Wednesday, July 30, 2014

Poster Session: 9:30 AM - 5:00 PM

Technology/Design Development

B-433

Determination of Aminothiol Adsorption Properties of Nano-Sized Titanium(IV) Oxide by HPLC-FLD

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Metal oxide nanoparticles are known for their optical, electrochemical, electrical, gravimetric, acoustic, and magnetic properties which make them appropriate for laboratory diagnostics. Among these, titanium(IV) oxide (a.k.a. titanium dioxide, TiO_2) nanoparticles display superior photocatalytic properties. In the present study, the adsorptive properties of eight TiO_2 polymorphs were evaluated when exposed to the toxic thiol-containing amino acid homocysteine. Homocysteine is an amino acid known to cause cardiovascular toxicity and neurodegenerative disorders and can be adsorbed to TiO_2 polymorphs to different degrees depending on the physicochemical characteristics of the polymorph. This adsorption quality may provide an alternative/point of care biosensing approach to homocysteine measurement.

A homocysteine standard solution was combined with dispersions of each TiO_2 polymorph under physiological conditions. After exposure, an HPLC fluorescence detection (HPLC-FLD) method optimized for quantification of total, unbound homocysteine was utilized and showed a range of results for the polymorphs studied. An initial centrifugation (1,280 RCF) step, followed by two microcentrifugation (11,152 RCF) steps were performed to ensure nanoparticle removal. The supernatants produced were used for homocysteine analysis. A thiol-specific derivatization agent, 7-fluorobenzofurazan-4-sulfonic acid (SBD-F) was employed to derivatize non-adsorbed homocysteine. The excitation wavelength was set to 385 nm and the emission wavelength was set to 515 nm. The peaks produced by the fluorescence detector were non-adsorbed homocysteine in the reduced form. It is possible that the homocysteine may have become oxidized by the nano-sized polymorphs upon contact, however tris-(2-carboxyethyl)phosphine (TCEP) was used to convert all homocysteine back to the reduced form for derivatization.

The nano-sized anatase polymorph adsorbed 2.92 µg of homocysteine per mg of polymorph, whereas amorphous TiO_2 only adsorbed 3.65 $x10^{-3}$ µg of homocysteine per mg of polymorph; both values were corrected for primary particle size. Results were determined by peak area comparison to the 5 µmol blank standard. Other polymorphs produced values between the anatase and amorphous polymorphs. Surface chemistries are distinguishing characteristics of these polymorphs and are responsible for their individual physicochemical properties. Variations in the observations were attributed to the unique combinations of size, surface area and polarity of each polymorph. The aminothiol adsorptive property of titanium dioxide polymorphs has potential applications in nanomedicine, biosensing, diagnostics, and amino acid tagging. This HPLC-FLD evaluation of homocysteine adsorption effectively determined which polymorphs may be best suited for applications in the clinical laboratory for diagnostic purposes.

B-434

Versatile Electrical Platform for Accelerated Development and Commercialization of In Vitro Diagnostic Assays

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Background: Diagnostic techniques have become critical to modern health care not only for patient diagnosis and optimization of treatment, but also for providing vital information regarding pathways for complex diseases. Ideal biosensors provide the maximum desirable data with the least amount of complexity. However, to date, most biosensors either yield very specialized data or are very complex and expensive. Researchers have attempted to address this need with the development of point-of-care sensors that can provide accurate data without the need for expensive equipment. Semiconductor manufacturing techniques offer a particularly attractive opportunity to design a biosensor that is inexpensive, scalable, easy to integrate with portable electronics, and highly sensitive to target analytes due to device size. Such fabrication techniques have been honed to perfection with optimization and standardization. Prototyping of new device designs in a semiconductor manufacturing manner can critically enable rapid development and commercialization of new in vitro diagnostic assays.

Methods: We present here a versatile electrical biosensor platform consisting of tens of thousands of devices fabricated with a 0.18 µm silicon-on-insulator technology. The platform consists of a unit cell transistor integrated seamlessly with control and read-out circuitry that is amenable for immediate commercialization. Each cell consists of a sub-micron FET Sensor with a near-Nernst pH sensitivity of around 56-59 mV/pH and a resolution of <0.01 pH. The gate oxide is directly exposed to the target analytes, instead of to the commonly employed floating gate architecture. This enables many critical advantages, including increased sensitivity due to the elimination of parasitic coupling capacitances and reduced vulnerability to crippling factors such as electrostatic discharge. The unit cell can be coupled to a variety of different surface chemistries for different target analytes and applications.

