS) disease, but a complex disorder with heterogeneous clinical presentation, disease courses, neuro-pathological, immunological and neuro-radiological features. MS pathogenesis is believed to be initiated by autoreactive T-cells crossing the blood brain barrier in response to the liberated myelin and non-myelin components in the perivascular space. The initial locally restricted CNS inflammatory process is subsequently amplified by a huge network of adhesion molecules, proinflammatory cytokines, chemokines and other cellular effector molecules. Antigen and epitope spreading is important to the propagation of inflammation. The search for biological markers in accessible body fluids (CSF, blood, urine) of MS patients has been a scientific focus over the past decades. While many studies have been conducted on cytokine expression in MS and healthy subjects; no consensus has been reached on whether the cytokine profile of MS subjects intrinsically differs from that of healthy people. Age, sex, and disease type (relapse remitting, primary progressive or secondary progressive) may all affect cytokine secretion, making useful comparisons difficult. However, the majority of these studies have examined only a few (4 – 6) cytokines in serum and very few in CSF. Millipore's MILLIPLEX Human cytokine kit based on Luminex xMAP technology allowed us to simultaneously measure 39 cytokines in the CSF of 4 patients with active MS, 11 normal patients, and two patients with known non-demyelinating CNS infection. The levels of several cytokines including FGF-2, GRO, GM-CSF, IL-1a, IP-10 and MDC were significantly elevated ($p < 0.005$) in MS patients. These studies suggest patients with MS demonstrate significant alteration of CSF cytokine profiles. These findings may have implications in the diagnosis and monitoring of chronic demyelinating disorders. In addition, reference ranges for CSF cytokines and the technical performance of this platform for measuring CSF cytokines in a clinical setting will be presented.

Poster 34

The development of particle based homogenous immunoassay using the SPARCL™ technology. Shapir, N¹, Salvati M¹, Odegaard BH¹, Kapsner KP¹, Handley RS², Lopac SK¹, Gaibor JE¹, McLernon TL¹, Akhavan-Tafti H². ¹Beckman Coulter Inc., Chaska, MN and ²Beckman Coulter Inc., Southfield, MI.

Minimizing the mechanical complexity and time requirement of washing has long been a goal of solid phase immunoassay development. SPARCL™ technology, incorporating proximity dependent chemiluminescent detection methods under development at Beckman Coulter, Inc., entirely eliminates the need for washing or separation. In SPARCL methods, the chemiluminescent substrate (acridan) is brought into proximity to an oxidative enzyme (horseradish peroxidase, HRP) through an analyte-specific interaction. To explore the SPARCL technology, both acridan and a specific capture antibody were coupled to a solid phase (microparticle), and a complementary antibody was conjugated to HRP. In the presence of analyte, the specific analyte/antibody interaction brings the two components (acridan and HRP) into proximity. At this point, a flash of light can be generated by the addition of trigger solution without the need to remove excess reactants. To further enhance signal to noise, a background reducing agent can be added to the reaction mixture. Utilizing the SPARCL technology, an assay with analytical sensitivity equivalent to a contemporary heterogeneous chemiluminescent assay has been developed*.

SPARCL technology was used to develop a sandwich format immunoassay, using cardiac troponin I (cTnI) as the model analyte. An assay using 15 uL of sample with a total reaction assay time of < 5 minutes demonstrated an analytical sensitivity of 0.005 ng/mL and a dynamic range from 0 to 35 ng/mL. A correlation study compared the model assay to the Beckman Coulter, Inc. AccuTnI™ assay and Deming regression analysis yielded the following statistics: $y = 0.974 \text{ AccuTnI} + 0.050$; $r = 0.958$, $N = 95$.

The SPARCL technology offers unique advantages that make it an attractive technology for developing point of care and combined immunoassay/chemistry work cells. Two distinct advantages inherent in the SPARCL technology are simplification of the assay mechanics (no requirement for separation and washing reduces time and requires less complicated instrumentation) and immediate generation of a flash signal upon triggering (reduces time). Thus, the SPARCL technology should enable faster, more reliable, less complex, and smaller robust immunodiagnostic devices.

*Internal feasibility assays, not for commercial distribution of diagnostic products



Poster 35

SPARCL™ in the absence of a solid phase: a novel homogeneous solution phase immunoassay methodology. Akhavan-Tafti H¹, Mendoza JD², Binger DG², Chen Y², de Silva R¹, Xie W¹, Salvati M², Odegaard BH², Kapsner KP², Shapir N². ¹Beckman Coulter Inc., Southfield, MI and ²Beckman Coulter Inc., Chaska, MN.

Solution phase immunoassays requiring no solid phase or particle reagents and no separation steps have been developed* utilizing SPARCL™ technology. In sandwich and competitive assay formats, analytical sensitivities similar to those obtained using contemporary solid phase chemiluminescent assays are described, marking a milestone in homogeneous immunoassay development. In both formats, an analyte-specific capture antibody is labeled with a chemiluminescent substrate (acridan). For sandwich assays, a complementary antibody is labeled with HRP, while for competitive assays, an analyte or homologue is labeled with the enzyme. The acridan-labeled antibody is brought into proximity with the HRP-conjugated moiety via the specific antibody/analyte interaction. At this point, a flash of light can be generated by the addition of trigger solution with no need to remove excess reactants prior to triggering. The addition of a background reducing agent improves signal to noise.

The SPARCL solution phase technology was used to develop a competitive assay for cyclic AMP (cAMP) and a sandwich assay for prostate specific antigen (PSA). Reagents for the cAMP assay were prepared by coupling acridan to anti-cAMP-MAb and cAMP to HRP. An assay using 4 µL of sample with a 30-minute incubation time achieved a dynamic range from 0 to 33 nM with an analytical sensitivity of 1.2 nM. Reagents for the PSA assay were prepared by coupling acridan to one anti-PSA-MAb and HRP to another, complementary anti-PSA-MAb. An assay using 4 µL of sample with a 30-minute incubation time achieved a dynamic range from 0 to 68 ng/mL with an analytical sensitivity of 3.0 pg/mL.

The homogeneous solution phase immunoassays using SPARCL offer four distinct advantages: i) No requirement for particle suspension, mixing, separation or washing reduces assay time and requires less complicated instrumentation, ii) No solid/solution reaction interface offers improved kinetics, iii) No solid/solution interface offers a more native biological environment, and iv) Immediate generation of the flash signal upon triggering reduces assay time. These SPARCL technology advantages should enable reliable, less complex and smaller robust immunodiagnostic devices with rapid, sensitive assays.

*Internal feasibility assays, not for commercial distribution of diagnostic products



Poster 36

Identification of somatic mitochondrial DNA variations in colorectal cancer by next generation sequencing. Luo JD¹ and Chiou CC^{1,2}. ¹Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan, and ²Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan.

Mutations in mitochondrial DNA are associated with tumorigenesis and cancer progression. However, these mutations are difficult to identify because of multiple copies and heteroplasmy of mitochondrial DNA (mtDNA) in the cells. In this study, we used next generation sequencing technology to profile the somatic mtDNA variations in colorectal cancer. Total RNA from paired normal and tumor tissues were extracted and the sequences of transcriptomes from the samples were obtained with genome sequencer (Illumina Solexa), which generated 8-12 million, 65-base reads for each sample. The sequencing data were then aligned to mitochondrial genome (from RefSeq database) with software MAQ (<http://maq.sourceforge.net/>). Variants of these sequences were then identified with software MapView (<http://evolution.sysu.edu.cn/mapview/>). Around 2 million reads correlated to mitochondrial genome were obtained from each sample. The reads covered almost all coding regions except tRNA genes and the D-loop region. After comparing the variation rates in tumor and normal tissues, 7 non-synonymous variations significantly enriched in tumor tissues were identified, which were located in COII (2), COIII (2), ND4L (1), ND5 (1) and ND6 (2) genes. On the other hand, 47 non-synonymous variations significantly decreased in tumor tissues were also discovered. Most of the variations had abundance lower than 10%, which is difficult to identify by conventional sequencing. In summary, we have demonstrated that using high throughput sequencing and bioinformatics analysis, the map of genetic shift of mtDNA during carcinogenesis can be sketched. Although further study is required to understand the biological effects of these variations, this method provides a powerful tool to identify the candidates of somatic mtDNA mutations that may be involved in cancer development.

Poster 37

Time-integrated fluorescence cumulant analysis for reagent characterization in immunodiagnosics. Skinner JP, Tetin SY. Abbott Diagnostics, Abbott Park, IL.

Demand for increased sensitivity and lower sample volumes in immunodiagnosics results in a push for reagent characterization and detection technologies that make use of single photon counting techniques. Here we explore time-integrated fluorescence cumulant analysis (TIFCA) as a method to measure antibody properties at the single molecule level. TIFCA resolves species using photon counts from randomly diffusing fluorophores in solution based on the molecular brightness and diffusion time through the observation volume. We show that TIFCA can resolve the number of free antigen molecules, single bound and double bound antibody in a mixture of antigen and antibody. Using the fluorescently labeled epitope of brain natriuretic peptide (BNP5-13, C10A) for mAb 106.3, we perform a titration and fit data to a model described by two independent binding sites on the antibody. The method may also be applied to determine antigen concentration in an unknown sample.