Results: We verified the device performance and potential applications by detection of urea level via an enzyme (urease)-catalyzed reaction. A high level of urea in blood plasma can indicate both potential kidney failure and the onset of kidney diseases. Compared to reported literature (Lai et al, 2001) and commercially available kits (SIGMA ALDRICH®), our devices exhibited a linear detection response in the hundreds of picomoles to nanomoles range with a sample volume of 0.1 µl, an improvement of one order in detection limit and three orders in required sample volume. In addition, we verified the device performance by the detection of DNA hybridization. As low as picomoles of DNA molecules were easily detectable, a limit that could be pushed down to femtomoles with integrated polymer microfluidics.

Conclusion: In summary, this work introduces a fully electronic biosensor platform produced using a semiconductor manufacturing foundry. As proofs of concept of the functionality of the unit cell, we demonstrated the detection of enzymatic reactions and DNA hybridization. Foundry fabricated FET sensors with integrated polymer microfluidics have the potential to enable highly cost effective, mass fabricated POC devices with better sensitivity and resolution than currently commercially available solutions. The finely tuned and high throughput nature of a semiconductor foundry can translate to immediate commercialization of electronic biomedical assays.

B-435

An easy, portable and rapid way for performing PCR reactions

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Background. Limits to the wide diffusion of molecular diagnostics are the elevated cost of thermalcyclers and the need of trained technicians that perform PCR reaction and analysis. Another limit is that PCR mixes, enzymes and components are typically stored at -20° or +2-8°C. Repeated freeze-thaw cycles could negatively impact on the assay's performance. We developed innovative technology to produce a freeze-dried mix for the amplification of nucleic acids, which is ready-to-use, pre-dispensed, flexible and room-temperature storable. This technology is successfully applicable to PCR or RT-PCR, End-Point PCR or Real-Time PCR, in singleplex or multiplex reactions. **Objective.** The aim of the present work was to couple two technologies, real-time chip technology and a technology that stabilize PCR reagents at room-temperature, in order to obtain a rapid, user-friendly and easy-to-store PCR system. **Methods.** A PCR mix was prepared as follows: reaction buffer, dNTPs, $MgCl_2$, DNA polymerase, primers, probe, preservatives and stabilizers. Around 5 µL of this mix was spotted in each chamber of a chip and freeze-dried using an Epsilon 2-12D freeze-dryer (Martin Christ). Performances were evaluated on a portable PCR system (STMicroelectronics). **Results and conclusions.** Real-time PCR performances evaluated on chip with the portable PCR system matched those obtained with a classical 7500 Real-time PCR System (Applied Biosystems). This suggests that it is possible to perform real-time PCR on a small, portable instrument with reduced test turnaround time, low-volume needs and with a user-friendly software; with freeze-dried reagents that are easy-to-store. This PCR system can be used at the point of impact in point-of-care molecular diagnostics.

B-437

Antioxidant profiles of human serum and biological fluids

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Background: Free radicals are strongly associated with numerous human diseases such as cancer, cardiovascular and neurodegenerative disorders.

Objective: The objective of the present study was to develop a test to assess the antioxidant profile of human serum and other biological fluids.

Methods: Human serum samples were subjected to serum protein electrophoresis in agarose gels using conditions similar for separation of lipoproteins or proteins (migration towards anode). The gels were then stained with an activity stain based on the ability of antioxidants in serum to reduce ferricyanide to ferrocyanide. In the presence of ferric ions, strong antioxidant components in serum yielded a dark blue band.

Results: Evaluation of the activity staining methodology gave positive bands only with strong antioxidants such as vitamin C, quercetin and Trolox (a water-soluble derivative of vitamin E). Uric acid and certain amino acids gave very weak bands. Twenty random serum samples were evaluated for their antioxidant profiles after serum electrophoresis. Different profiles were observed for various samples suggesting that this test may be potentially used for diagnosis and management of human diseases that are strongly associated with free radicals. Some of the antioxidant bands corresponded to LDL, VLDL and HDL. However, there was no direct correlation between the intensity of the antioxidant and lipoprotein bands. For example, some samples showed weak lipoprotein bands but strong antioxidant activities. All samples also showed a strong reducing activity at the point of origin (gamma globulin region).