April 22 & 23, 2010 San José, Calif.

Poster 38

WITHDRAWN



April 22 & 23, 2010 San José, Calif.

Poster 39

A novel microfluidic PCR method for clinical pathogen detection. Bousse L, Zhang JP, Li C, Chan S, Wu W, Eto D, Chien RL, Wada HG, Ishikawa T, Hamada T, Kawabata T, Watanabe M, and Satomura S, Wako Pure Chemical Industries, Mountain View, CA, and Amagasaki, Japan.

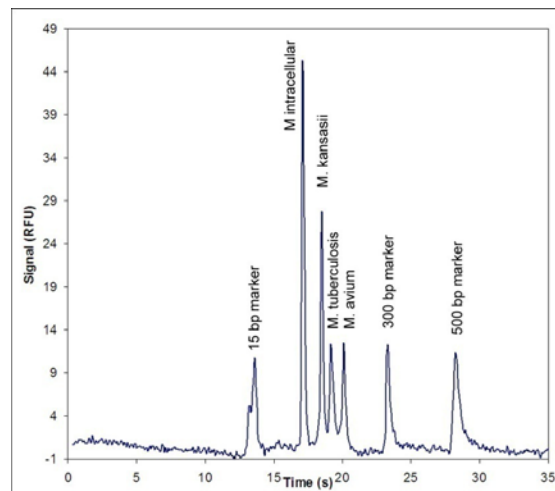
A successful PCR system for detection of infectious agents must combine very high sensitivity with low-cost disposables and detection system. We have developed a microfluidic device that achieves these goals by using a large volume (20 μ l) chamber for PCR cycling, combined with capillary electrophoresis (CE) separation, and on-chip detection. The device is fabricated by polymer molding followed by lamination with a thin polymer film, which provides good thermal contact to the solution being thermally cycled.

CE separation and detection of amplicons using intercalating fluorescent dye has the advantage of allowing simultaneous detection of multiple PCR targets without multiple expensive fluorescent probes. Our chip uses a network of microfluidic CE channels with dimensions much smaller than the PCR chamber itself. Since the flow resistance of these channels is more than 10^6 times higher than that of the PCR chamber, there is no need for integrated valves, leading to an inexpensive disposable chip.

During thermal cycling, all wells in the chip are connected to a single manifold, to avoid pressure differentials, and an external pressure of 1 to 3 atmospheres is applied to suppress the formation of vapor bubbles.

DNA samples from the PCR chamber can be electrophoretically injected in real time during thermal cycling. In this way, we have demonstrated both endpoint and quantitative PCR amplifications. The figure below shows an example of endpoint PCR detection with four multiplexed amplicons.

Using this method we successfully demonstrated that four species of mycobacterium pathogens were simultaneously detected by PCR followed by CE separation and identification on the same chip. The theoretical CE peak capacity of these DNA separations is greater than 20, which suggests that an equivalent degree of multiplexing could be achieved.



Endpoint CE separation of a multiplexed PCR separation of four mycobacterium pathogens: M. intracellular (101 bp); M kansasii (143 bp); M. tuberculosis (169 bp) and M. avium (192 bp).

Poster 40

Universal HPV genotyping assay using hybrid capture. Agarwal S, O'Neil D, Basham H, Nazarenko I. R&D Department, QIAGEN Inc., Gaithersburg, MD.

Currently, a single HPV test that can genotype all high-risk HPV types (found in malignant lesions) and low-risk HPV types (found in benign lesions) does not exist. In addition, existing HPV genotyping assays that are performed with consensus primer sets (e.g. GP5+/6+, MY09/MY11, etc.) in PCR amplification can suffer from amplification bias, and they are limited in multiplexing capability by the constraints of the consensus primer design. This study aims to develop a highly specific universal HPV genotyping assay which is capable of detecting and distinguishing all 56 high-risk and low risk HPV types of the alpha-papillomavirus family which are found in mucosal and cutaneous lesions. The applications presented here are for research purposes. They are not intended for diagnostic use.

The assay process includes extraction of target DNA from samples using QIAGEN's Hybrid Capture[®] (HC) Technology as a sample preparation method, followed by isothermal whole genome amplification (WGA) and detection using Liquichip[®] Technology (LMX). HC sample preparation uses sequence-specific RNA probes that bind to DNA targets to form DNA/RNA hybrids. DNA/RNA hybrid-specific antibodies conjugated to magnetic beads then capture these hybrids. Using short sequence-specific RNA probes improves the specificity of the assay by capturing only desired targets and increases amplification efficiency by allowing all extraneous material to be washed away.

WGA of the bead-captured DNA strand takes place in a two hour reaction, resulting in a high concentration of amplification products. Denaturation and hybridization of amplification products to uniquely classified beads, each corresponding to a specific HPV type, takes place after amplification. Each bead is conjugated with two oligonucleotide probes (one each in the E6/E7 and L1 regions) specific to that bead's HPV type. Targeting both the E6/E7 and L1 regions minimizes the probability of false results due to mutations or deletions/insertions present in one region. Specific biotinylated probes bind to the target, and are subsequently labeled with streptavidin-phycoerythrin (SA-PE). Target detection is achieved when PE is excited by a laser and a fluorescent signal is observed.

HC sample preparation allows the isolation of HPV target from as much as 5 µg of human genomic DNA. Initially, each HPV type was characterized individually for the E6/E7 and L1 region to verify that these regions can be independently detected. After initial verification, dual characterization (E6 and L1 together) was performed, followed by multiplex detection. Multiplex experiments with multiple HPV types (including all high-risk types) using this protocol demonstrated a sensitivity of 100 copies for each type while still being specific against other types at up to 10⁷ copies. These results demonstrate the potential of this assay to achieve full genotyping of the entire alpha-papillomavirus family in a single multiplex assay.



Poster 41

A low-cost, semi-quantitative point-of-care diagnostic for monitoring liver function in low-resource settings using patterned paper. Beattie P¹, Vella S², Laromaine A², Cademartiri R², Mirica K², Phillips S³, Sindi H², Whitesides G². ¹Diagnostics For All, Cambridge, MA; ²Harvard University, Cambridge, MA; and ³Pennsylvania State University, State College, PA.

This work focuses on the development of a disposable, readerless point-of-care diagnostic for monitoring liver function from a fingerstick (20uL) amount of blood for use in low-resource settings. It is estimated that a new diagnostic for malaria could save over 2 million lives annually and avoid tens of millions of unnecessary treatments. As treatment options in developing countries have increased, the ability to easily monitor the health status of patients in decentralized settings has become increasingly important. This is especially true for patients taking anti-retroviral and anti-tuberculosis medications, whose treatments are related to a high incidence of hepatotoxicity, yet few clinics have the capacity to test for liver function, especially in remote settings.

Using our patterned paper platform, we are developing an inexpensive, disposable diagnostic for monitoring liver function at the point of care. Wax printing technology allows us to easily and inexpensively (<\$0.005/device) pattern microfluidic channels and multiple reaction zones into small (1"x2") pieces of chromatography paper. Each reaction zone can be a unique test, a verification, or control. A single \$800 printer can pattern up to 71 million devices per year by itself, and the entire process of patterning the paper takes approximately 2 minutes.

Our LFT device is postage-stamp sized, requires no more than 20uL of whole blood, and can test up to five separate analytes. Colorimetric assays in development are for alanine transaminase (ALT), aspartate transaminase (AST), Alkaline phosphatase (AP), and total protein. Assays have been tuned to be semi-quantitative with visually distinct colors relating to clinical decision levels. Additionally, color intensity analysis from scanned images has been shown to correlate to quantitative levels of analyte, proving compatibility of our diagnostic with the emerging field of telemedicine.

Our device has the potential to radically expand the capabilities of clinicians and community health workers in remote settings by providing important liver function information rapidly (~5minutes) and cheaply without any external reader or power requirements. With millions of needed LFTs not being performed each year in developing countries, our diagnostic has the potential to save hundreds of thousands of lives a year by warning them of liver toxicity before symptomatic diagnosis is possible. Furthermore, this diagnostic proves the viability of incredibly low-cost patterned paper as a platform for appropriate point of care diagnostics.



Poster 42

Comparison of a point-of-care CD4 monitoring system to flow cytometry in determining absolute CD4, total lymphocyte counts and CD4 percentage in whole blood. Jacobson JW, Bhagwandin BD, LabNow Inc., Austin, TX.

Obtaining timely CD4 test results continues to be a major problem for determining treatment of HIV/AIDS patients worldwide, especially in resource limited settings. Flow cytometry (FC) in central laboratories is still the standard for providing CD4 counts. However, the paucity of skilled labor, high capital costs and infrastructure requirements all limit FC utilization. In addition, patients often live far from a central laboratory, necessitating a logistical infrastructure to transport samples and return results to the clinic. Consequently, many CD4 test results are not matched to the patient causing further delay in treatment and additional burden to the HIV/AIDS healthcare system.

The ability to assess CD4 immune status at the point of care – when the patient, the care giver and the therapy are co-localized – would greatly enhance the timely identification, treatment and management of HIV/AIDS patients. To that end, we have developed a portable, battery-operated CD4+ monitoring system utilizing low-cost LEDs, CCD-based imaging and proprietary software algorithms for data analyses. The system integrates a self-contained, single use, disposable credit-card sized BioChip to process the sample, combine assay components and carry out assay steps. No cold-chain is required. The sample is a drop of whole blood and time to results (CD4+ count, Total Lymphocyte Count, and %CD4+) is under 15 minutes.

In this study, we compared the results for absolute CD4+ counts, Total Lymphocyte Counts and Percentage CD4+ obtained with the LabNow CD4 system with FC on the same samples. Samples were analyzed from 111 subjects (84 HIV Positive, 27 HIV Negative). Subject ages ranged from 22 to 76 years and included 86 males and 25 females and CD4+ counts ranged from <50 to >500 per microliter. Whole blood specimens were collected into EDTA vacutainer tubes and kept at room temperature until analyzed. Samples were analyzed using the LabNow CD4 System within 8 hours of collection and within the current accepted standard of 48 hours for FC. Data analysis included regression to determine correlation between methods and Bland-Altman plots to assess bias between methods.

The correlation coefficient (r) between the two methods was 0.92 for absolute CD4+ counts, 0.91 for total lymphocyte counts and 0.94 for percent CD4+ ($r = 0.94$). When the two assay methods were compared with Bland-Altman Plots, the bias was 25.5 counts (95% CI, 3.5 to 47.6) for absolute CD4+ counts, 48.4 counts (95% CI, -5.2 to 102) for total lymphocyte counts, and 0.30% (95% CI, -0.58% to 1.17%) for percent CD4+.

Results of this study indicate that the LabNow CD4 System provides test results comparable to the flow cytometry standard across the clinically relevant range. These results, along with system portability, minimal sample requirements, consolidated all-in-one assay biochip and minimal infrastructure requirements suggest that CD4 test results can be provided at the point of care and improve ongoing care for HIV/AIDS patients.

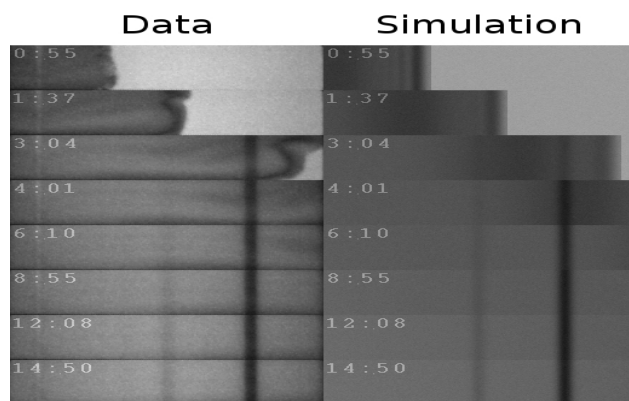
Poster 43

A novel simulation toolkit for lateral flow assays. Dylewski S, PhD. Alverix, Inc., San Jose, CA.

Most lateral-flow assays are measured in a very simplistic way: Apply the sample, wait a few minutes, and then look for the presence or disappearance of a line. Similar methods are used during the assay development process, even though the results may be recorded with a scanning or imaging reader. Even when such readers are used, typically the endpoint data is all that is measured; the kinetic information is generally ignored. Our objective is to measure as much information as the assay provides, and then match that information with a working mathematical model to extract additional assay parameters like binding rate constants and total conjugate. These new simulations could help biochemists isolate specific assay problems and improve performance, thus enabling better and more sophisticated point-of-care tests.

An infectious disease test was run according to the manufacturer's directions with a known positive sample. Using the Alverix Assay Development System, images of the assay membrane were acquired every 5 seconds until 15 minutes elapsed. These images were then compared with a mathematical model based upon original work from Qian and Bau¹. Their model was extended to include non-specific binding, variable flow rate, dissolving conjugate, and realistic capture-line profiles. A multi-parameter fit was then performed in order to estimate the parameters that, in turn, simulate images that best match the real assay.

The multi-parameter model matches very well with the live assay results. We will show side-by-side comparisons and analytical results for assays run with a sample dilution series along with statistics showing the overall variance of each fit parameter. Fit parameters include total conjugate, capture line ligand quantity, and binding rate constants.



¹ S. Qian, H.H. Bau, Analytical Biochemistry 322 (2003) 89-98

Poster 44

Direct detection of single bacterium in blood using a miniature NMR detector. Dryga SA¹, Clarizia L-J¹, Adams EA¹, Sitdikov RA¹, Crawford CW¹, McDowell AF¹, Young S.², Overturf G², Esch VC¹.
¹NanoMR, Inc., Albuquerque, NM, and ²TriCore Reference Laboratories, Albuquerque, NM.

Sepsis due to blood-stream infection is a severe clinical condition that requires extensive hospitalization and is the 10th leading cause of death in the US. Delayed diagnosis is the major contributor to disease progression. Currently, bacterial and fungal infections are diagnosed by blood culture, requiring an initial incubation of 12-72 hr to detect growth, followed by subsequent 12-36 hr incubation for organism identification. We have developed a new system for detection of infections directly in patient blood with single-cell sensitivity. The process involves labeling blood-borne bacterial cells with bacteria-specific magnetic nanoparticles, separation of blood and blood components from the labeled targets, then detection of the microbial cells by Nuclear Magnetic Resonance (NMR). Selective perturbation of the magnetic field by the immunolabeled targets results in single-cell sensitivity.

Methods. Blood samples from healthy volunteers were spiked with clinically relevant concentrations of bacteria (1-10 CFU/mL) including both laboratory strains and clinical isolates of the bacterial species most frequently found in bloodstream infections. Goat IgG antibodies were raised against specific bacteria, purified using protein G affinity chromatography, and absorbed against non-specific targets. Superparamagnetic beads were synthesized by encapsulating iron oxide nanoparticles (5-15 nm diameter) in a latex core and labeling with goat IgG. The spiked samples were diluted 3-fold with a Tris-based binding buffer and target-specific beads. After incubation, the labeled targets were magnetically separated followed by a wash step designed to remove blood products. Following a second magnetic separation step, labeled bacteria were resuspended in detection buffer and loaded onto NMR detector. All steps of the sample processing and detection are integrated into microfluidic components.

The unique NMR detector pairs a compact, high-homogeneity permanent magnet (1.7 Tesla, <0.1ppm variation) with a nanoliter scale detection coil, a combination facilitated by our patented tuning circuitry. As sample passes through the detector coil, a hydrogen resonance signal is observed at a sharply defined frequency (~70 MHz). Immunomagnetically labeled bacterial cells create local disturbances in the field, causing a broadening of the resonance frequency and a reduction of measured signal intensity which creates a detection event.

The entire NMR system has been integrated into working benchtop devices measuring 35x35x60 cm³. This includes the magnet, which weighs less than 4 kg, as well as the user interface and control computer, radiofrequency electronics, power source and controller for field homogeneity adjustment coils, temperature controller, and syringe pump for fluid pumping.

Results. Superparamagnetic beads 250 nm in diameter were synthesized by incorporating iron oxide nanoparticles into latex cores, followed by attachment of bacteria-specific IgG. Samples (1 to 10 mL of blood) were spiked with bacteria at clinically relevant concentration (1-10 CFU/mL). Initial studies using Gram-positive bacteria confirm analytical sensitivity comparable with bacterial concentrations found in clinical samples (1-10 CFU/mL). Evaluation of the system with clinical samples has been initiated.

Poster 45

Locally-smoothed (LS) median and maximum absolute difference (MAD, MaxAD): theory, application, and clinical boundary conditions: Singh S, Tran NK, Kost GJ, UC Davis-LLNL Point-of-Care Technologies Center, Point-of-Care Testing Center for Teaching and Research (POCT-CTR), Medical Pathology and Laboratory Medicine, School of Medicine, University of California, Davis.

Objective: Point-of-care (POC) glucose meters are crucial for maintaining blood glucose within an acceptable range in both diabetics and critically ill patients. Standards such as ISO 15197 now are inadequate for performance assessment. We propose the use of a new approach to improve existing guidelines for licensing POC devices.

Methods: We introduce the locally-smoothed maximum absolute difference (LS MaxAD) curve to identify maximum errors. This approach complements locally-smoothed median absolute difference (LS MAD) curves (*Clin Chim Acta* 2008;389:31). We implemented these methods using Matlab (Natick, MA). Data were extracted from multi center (n=2,767) and multi system (n=613, n=100) studies of glucose meter system (GMS) performance. We use an error tolerance limit (ETL, 5mg/dL) for the LS MAD curve, 95% non-parametric confidence intervals, “breakout points”, “knockout intervals”, and Class I and II discrepant values. Here, we also introduce a maximum error tolerance limit (METL) to the maximum error envelope and apply it to the LS MaxAD curve.