Conclusion: This is the first report of visualizing antioxidant activities of serum components. Profiles of other human biological fluids will also be presented. Studies are currently underway to assess the serum antioxidant profiles of patients with various diseases compared to a healthy population.

B-438

Bioenergetic health index: a predictive biomarker of human health that combines the effects of systemic stress and disease susceptibility

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Background: The increasing incidence of bioenergetics-related diseases and the personalized nature of these disease progression is one of the major health concerns worldwide. Emerging literature suggest the existence of substantial diversity in individual susceptibility to diseases associated with energetic dysfunction such as diabetes, alcoholoc liver disease and cancer. This opens up a valuable avenue to develop a personalized predicitive biomarker in the diagnosis and management of diseases involving bioenergetic alterations. However, no clinical test capable of determining "bioenergetic health" exists to stratify these patients. Several studies studies have demonstrated the influence of genetic, environmental and lifestyle factors and age in disease susceptibility and progression and also in the individual bioenergetic function. Our recent findings support an emerging concept that circulating leukocytes and platelets can act as sensors or biomarkers of bioenergetic dysfunction that occurs in chronic diseases. It is proposed that chronic disease-induced systemic stress (inflammation, oxidative stress etc) will cause alterations in leukocyte bioenergetics, which is the resultant of the intensity of stress and individual health. This suggests that oxidative stress induced typical changes in leukocyte mitochondrial function can be used as an indicator of bioenergetic health of individuals. Hence it is hypothesized that systemic stress alters the bioenergetic capability in leukocytes and platelets and these alterations can be used to assess individual health and disease susceptibility.

Methods: In this study, by inducing oxidative stress, the impact of stress on bioenergetic capability of human leukocytes and platelets isolated from different individuals is demonstrated. The bioenergetic capability of cells is determined using the extracellular flux analyzer (Seahorse Biosciences). Oxidative stress was induced in isolated leukocytes (monocytes, lymphocytes and neutrophils) and platelets using well characterized oxidants, such as dimethylnaphthoquinone and lipid peroxidation product 4-hydroxynonenal. Using the individual parameters of bioenergetic assay, 'the bioenergetic index(BHI)' is calculated for each cell type.

Results: We demonstrate that oxidative stress induces different degrees of mitochondrial dysfunction in different individuals. Our results also show that these oxidant-induced changes in the profiles of bioenergetic parameters is characterized by increase in basal respiration, proton leak and non-mitochondrial respiration and a decrease in ATP-linked and maximal respiration in freshly isolated monocytes, lymphocytes, neutrophils and platelets. These alterations also show typical profiles for different individuals. In addition, different degrees of oxidative stress is required for bioenergetic dysfunction in different individuals suggesting the personalized nature of this parameter. Using the bioenergetic parameters of oxidant-treated leukocytes and platelets we also demonstrate the development of the bioenergetic health index, a functional measure that can define the health of individuals.

Conclusion: These novel findings suggest that peripheral blood leukocytes from different individuals significantly differ in their response to oxidative stress which is reflected in the bioenergetic health index in monocytes, lymphocytes, neutrophils and platelets. Taken together, bioenergetic health index is a novel parameter that is derived from the mitochondrial function of cells and demonstrates the interplay between innate health and acquired risk factors. It is also suggested that the 'bioenergetic health index' has the potential to be used as a clinical test in developing personalized management/therapeutic strategies in patients.

B-439

Development of an HCV Ag-Ab COMB ELISA Kit and Evaluation in Taiwan Population

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Background: Worldwide infection statistics showed 184 million people with antibodies to Hepatitis C (HCV). HCV infection is important risk factor of hepatocellular carcinoma, which was ranked in the second place among worldwide cancer incidences. There is still no vaccine or immune globulin products specific to HCV infection, so the prevention is relied on screening. To provide a screening method that reduces window period and testing cost, we developed an enzyme-linked immunosorbent assay (ELISA) kit that can detect HCV antigen and antibody simultaneously.