Results: The GMS from the multi center study performed satisfactorily up to the breakout point at 179 mg/dL by the LS MAD and the ETL conditions. Analysis from the modified Bland-Atlman plot illustrated a mostly negative locally-smoothed mean bias (LS MB). From the multi system study, LS MAD curves showed that GMS 1a and 2a performed best around the tight glycemic control (TGC) interval than in the higher measurement range. The LS MAD curve for GMS 5 remained below the ETL throughout the measurement range, except for two outliers present at the reference values of 358 and 406 mg/dL. For all systems, we propose an ETL_{MAX} of 20 mg/dL above which due consideration must be given to assigning knock-out ranges.

Conclusions: The LS MAD and LS MaxAD curves with error tolerance limits when combined with the modified Bland-Atlman plot provide an integrated package for clinically relevant assessment of POC devices.

Poster 46

Point-of-care pathogen detection for critical care using highly multiplex linear-after-the-exponential polymerase chain reaction. Gentile NL¹, Czajka JW², Lewington J³, Williams GV², Reis AH⁴, Barry PA¹, Polage CR¹, Kost GJ¹. ¹University of California, Davis, Davis, CA; ²Smiths Detection Diagnostics, Edgewood, MD; ³Smiths Detection Diagnostics, Watford, UK and ⁴Brandeis University, Waltham, MA.

Objective: Our goals are to validate linear-after-the-exponential polymerase chain reaction (LATE-PCR) assays for the detection of bacteremia and to assess their feasibility for point-of-care testing. We target eight pathogens in critically ill patients to facilitate early, goal-directed therapy. The eight target organisms are *Staphylococcus aureus*, including methicillin resistant strains, coagulase negative *Staphylococcus*, *Pseudomonas aeruginosa*, *Enterococcus* species, *Acinetobacter baumannii*, *Klebsiella* species, *Enterobacter* species, and *Candida* species. The project features the Clinical Bio-SeqTM System (Smiths Detection, Edgewood, MD), a “sample in—answer out” platform that utilizes automated sample preparation cartridges. This novel asymmetric PCR technique provides significant performance benefits over real time PCR.

Methods: LATE-PCR, a novel form of asymmetric PCR, combines both exponential and asymmetric amplification, resulting in an excess of single stranded PCR product. Excess ssDNA amplicons allow probe hybridization to occur at lower temperatures without competition from complementary amplicon strands. Experiments consist of two components: a) bench (analytical) validation and b) clinical evaluation. Bench validation involves five individual experiments that determine assay limit of detection (LoD), dynamic range, analytical specificity, multiplexing capabilities, and potential white blood cell (WBC) genomic DNA interference. We use 2 mL aliquots of whole blood obtained from healthy asymptomatic volunteers. Blood is spiked with known concentrations of target pathogens. Clinical validation involves testing whole blood from 50 adult (age ≥ 18 years) critically ill patients at the University of California, Davis Medical Center. Results are compared to blood culture and when feasible, MRSA GeneXpert PCR System (Cepheid, Sunnyvale, CA) results. Arbitrated case review determines the clinical significance of LATE-PCR results versus cultures. The study is IRB approved.

Results: Experiments are in progress. We expect that LATE-PCR will compete with, or prove better than other PCR assays when detecting eight target organisms. LoD and dynamic range will be equivalent or better than the LoD and dynamic range of current PCR assays. Analytical specificity will be sufficient to prevent cross reactivity. LATE-PCR will not exhibit interference when challenged with very high or very low genomic DNA from white blood cells.

Conclusion: LATE-PCR using the Clinical Bio-SeqTM System demonstrates the potential for rapid point-of-care diagnosis of bacteremia in critically ill patients and represents a valuable nucleic acid recognition technique that serves as an adjunct to blood culture in diagnosing bloodstream infections. Fast bedside information will aid in early evidence-based treatment decisions of high value in critical care medicine.



Poster 47

Low cost molecular diagnosis of hospital acquired infection. Hicke B¹, Pasko C¹, Dunn J¹, Jaeckel H¹, La H¹, Wright D¹, West M¹, Rea L¹, Zheng X² and Jenison R¹. ¹Great Basin Scientific, Longmont, CO and ²Children's Memorial Hospital, Chicago, IL.

Appropriate early treatment of nosocomial, or hospital acquired, infections have been associated with lower treatment costs and improved patient outcomes. Staphylococcal species are the main causative pathogen of bloodstream infections including the superbug, methicillin-resistant *Staphylococcus aureus* (MRSA). For a patient suspected of having a BSI, blood is drawn and placed in a continuous-monitoring blood culture instrument. Standard microbiological methods for identifying *Staphylococcus* from a patient's blood culture require 24 to 72 hours. Molecular diagnostic approaches utilizing real-time PCR, have shortened the time to identification of MRSA and MSSA to less than 2 hours after indication of a positive blood culture. No information is provided about other Staphylococcal species, the so-called coagulase-negative Staphylococci (CoNS), because they are commensal organisms and are widely disregarded as contaminants in positive blood cultures. However, MRCoNS and CoNS spp are increasingly being recognized as true infections due to the use of indwelling catheters, which can harbor these organisms. This article describes a sample in/result out molecular diagnostic platform approach that combines the sensitivity of nucleic acid amplification with the multiplex capability of chip-based detection on a disposable card format. The approach is low cost by using temperature tolerant isothermal amplification, allowing for a low cost heater, and human eye visible signal on the chip surface, allowing for inexpensive or even no imaging equipment. The instrument utilizes simplified fluidic channel design and pressure changes to move fluid. Combined the overall cost and complexity is low. Validation of the approach will be provided with data from retrospective clinical specimens.

Poster 48

Single domain nanobody-based microarrays for detection of cancer metastasis. Malecki M¹, Malecki RP². ¹Western University, Pomona, CA and ²PBMEC, San Francisco, CA

Background: Cancer metastasis is the most advanced stage of the disease's progression; it is when cancer cells spread via blood and/or lymph from the primary tumor into the distal organs. The most common homing patterns for cancer metastasis involve: lungs, liver, brain, and bones. At the initial stages, small foci of metastatic cancer cells are beyond detection with physical examination and radiological techniques. They are also beyond the means of local therapies. Therefore, a simple clinical laboratory test detecting metastasizing cells in the blood or lymph circulation would be of great diagnostic and prognostic significance, as well as, guidance to their elimination.

Objectives: The ultimate objective of this work is to develop a genetically-engineered, single domain-antibody based microarray, which would facilitate detection of cancer cell specific biomarkers in the patient's blood or lymph.

Methods: We have developed libraries of single domain nanobodies (SDN) targeting known cancer cell biomarkers. In this project, SDNs included those targeting: epidermal growth factor receptor (EGFR, HER1), truncated epidermal growth factor receptor (EGFRvIII), epidermal growth factor receptor 2 (EGFR2, HER2), and its truncated mutant, prostate-specific membrane antigen (PSMA). We have also developed thin-film semiconductor microarrays allowing us selective deposition of the single domain nanobodies in the defined fields of these microarrays. Therefore, we created the single domain nanobody-based microarrays.

Results: Initially, we tested specificity of our microarrays by dripping suspensions of the ATCC well defined prostate and breast cancer cell lines onto the microarrays. Recently, based upon our IRB protocols, we started to collect blood samples from patients. We were using a simple microarray reader to score these samples. Cancer cells expressing specific biomarkers were captured onto the defined fields of the microarrays with utmost accuracy. As the figures show, the breast cancer cells were not anchored to PSMA fields. Vice versa, prostate cancer cells were captured onto the PSMA fields.

Discussion/Conclusions: The main advantage of the microarrays, which we designed and manufactured, relies in their ability to reveal display many biomarkers of cancer cells very quickly and accurately. This should facilitate to diagnose the cancer and to select targeted therapy (e.g., only 30% of breast cancers are Her2 positive, thus responsive to Herceptin). As such this new technology opens a new avenue for diagnosis and therapy of advanced cancer.



April 22 & 23, 2010 San José, Calif.

Poster 49

Automated label-free cell sorting using dielectrophoresis and centrifugal microfluidics.

Martinez-Duarte R, Madou MJ. University of California, Irvine, Irvine, CA.

The objective of this work is to develop an automated platform for label-free point-of-care diagnostics. Here we present the initial prototype. The use of dielectrophoresis (DEP) obviates the need for labels during bioparticle sorting. The integration of DEP with compact disk (CD)-like centrifugal microfluidics permits assay automation. Some of the most important diagnostic assays rely on cell sorting to obtain a result, for example white blood counts. Current cell sorting techniques heavily rely on the use of antibodies which prohibits them from having an impact in resource-limited settings. For example, the use of antibodies in diagnostic tests often requires refrigeration, skilled personnel and large laboratory infrastructure. Antibody-based diagnostic assays are expensive and hinder the continuous monitoring of patients, even in developed countries. Many infections and diseases such as kidney infections and ovarian cancer can be cured if diagnosed early. DEP eliminates the use of antibodies because it only uses the intrinsic dielectric properties (given by cell morphology) of targeted cells to isolate them from a background. Cell discrimination is based on the characteristic interaction that different cells have with an electric field. This electric field is created across the sample using a polarized electrode array. DEP proves successful in the selective isolation of relevant cells such as white blood, cancer and stem cells. DEP also enables the sorting of bacteria to rapidly detect the cause of an infection and represents an alternative to culture methods. However, current DEP platforms are too bulky and complex to allow their practical use in a clinical setting. The integration of DEP chips on an electrically-interfaced CD platform significantly reduces the footprint, complexity and cost of a DEP system. Here the entire diagnostic assay is contained in modular monolithic chips that can be easily interchanged as needed. This approach eliminates the use of precision pumps, syringes, external valves, tubing and other supporting fluidic elements used now in DEP settings. The resultant platform has the footprint of a traditional data CD. The use of centrifugal forces allows for programmable fluid manipulation by optimizing the dimensions and geometry of the microfluidic network and the platform spin direction, velocity and acceleration. CD platforms are successful in implementing automated sample preparation and several fluidic functions including valving, decanting and mixing have been demonstrated.

The proof-of-principle of this prototype is a cell viability assay. The test sample is introduced directly into the chip. A spin protocol then drives the sample through an electrode array which is polarized to trap viable yeast cells and repel non viable ones. Viable cells are separated from non viable ones with efficiency of 0.75 ± 0.05 . Results are obtained within 10 minutes when working with sample volumes of 50 μl .

The prototype demonstrated here has the potential to 1) eliminate the use of antibodies in cell sorting and 2) enable rapid detection of pathogens. The integration of DEP with centrifugal microfluidics derives in a stand-alone platform amenable to be used in a clinical setting.



April 22 & 23, 2010 San José, Calif.

Poster 50

A rapid test for malaria utilizing a “smart” microfluidic concentrator and immunoassay.
Golden AL¹, Lai JJ¹, Battrell CF², Hoffman AS¹, and Stayton PS¹. ¹University of Washington, Seattle, WA and ²Micronics, Inc., Redmond, WA

Point-of-care (POC) immunoassays (*i.e.*, lateral-flow tests) are rapid and cost-effective analytical tests, which can be performed outside of centralized laboratories with untrained users and minimum instrumentation. POC immunoassays exhibit significant potential for infectious disease diagnostics but have not yet been fully utilized because detecting dilute biomarkers from complex human samples remains a challenge in the field of rapid diagnostics for resource-limited settings. To address this issue, we have developed phase-transitioning, “smart”, reagents, suitable for capturing antigen, by covalently conjugate poly(N-isopropylacrylamide), pNIPAAm, to antibody using carbodiimide chemistry. Additionally, a microfluidic concentrator is developed to capture and concentrate antibody-bound antigen through aggregation of smart reagents at a porous membrane surface heated above the lower critical solution temperature. This technique is utilized for capturing and detecting malarial antigen *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) from spiked human plasma, which matches to clinically-relevant antigen concentrations. Antibody conjugates are efficiently captured at the membrane surface, even from such complex fluids as human plasma. Conjugation and purification of the IgM antibody against PfHRP2 antigen did not adversely affect binding of antigen as compared to an identical non-conjugated antibody. Using gold colloids as detection reagents, a stable, visually-detectable signal is generated at the membrane surface (Figure 1). The limit of detection for full-stack immunoassay from 50% human plasma is comparable to ELISA and is achieved in a total time of less than 10 minutes from a 50 μ l sample. Using larger sample volumes, visual detection of dilute antigen samples is enhanced and color intensity increases as the immunocomplex-bound gold colloid is accumulated at the membrane. This technology can concentrate dilute biomarkers from complex fluids to improve detection ranges and can also be applied to sample preconditioning to remove specific unwanted elements from complex mixtures without further diluting the sample.

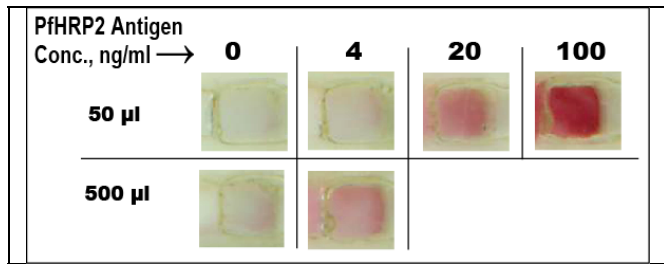


Figure 1. Smart microfluidic immunoassay with gold colloid (40 nm) detection reagent. Color intensity (signal) generated by gold colloids accumulated on membrane is dependent on PfHRP2 concentration (0-100 ng/ml) and total sample volume (50 or 500 μ l sample of 50% human plasma spiked with PfHRP2). Membrane area shown is 0.5 mm \times 0.5 mm. A typical ELISA detection limit, using identical antibodies, is approximately 4 ng/ml.

Poster 51

Quantitative analysis in a multiplex universal Real-Time TMA assay using a master calibration approach. Nelson NC, Peng H, Miick SM, Phelps SS, Chelliserrykattil J, Kaminsky MB, Darby PM, Jackson JA, Fors ZL, James AL, Turner SM, Ong E and Groskopf J. Gen-Probe Incorporated, San Diego, CA.

Recently a quantitative, multiplex Real-Time Transcription-Mediated Amplification (TMA) assay format has been described. This format utilizes universal sequence tags that are incorporated into the amplicon via a unique target capture-mediated process. After the initiation step, amplification is driven by a set of universal tag sequences. Quantitation is typically achieved using an external calibration curve generated with each run of the assay. The objective of this study was to determine if accurate and precise quantitation could be achieved using a stored master calibration curve.

In the study presented here, a version of the assay referred to as Half Universal Reverse TMA (HUR-TMA) was utilized to detect and quantitate both prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) mRNA from a clinical urine specimen in a single multiplex reaction (internal control also included). Standard quantitation was achieved using an in-run duplex calibration curve comprised of PCA3 and PSA in vitro transcripts. Using this curve, the PCA3 and PSA copy numbers for each specimen tested were determined and the PCA3 score ($[\text{PCA3}]/[\text{PSA}] \times 1000$) was calculated. Copy levels and PCA3 scores for each sample were then recalculated using a stored duplex calibration curve and a single in-run adjuster.

Quantitation of PCA3 and PSA using either the standard in-run calibration method or the stored calibrator approach using an in-run adjuster yielded essentially equivalent results, both demonstrating good accuracy (± 0.25 log copy) and precision (standard deviation ≤ 0.15 log copy) over the biologically relevant dynamic range (2.01 – 5.35 log copies/rxn PCA3 and 3.47 – 6.79 log copies/rxn PSA). Furthermore, both quantitation methods yielded essentially equivalent PCA3 scores to those generated using the PROGENSA® PCA3 Assay, a CE marked uniplex end-point TMA assay.

In conclusion, the stored calibration curve method of quantitation can be used with no loss of performance compared to the standard in-run calibration method. This yields a significant advantage in that only a single adjuster needs to be included per assay run compared with an entire set of calibrators in the standard method. This not only saves on time and reagent usage, but also allows more clinical samples to be analyzed per run. Furthermore, overall performance of the universal, multiplex Real-Time TMA assay was excellent. This assay shows great promise for use in a variety of molecular diagnostic applications.

Poster 52

An improved hydrophilic magnetic solid support for immunoassays. Finne, E.S., Manger I.F., Songe P.H., Life Technologies, Invitrogen Dynal AS, Oslo, Norway.

Magnetic beads are used as a solid-phase for presenting antibodies or antigens in the majority of automated clinical immunoassay systems, and Dynabeads[®] are well renowned in the IVD field for this purpose.

With the introduction of new generations of assays there is a need for a continuous optimization of the magnetic solid phase in order to meet the demand for increased sensitivity.

Invitrogen Dynal AS has recently developed a new version of Dynabeads[®] with a surface chemistry designed to improve the signal-to-noise ratio. The first product has been launched for use in Immunoprecipitation (IP) and this new Dynabeads platform is also available for use in development of new immunoassays. The activated beads are uncharged at neutral pH and have a high binding capacity for proteins and antibodies. After coating with ligands the beads provide low background without further need for treatment, avoiding known variations due to e.g. the quality of BSA used for blocking.

Data from the use of the beads in a 96-well plate assay using an automated plate washer with a magnetic bead separation option, will be presented.

The sensitivity and activity of immobilized antibody obtained with the new surface coating is compared with hydrophobic and carboxylic acid bead surfaces in a side-by-side study using a model Troponin I assay.

A 2-fold increase in sensitivity is obtained using the same amount antibody immobilized on the beads. The increased activity of the bound antibody is shown to correlate with an increase in the apparent affinity constant of the antibody immobilized on the new surface.

Poster 53

Point-of-care surface acoustic wave biosensors for blood donor screening: Keeping the blood supply safe and available for emergency and disaster. Sonu R¹, Larson RS², Bisoffi M², Kost GJ¹. ¹University of California, Davis – Lawrence Livermore National Laboratory Point-of-Care Technologies Center, and ²University of New Mexico School of Medicine, Albuquerque, NM.