Methods and results: We used 218 positive and 204 negative serosamples of Taiwanese population to compare the sensitivity and specificity of our HCV Ag-Ab COMB kit and Monolisa HCV Ag/Ab kit. The result shows as table; the sensitivity and specificity of our kit were 100% and 100%, which were better than Monolisa kit. We also used seroconvertion panels and British Working Standard to evaluate and compare the performance of our kit and Monolisa kit. The test HCV seroconversion panels included BBI: PHV901, PHV906, PHV914, PHV917, PHV919, PHV920 and BCP: 6214, 6215, 6227, total 9 panels. When our kit was compared to the Monolisa kit, 6 of the 9 seroconversion panels tested gave the same results on both kits. For 2 of the 9 panels tested, our kit detected HCV infection earlier than Monolisa kit. The analytical sensitivity of our kit (1:32 dilution) was higher than Monolisa kit (1:2 dilution) by using serial dilutions of the NIBSC British Working Standard for Anti-HCV.

Conclusion: Although previous data shown the antigen detection limit of our kit was still higher than compared one, our kit shown a lower antibody detection limit, a better clinical sensitivity and a better specificity. Besides, the total reaction time only takes 105 minutes. We therefore provide a useful HCV infection screening choice.

		Our HCV Ag-Ab COMB kit		
		HCV+	HCV-	Total
Monolisa HCV Ag/Ab kit	HCV+	215	0	215
	HCV-	3	204	207
	Total	218	204	422

B-441

Integration of Microarray and qPCR system for quantitative and multi-qualitative analysis of MTB, NTM and MDR

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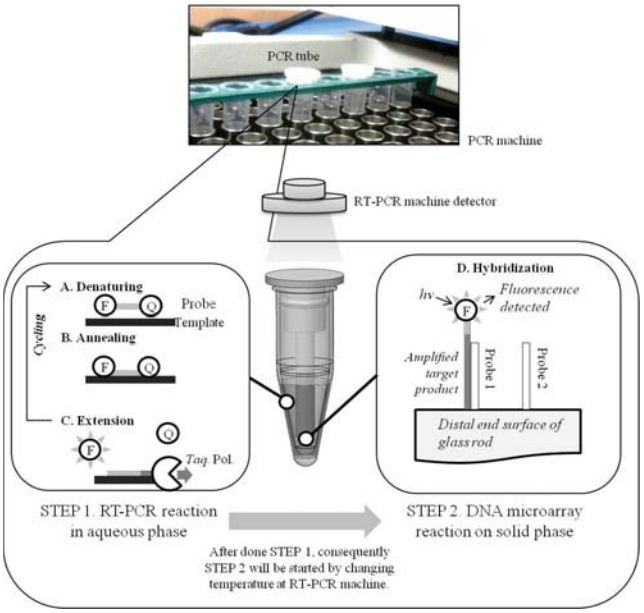
Background: qPCR and DNA Chip analysis have been widely used for molecular diagnosis. We developed a novel platform for molecular diagnosis, called Ampli & Array system, which integrates the qPCR and hybridization in a DNA microarray. In this system, entire reaction of qPCR and microarray are carried out in same container. So, quantitative and multi-qualitative analysis can be achieved at the same time. In this study, the Ampli & Array system was applied to the detection of *Mycobacterium tuberculosis* (MTB) to verify the usefulness in diagnosis.

Methods: Probes identifying mutation genes and genotyping of MTB/NTM are immobilized on the surface of Ampli & Array platform. We can recognize clinically important target genes, rpoB, katG and inhA that are related with rifampin and

isoniazid by using these probes. Amplification of the target DNA was carried out in a normal qPCR machine. After qPCR step, hybridization was performed sequentially by simply changing temperature at the qPCR machine.

Results: We measured 10 copies of MTB in qPCR process, and detected NTM genotypes and mutation genes of rpoB, katG and inhA specifically. In hybridization, we screened 94% of NTM by detecting 5 types of NTM. And, we also classified the mutation related to MDR. In rifampin related genes, we have screened L511P, D516V, D516Y, H526Y, H526D and S531L. Also, we have detected 2 types of mutation in isoniazid related genes.

Conclusion: With the Ampli & Array system, we could quantitatively analyze various genotypes in samples of large number at once. Also, more accurate results could be provided with more economical manner. The system encouraged us to overcome the limitations of current molecular diagnosis/DNA analysis in revolutionary way. For this reason, we expect that the Ampli &Array system will replace the existing qPCR and microarray in molecular diagnosis market.



B-442

A Novel and Robust Real-time PCR System for DNA Polymorphism Analysis Directly from Crude Clinical Samples

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Background: Real-time quantitative PCR (qPCR), a powerful technology utilized in many scientific disciplines, is also used for analysis of clinical samples. However, conventional qPCR systems using Taq DNA polymerase suffer from the following problems: (1) target size limitations (<200 bp), (2) poor amplification of GC-rich targets, and (3) PCR inhibition from the biogenic substances present in clinical samples. To circumvent these problems, we developed a novel qPCR system (KOD-SYBR) that uses KOD exo(-) DNA polymerase and SYBR Green I dye. Thus far, KOD-SYBR has been used to amplify large targets (<2 kb), GC-rich targets, and targets from crude clinical samples (whole blood, tissue lysates, hair roots, Gram-negative and Gram-positive microorganisms).

Objective: To determine the effectiveness of the KOD-SYBR system for DNA polymorphism analysis of crude clinical samples.

Methods: First, we evaluated the KOD-SYBR system using amplified fragment length polymorphism (AFLP) PCR with melting curve analysis at the end point. We used three primers to generate fragments of two different sizes [wild type (WT) allele: 100 bp; Inserted allele (IN): 341 bp]. Mouse tissue lysates prepared by a rapid method were used as templates. Next, single nucleotide polymorphisms (SNPs) in the alcohol dehydrogenase gene were detected using allele-specific primers containing 3' end mismatched bases followed by melting curve analysis. Diluted whole blood and oral mucosa specimens were used. A G-specific primer-bearing tail sequence at the 5' end was used to obtain a larger fragment (57 bp) than that of the other primer set (an A-specific primer and a common primer), which had no tail sequence (45 bp). The G-specific and common primer set detects an A>G SNP that leads to a missense mutation (E487K).

Results: On melting curve analysis, the 100- and 341-bp-amplicons obtained from crude mouse tissue lysates gave distinct peaks at 81°C and 84°C, respectively. Additionally, all of the variants tested (WT/WT, WT/IN and IN/IN) were distinguishable from each other. In the SNP assay, A/A, A/G, and G/G variants were detected in the crude specimens. Peaks from 45- and 57-bp-amplicons were detected by melting curve analysis at 73°C and 80°C, respectively. However, when tested, the conventional qPCR system failed to amplify any of the target DNA molecules. We found that to obtain separation of distinct peaks on melting curve analysis required melting temperature differences of at least 3°C between the amplicons, and amplicon sizes of 45 bp to 1 kb.

Conclusions: KOD-SYBR is a robust system for polymorphism analysis of crude clinical samples. The flexibility of being able to use relatively large amplicon sizes (<2 kb) for melting curve analysis is a distinct advantage of this system. KOD-SYBR has potential to be a powerful tool for high-throughput genotyping of clinical samples.

B-444

Highly sensitive quantification of oxalate in plasma and dialysate fluid by ion chromatography.

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Background: Oxalate is an end product of glyoxalate and glycerate metabolism that is excreted in the urine. This dicarboxylic acid is a common component of up to 75% of kidney stones. In addition to kidney stones, more extreme hyperoxaluria due to either genetic (primary hyperoxaluria) or acquired/secondary causes (enteric hyperoxaluria) can cause nephrocalcinosis and/or renal failure. If patients with primary (or sometimes enteric) hyperoxaluria develop renal failure, plasma oxalate levels and removal rates in dialysate need to be monitored closely to prevent oxalosis and/or rapid loss of a transplanted kidney. Previously we have validated enzymatic assays of oxalate in plasma and dialysate. Use of ion chromatography to quantify oxalate has the potential advantages of improved precision, automation, and integration with the laboratory information system.