Objective: The objective of the study “Point-of-care (POC) surface acoustic wave biosensors (SAWB) for blood donor screening: Keeping the blood supply safe and available for emergency and disaster” by Sonu R, Larson RS, Bisoffi M and Kost GJ, is to demonstrate a novel POC surface acoustic wave biosensor (SAWB) device for detecting Human Immunodeficiency Virus (HIV) -1, -2, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) for emergency and disaster. This rapid, multiplex POC pathogen detection device would provide a solution for keeping the blood supply safe and available during sustained disaster situations. During disasters, blood supply systems are not adequately structured to fulfill the demands of immediate massive transfusion.¹ Processing of blood donations and screening for transfusion-transmissible pathogens requires several days and the most common method, nucleic acid testing (NAT), requires large, expensive analyzers that require pre-processing, trained personnel, and electrical power. Therefore, the current method for blood donor screening is impractical for disaster situations. In addition, investment is needed for research and development to enable developing countries to implement innovative methodologies and appropriate technologies for blood safety.²

Methodology: The surface acoustic wave biosensors are fabricated at the Sandia National Laboratory. Specific aim 1 (University of New Mexico (UNM)): Development of an antibody-based multiplex sensing capability for HIV-1, -2, HBV and HCV in defined buffer conditions. Specific aim 2 (UNM): Demonstration of viral detection in human blood and bench validation studies. Specific aim 3 (UC Davis (UCD)): Environmental stress testing under different temperature/humidity profiles to test for device robustness. Specific aim 4 (UNM, UCD): Multi-center clinical validation study to determine the clinical specificity and sensitivity to an FDA-approved NAT method.

Preliminary Data: We have been able to demonstrate the specific and sensitive detection of HIV-1, HIV-2, HBV, and HCV with this hand held device. The results are available in less than 5 minutes after application. We have also shown a linear response ($R^2 > 0.99$) in the detection of these analytes. We have further configured the device so that multianalyte detection of this device is possible.

Conclusion. Preliminary results show that the SAWB technology is capable of viral detection and therefore, may have the potential to be used as a rapid, multiplex pathogen device specific for blood donor screening in the emergency and disaster field.

References:

¹Erickson ML, Champion MH, Klein R, Ross RL, Neal ZM, and Snyder EL. Management of blood shortages in a tertiary care academic medical center: the Yale-New Haven Hospital frozen blood reserve. *Transfusion* 2008;48:2252-2263.

²Dhingra N. World Health Organization Statement: Making Safe Blood Available in Africa. June 27, 2006. Available <http://www.who.int/bloodsafety/en/>



April 22 & 23, 2010 San José, Calif.

Poster 54

Novel time resolved fluorescence platform for near patient diagnostics. McHugh S, Hainesworth J, Burnell S, Shenhav S, Svarovsky S, Manneh V. Xen Biofluidx, Inc & Cambridge Consultants, Ltd., San Diego, CA.

A new immunoassay platform for a variety of biomarkers based on a TRF label and a low cost portable reader is reported. We have previously used this platform to demonstrate a sensitivity improvement of orders of magnitude over gold-label based assays, with good repeatability. The system has several benefits in addition to its high sensitivity – the core components are low-cost and compact, which offers the potential to place the platform at the core of highly commercially attractive low-cost rapid diagnostic systems.

The results show that the platform is capable of detecting NT-proBNP and hGCH concentrations of 0.1 pM, and under certain conditions as low as 0.01 pM. The platform can be used for monitoring heart failure and cancer biomarkers and has the potential to achieve a high sensitivity within an optimized system. The low cost of the platform and its suitability for the Point-of-Care and Rapid Diagnostic Test markets make it an attractive candidate for the near patient markets.



April 22 & 23, 2010 San José, Calif.

Poster 55

System Verification of Zplex[®], a multiplex gene expression system for translational research and clinical diagnostics. Englert DF¹, Wilson DJ¹, Dean D¹, Liederman J¹, Zafarana G², Albert M², Meng A², Bristow RG². ¹Axela, Inc., Toronto, ON and ²STTARR Innovation Centre, Departments of Radiation Oncology, University of Toronto and Radiation Medicine Program, Princess Margaret Hospital-University Health Network, Toronto, ON.

Panels of genes differentially expressed between disease states or treatment regimes are under investigation by many laboratories with the expectation that these investigations will lead to the development of clinical tests. As research progresses from discovery to clinical implementation, confirmation of an expression profile with a different technology and/or different probes for the same nominal targets is an important step in defining a test for further development and clinical validation. Validation of a test requires the processing of a large number of samples with a reproducible and reliable system in multiple clinical diagnostic laboratories where ease-of-use and robust performance are paramount. We have developed an automated multiplex gene expression system, called Zplex, intended for translational research and clinical diagnostics. We report on an example of cross platform validation of a signature under development for clinical research and on system verification studies of system performance and robustness.

1) Concordance of differential expression between Zplex and the NanoString nCounter[™] system. Probes for 135 genes were assayed on both systems. There were two probes for each target on Zplex, and one probe set for each target on the nCounter system. The data from both platforms were normalized to the geometric mean of the genes ACTB and GAPDH. There were robust signals for about 70-80% of the target genes on both systems. Neither system reported robust signals for about 3-5% of the genes, and only one system reported robust signals for 8-16% of the genes depending on the sample. The dynamic range measured from the samples in the study was similar for Zplex and Nanostring (> 3,000-fold). Differential expression was concordant between the two systems in many cases - e.g., exposure of a cell line to irradiation resulted in increased expression of between 1.5- and 6-fold for seven genes on both systems. Cases of discordance were also observed - e.g., differential expression greater than two-fold on one system but not on the other in a knockdown experiment.

2) Verification of Zplex system performance and robustness. Exogenous RNA was spiked into human total RNA and processed with standard procedures. The linear dynamic range observed from a dilution series of the spiked-in RNA was greater than 10^4 . Two-fold differences in spike amount were resolved at the extremes of the dilution series. The median coefficient of variation across three instruments and multiple array lots was 12% for the raw data and 7% when the data were normalized. Selectivity which depends on hybridization temperature was not significantly affected when the instrument was operated at extremes of ambient temperature.

Conclusion: Expression signatures can be reproduced between Zplex and the nCounter system, although cases of significant discordance were noted. Zplex meets or exceeds the minimum performance and robustness requirements for which it was designed.



Poster 56

Cold chain technologies improve diagnostic test shelf life. LaBarre, P. PATH, Seattle WA.

With the number of molecular diagnostic tests and rapid diagnostic tests expected to be prequalified for the World Health Organization (WHO) over the next ten years, developing countries that choose to introduce these new tests will face an enormous increase in cold chain storage requirements and transport volumes. Diagnostic manufacturers are beginning to understand the importance of limiting the size of new reagent packaging and presentations. However, ease of use takes precedence over small packaging as a priority product characteristic in industrialized countries. Thus, new tests that have entered global markets demand significant space in the cold chain. As a result, when countries adopt new diagnostic tests, space needed in the cold chain increases dramatically. Thus, the stakes have increased for countries to maintain reliable cold chain systems. PATH's research and development activities related to the vaccine cold chain offer some technical options for stable storage of diagnostic tests. Recent advances in eutectic materials, colorimetric time/temperature indicators, hybrid power sources and vacuum insulation are ready for more broad scale application in resource-limited settings. By appropriately matching these new cold chain developments to diagnostic transport and storage needs, the incidence of wastage and poor test performance due to out-of-thermal-specification storage can be decreased leading to decreased per-accurate-diagnosis cost and to increased accuracy.



April 22 & 23, 2010 San José, Calif.

Poster 57

A single use plasma separation device, facilitating molecular diagnostics in resource limited settings. Gruebl T¹, Heinrich M¹, Pasche JP¹, Will S¹. ¹Roche Diagnostics Ltd., Rotkreuz, Switzerland.

While Molecular Diagnostics tests are becoming standard practice in high-income countries, they are rarely utilized in developing countries because of costs and infrastructural barriers. In contrast, medical treatment has become more accessible in resource limited settings, enhancing the acceptance of pharmaceutical treatment in these populations. From this divergence, an increasing need for low cost and low complexity solutions for molecular diagnostic testing in resource limited settings has emerged. The enabling of quantitative molecular diagnostic testing in resource limited settings would not only facilitate determination of necessary treatment decisions but would also be a tool for monitoring adherence to treatment and for sentinel surveillance.

Remaining challenges to the introduction of molecular diagnostic testing are 1) remote primary sampling, 2) transportation of specimens to centralized diagnostic units and 3) the stabilization of samples under uncontrolled conditions. The remote separation of plasma without centrifugation, providing a stable sample matrix compatible with standard quantitative molecular diagnostic assays, would be a considerable advance in the availability of such testing to those in resource limited settings.

We describe the production of a prototype single use device for the separation of plasma from whole blood in resource limited settings. Such a device could be used for the field collection of plasma, where access to standard centrifugation and refrigeration protocols is limited. We have shown that such a device can effectively separate plasma from whole blood in around 4 minutes, with a minimum input volume of 200 microliters. Plasma is captured and stabilized in a way that is suitable for storage and/or subsequent shipment to a central location. There it can be further analyzed with existing molecular diagnostic testing procedures. The design of the device is based on a combination of depth filtration and size exclusion membrane technology in a convenient and low cost format.

To ensure sample to patient tracking from primary sampling through to the final results, we have designed a simple and low complexity workflow in combination with sample barcode labeling options enabling fully automated downstream diagnostics and LIMS based data management.



Poster 58

Evaluation of a multiplexed immunoassay system for robustness. Matson RS, Rampal JB.
QuantiScientifics, LLC, Irvine, CA

Multiplex immunoassays are in use for biomarker validation, clinical research and diagnostics applications. It is important to characterize such assay systems in order to understand their advantage, as well as, their limitations. Here we evaluate the A2® MicroArray System in a fully multiplexed cytokine assay. This platform utilizes a 96-well microplate (A2 plate) in which a standardized array of sequence specific “universal” capture oligonucleotides are immobilized in each well. Complementary oligonucleotides are subsequently conjugated (“oligo-linked”) to the antibodies or biomarkers of choice. The oligo-antibody conjugates are then mixed together and applied to the wells of the plate. Through the process of hybridization the antibody array is created by self-assembly. Up to 13 different conjugates can be immobilized to create a multiplexed immunoassay. To evaluate performance (robustness) for this platform we examined analytes in a serum matrix. 13 human cytokine oligo-antibody conjugates were prepared by solid-phase coupling chemistry and applied to the A2 plate. We performed 13 fluorescent-based ELISAs simultaneously in 80 wells per plate and compared results obtained across 3 plates. Inter- and Intra-imprecision was determined for each of the 13 immunoassays. A total of 30 standard dose-response curves generated for each of the analytes were analyzed. Examination of curve fits at low analyte levels revealed that a serum diluent contained traces of IL-6 but no other cytokine. Plate to plate variation based upon mean dose response remained under 20% CV. For example, IL-4 ranged 1.3% to 16.6% over 4 pg/mL – 2900 pg/mL; IL-6, 0.3% to 9.7% over 7 pg/mL – 5200 pg/mL; and IFN-g, 0.7% to 6.7% over 2.7 pg/mL – 2000 pg.



April 22 & 23, 2010 San José, Calif.

Poster 59

Label-free DNA sensing based on GaAs/AlGaAs/InGaAs modulation-doped field-effect

transistors. Klemer DP¹, Pietz BC², Viar H³, Wagner J³, Mikelson H³, Carlson K³. ¹University of Wisconsin-Milwaukee, Milwaukee, WI, ²BloodCenter of Wisconsin, Milwaukee, WI, and ³Chippewa Valley Technical College/NanoRite Nanotechnology Innovation Center, Eau Claire, WI.

Background: Solid-state microelectronic devices based on compound semiconductors such as gallium arsenide can serve as label-free nucleic acid biosensors, allowing for direct conversion of DNA hybridization events into an electronic signal and facilitating the development of low-cost *in vitro* molecular diagnostics. The ability to precisely grow compound semiconductor epitaxial wafers with GaAs/AlGaAs/InGaAs heterointerfaces allows for confinement of electron current flow very close to the material surface, within hundreds of angstroms. This permits sensing of small changes in surface electrostatic charge which occur during hybridization of target DNA with probe oligonucleotides immobilized onto the wafer surface.

Methodology: We describe the design, fabrication and testing of two high-frequency modulation-doped (AlGaAs/InGaAs) field-effect transistor (ModFET) biosensor designs. The first design is fabricated with interdigitated electrodes serving as transistor source and drain. A thin-film titanium-gold gate electrode is patterned between source and drain, with disease-specific probe oligonucleotides immobilized onto the gate metallization using gold-thiol immobilization chemistry. A second, simpler design eliminates the gate metallization by direct immobilization of thiolated probe oligonucleotides onto arsenic atoms exposed by a suitable chemical treatment of the GaAs wafer surface.

Results: To demonstrate proof-of-concept, ModFET transistors were fabricated onto gallium arsenide material incorporating an AlGaAs/InGaAs heterointerface 550 angstroms below the wafer surface. Oligonucleotide primers specific for human platelet antigen genotypes HPA-1a and HPA-1b were immobilized onto the gate electrode, and transistor impedance was measured with an Agilent E5071B microwave impedance analyzer. Variations in impedance over the frequency range 0.1 to 8GHz are associated with probe/target oligonucleotide binding.

Conclusions: Microwave GaAs ModFET transistors can be fabricated as sensitive detectors of oligonucleotide binding, facilitating the development of inexpensive semiconductor-based molecular diagnostics.

Acknowledgements: Support from the Lynde and Harry Bradley Foundation and the Medical College of Wisconsin Clinical and Translational Science Institute is gratefully acknowledged.



April 22 & 23, 2010 San José, Calif.

Poster 60

A rapid, multiplexed diagnostic platform for detection of antibodies, proteins and nucleic acids in low-resource settings. Cull MC, Roark CL, Cull TE, West AB. Beacon Biotechnology, LLC., Aurora, CO.

Beacon Biotechnology is developing a portable, hand-held device (BrightSPOT) that can be used in low-resource settings to provide rapid diagnosis of infectious disease, auto-immune diseases, and detection of pathogens in the environment. The BrightSPOT platform is an ultra-sensitive light detector that is a highly mobile, rapid, simple, inexpensive system capable of multiplexing up to 112 assays performed simultaneously on a single sample without the use of super-cooled cameras, lasers or optics systems required in current high-sensitivity diagnostic devices. The detection molecules are coupled to Gaussia luciferase enzyme that generates a very bright bioluminescent light. The BrightSPOT hardware consists of 112 individual, extraordinarily sensitive, light-detecting pixels that detect the bioluminescent light generated at the surface of the silicon chip. Using the BrightSPOT platform we demonstrate 1) rapid, multiplexed detection of anti-HIV antibodies, 2) detection of autoantibodies, 3) detection of nucleic acid targets (either RNA or DNA) that can identify and differentiate bacterial or viral pathogens.

Specifically, we detect antibodies against multiple HIV antigens in whole blood in a 13 minute assay that was sensitive to levels that gave a false negative by a commercially available ELISA format. Furthermore, the BrightSPOT platform was used to detect autoantibodies to the GAD65 auto-antigen, recognized as an early predictor of development of Type I diabetes. In these studies, GAD65 autoantibody was detected in patient serum diluted 1×10^6 fold. When this same serum was assayed by the Barbara Davis Center in their traditional fluid phase radioactive test (2 days), detectable levels of anti-GAD antibody are measurable only to a 1:62,500 final dilution, making the BrightSPOT platform 16 times more sensitive. Finally, anti-GAD 65 antibodies are detectable in saliva samples, allowing for non-invasive sample collection when using this platform. We also demonstrate highly sensitive detection of nucleic acid targets using our proximity-based NAPA technology. Our data shows that the platform can detect less than 0.4 attomoles of duplexed DNA. Thus, the extremely sensitive BrightSPOT platform works in multiple diagnostic assay configurations for the rapid detection of disease in point-of-contact and low-resource settings.



Poster 61

An automated low cost instrument for simultaneous multi-sample tissue homogenization.

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Tissue homogenization is a common sample preparation procedure in the analysis of clinical samples, but to date there has been no good way to incorporate it into high throughput assays. The classic mortar and pestle is still in common use in laboratories for homogenizing small samples in low quantities. Blenders and high pressure valve processors are used when large quantities of tissue must be homogenized. There are two technologies on the market that process multiple samples simultaneously: bead beating and sonication. Both have been implemented on a standard microplate form factor, but have their drawbacks. Bead beating requires the careful addition and subsequent removal and sanitizing of the beads, which can be a costly and time-intensive process. Sonication works well only with very small samples and does not always give consistent results. We describe the development of a new instrument that is capable of quickly homogenizing an array of unique tissue samples directly in a microplate. The instrument requires no special training to achieve uniform, repeatable results, and is thus adaptable to semi- and fully automated equipment. Additionally, the system is easy to clean and sterilize, has adjustable speed and force to control shear and unwanted heating, and is useful for sample sizes ranging the entire breadth needed for clinical samples (microliters to milliliters).



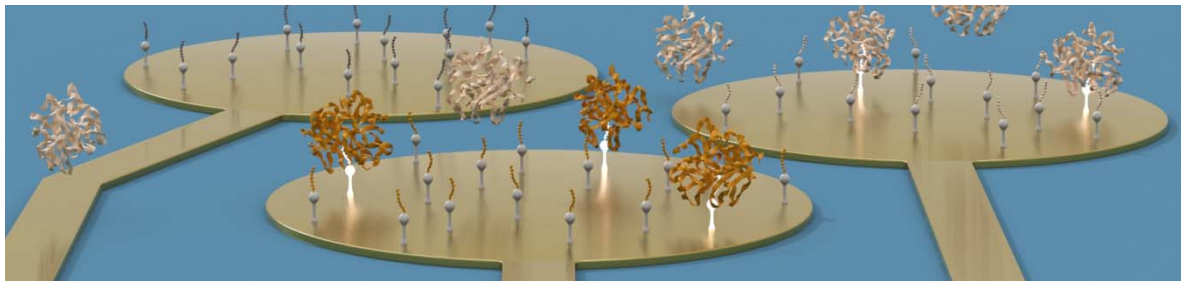
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Poster 62

Patient monitoring at home: an approach to chronic disease management and risk profiling. Georganopoulou D. Ohmx Corporation, Evanston Ill.

Ohmx is redefining e-Health by developing a home monitoring device that can quantify biomarkers, blood gases, metabolites, electrolytes, and other important disease indicators from the comfort of a patient's home. With the exception of diabetes management, there are no available devices that can monitor chronic diseases or diagnose/ risk stratify patients remotely. Ohmx Corporation proposes to fill this void by offering a comprehensive menu of critical tests that are easy to use, fast and require only a few drops of blood. By moving these routine but essential tests from the hospital to the home, we aim to improve the patients' quality of life and drastically reduce healthcare costs.

Ohmx will present data for current feasibility assays that include oncology biomarker Prostate Specific Antigen (PSA), cardiovascular biomarker sP-Selectin, sepsis biomarker Staphylococcus enterotoxin B (SEB) and diabetes biomarkers: glucose, hemoglobin A1C and fructosamine. Advantages of the Ohmx analyzer will be presented that include: 1. Bioelectronic detection 2. Handheld electronic instrument 3. Low sample volume obtained by a finger prick 4. Reagentless assay 5. Disposable biochip with multiple tests available within the platform 6. Simple to operate and availability as a consumer diagnostic 7. Capability to interface as a front-end diagnostic of e-Health.



Poster 63

Isolation of omnipotent, non-embryonic stem cells with superparamagnetic, stage-specific, genetically engineering single chain variable fragments (scFv). Malecki M, Malecki B. Western University, Pomona, CA and Phoenix Biomolecular, Tempe, AZ.

One of the essential problems for streamlining omnipotent stem cells (OSC) based therapies into regenerative medicine is immunological response of the patients. Autologous transplants would clearly resolve this problem. In particular, that recent discoveries have validated omnipotency adult stem cells. However, this would require effective isolation and propagation of OSC. Small populations of SC residing in bone marrow (BMSC), blood, skin, and other tissues have been found to express certain markers of omnipotency such as SSEAs, Oct4 and Nanog. In the past, we genetically engineered single chain variable fragments (scFv) against these OSC markers. Using them, we were able to identify OSC *in vivo*, *in situ*, in bone marrow and other tissues. Using these constructs for scFv targeting SSEAs as the start-ups, we genetically engineered the constructs for their fusions with the domains chelating superparamagnetic ions. They were linked via proteolysis sensitive linkers. After propagation, we expressed them in human myelomas, affinity purified, and chelated. In the immunoblots from OSC native lysates and in the high resolution EDX, these superparamagnetic scFv demonstrated high specificity and affinity towards SSEAs. In the mixed cultures and bone marrow aspirates, these scFv efficiently labeled OSC and allowed us to pull them out on the magnets. These new scFv offer a new route for a fast isolation and evaluation of OSC for regenerative medicine trials.



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Poster 64

Detection of aggregated proteins using synthetic polymeric ligands. Stanley CJ, Lane A, Wilson SM. Microsens Biotechnologies, London, UK.

Proteins can change structure, mis-fold, oligomerise and eventually aggregate. Rogue, misfolded proteins can be infectious (prion diseases), neurotoxic (leading to neurodegenerative disease) or have negative economic consequences (in biopharmaceutical manufacturing). Detection of a protein aggregation process at an early stage has proved to be a significant technical challenge. The first steps of oligomerisation are poorly characterized and the early stages of the misfolding process itself are not well understood. A particularly difficult analytical problem is the detection of rare abnormal protein structures against a background of the predominant normal globular state. For prion proteins this has been achieved by exploiting the different protease sensitivities of the two forms. The normal form of the protein is removed by digestion before carrying out an immunoassay to quantify the abnormal form that remains. This property of differential resistance to protease is unique to the prion protein however and other proteins, such as beta amyloid, alpha synuclein and huntingtin (all implicated in Alzheimer's Disease, Parkinson's Disease and Huntington's Disease respectively) do not exhibit rogue form protease resistance. Protein aggregation phenomena are not just restricted to the human and animal health fields. Aggregation of a recombinant therapeutic protein or monoclonal antibody during downstream processing is well known and aggregates of these proteins can develop later in the finished product; sometimes weeks or months after the final stages of production. This can lead to loss of product or even total batch failure.

An alternative approach to detection of protein aggregation has been to use synthetic polymeric ligands (the 'Seprion' polymers) that can, under appropriate conditions, be selective in binding to the abnormal forms (1). Such a system does not exhibit specificity for any particular aggregated protein; it is instead a general approach to binding this class of target. To make the assay specific for each particular protein an appropriate monoclonal antibody is used to probe the various aggregated species bound to the polymeric ligand. The Seprion technology has been applied to the detection of the prion diseases CWD, BSE, scrapie and CJD in a 96 well ELISA-type format and it is now widely used for post mortem tissue analysis. Recently the Seprion ligands have been used to detect a) the abnormal form of the huntingtin protein in brain tissue from a mouse disease model (2), b) the aggregated beta amyloid protein in both brain tissue from a mouse disease model and Alzheimer's Disease patients and c) the early stages of aggregation of a model protein, beta lactoglobulin.

1. Lane *et al.* 2003. *Clinical Chemistry* 49:1774-1775
2. Sathasivam *et al.* 2010. *Human Molecular Genetics* 19: 65-78



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Poster 65

Rapid, sensitive colorimetric assay for routine analysis of multiplex assays in 96-well array plates using the CLAIR™ system. Hattara L¹, Mueller HW², Saviranta P¹. ¹VTT Medical Biotechnology, Turku, Finland, ²Sensovation Corporation, Stockach, Germany

We evaluated the CLAIR colorimetric array imaging reader for routine analysis of multiplex assays in 96-well plates using VTT's Arrays-in-Wells plates. For the evaluation, we used a previously developed multiplexed sandwich assay detecting seven common respiratory viruses: Adenovirus, Respiratory Syncytial Virus (RSV), Influenza A (InfA), Influenza B (InfB), Parainfluenza 1 (PIV1), Parainfluenza 2 (PIV2), and Parainfluenza 3 (PIV3).

In initial proof-of-concept tests, we assayed 46 control samples containing varying dilutions of the virus-infected cell lysates. The array at the bottom of each well consisted of seven virus-specific capture antibodies in triplicate, while the assay buffer contained a mixture of seven HRP-conjugated antibodies against that set of viral antigens. The presence of viral antigen in the sample led to antibody sandwich formation at the corresponding spots which was detected by HRP-catalyzed blue color formation at the spot. Array imaging and spot signal quantitation were automatically performed with the CLAIR™ system. The multiplex virus assay successfully detected and correctly classified all 46 control samples. The readout with CLAIR was rapid and easy.

We next tested CLAIR in the same multiplexed viral antigen assay using clinical patient samples. An 8 x 8 spot array format was used. Each capture antibody was present in six spots in the array: three replicate spots at high surface density and three spots at low surface density to increase the dynamic range of the assay. In addition, the array contained positive control spots (anti-mouse IgG) which also served as reference spots for grid alignment, and negative control spots (unspecific mouse IgG) to detect non-specific background binding.

The clinical samples were obtained from the Department of Virology, University of Turku. We selected 88 samples with previously determined viral antigen status- half were antigen negative, the other half antigen positive. The samples were analyzed with the colorimetric assay using CLAIR as the reader. For comparison, the whole set was analyzed in parallel with an amplified fluorescence assay on the TECAN LS400 laser scanner.

The results obtained with the colorimetric reader (CLAIR) compared very well to amplified fluorescence measured (Tecan LS400). Fluorescence/Tecan characterized 82 of 88 correctly (compared to TR-FIA reference method). CLAIR/colorimetry characterized 77 of 88 correctly (compared to TR-FIA reference method). Mischaracterized samples were PIV 3 samples which had degraded over time (TR-FIA measurements were performed several weeks prior). The LS400 detected them just above cut-off.

We showed that colorimetric detection is comparable to fluorescence-based detection in microarrays, in terms of sensitivity and linear range. Amplified fluorescence (tyramide signal amplification) showed slightly better sensitivity than colorimetry.

The advantage of a colorimetric multiplexed assay is the less complicated and costly equipment than that used for fluorescent detection. Therefore, the colorimetric microarray is ideal for routine testing. It proved to be easy to convert existing fluorescent assays to the colorimetric format.

In conclusion, the results of the clinical sample panel show that colorimetric microarray-based multiplex assays have great potential to replace traditional single-analyte immunoassays in situations where it is desirable to measure several analytes from one sample.