Methods: A Dionex ion chromatography system and IonPac column were used to measure oxalate. Dionex ion chromatography system was modified to accommodate a Boric Acid eluent. Waste dialysate and plasma samples from patients with and without hyperoxaluric diseases were obtained for clinical and analytic validation. All samples were acidified to a pH 2.5 - 3.0 within 1 hour of collection, previously shown necessary for oxalate stability. Accuracy was assessed via oxalate spike recovery and comparison to our laboratory's current enzymatic oxalate oxidase method that is based on the Trinity Biotech oxalate kit (Trinity Biotech plc, Bray, Co. Wicklow, Ireland).

Results: During initial development it was demonstrated that oxalate levels increased in plasma and dialysate samples, even after acidification. This is consistent with previous observations that oxalate levels can increase in samples exposed to basic conditions, perhaps due to conversion from ascorbic acid. Thus the Dionex ion chromatography system and IonPac column were modified to accommodate a Boric Acid eluent. Using this set-up, plasma and dialysate oxalate measurements were linear over the range of 1- 50 mcmol/L. Intrassay precision was acceptable (10%) at 1 mcmol/L, and improved to <2 % for values above 10 mcmol/L. Average recovery with serial dilutions was 102%. Oxalate was stable refrigerated or frozen (-20°C or -80°C) when plasma or dialysate was acidified (pH 2.5), but oxalate levels variably increased in samples that were stored refrigerated or frozen but unacidified. Results for 48 plasma samples across the normal and abnormal range compared well with the current assay with a mean difference of only 2% between the two methods.

Conclusion: Ion chromatography using borate buffer can be used to reproducibly quantitate oxalate in plasma and hemodialysate fluid. Use of common methods based on aqueous and carbonate eluents are not acceptable for highly sensitive quantification of oxalate. Results of a new chromatographic method compare well with a previous enzymatic oxalate oxidase method.

B-445

Evaluation of the Sebia Capillarys 2 Flex Piercing hemoglobin A_{1c} (HbA_{1c}) assay

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Background: Hemoglobin A_{1c} (HbA_{1c}) is used to monitor long-term glycemic control in patients with diabetes, guide therapy, predict the risk of microvascular complications, and diagnose diabetes. It is vital that methods to measure HbA_{1c} be accurate, precise, reliable and subject to minimal interference. Recently, Sebia (Lisse, France) developed an automated liquid-flow capillary electrophoresis method to

measure HbA_{1c} on the CAPILLARYS 2 Flex Piercing instrument. We evaluated the performance of the Capillarys for the measurement of HbA_{1c}.

Methods: Technical evaluation was performed in two clinical centers (center 1, a NGSP Secondary Reference Laboratory (SRL9) and center 2, a clinical laboratory). The instruments and reagents were provided by Sebia and used according to the manufacturer's procedures. Linearity, carryover, and interference studies were performed on fresh or fresh frozen samples collected in each center. Blood samples were shared between the centers for precision and accuracy studies. Precision evaluation was performed following CLSI EP-5 using pooled whole blood daily-use aliquots frozen at -70ºC at four HbA_{1c} levels (4.7%, 6.3%, 7.4% and 11.1%) and compared to National Academy of Clinical Biochemistry (NACB) recommendations. Accuracy was assessed using 100 single-donor patient samples with ≤ 16 samples analyzed in duplicate each day. HbA_{1c} values obtained with the Capillarys were compared to those obtained with two other analyzers used routinely at the two centers: NGSP SRL9, G8 (Tosoh Bioscience, South San Francisco, CA) in center 1 and D-10 (Bio-Rad Laboratories, Hercules, CA) in center 2. For interference studies, a difference of ±0.2% HbA_{1c} was used as an acceptable limit.

Results: The Capillarys was linear for HbA_{1c} results from 4.2 to 17.6%. There was no carryover when samples with HbA_{1c} of 4.7 and 14% were analyzed alternately. There was no interference from labile HbA_{1c} as high as 11%, carbamylated hemoglobin (blood samples incubated with 0.15 to 1 mmol/L of KCNO at 37ºC for 3 hours), or triglycerides (93 to 4666 mg/dL). No additional peaks (i.e. for labile, carbamylated, etc) were observed on the Capillarys electropherograms. Total CVs were < 2.0% in both centers. HbA_{1c} values obtained with the Capillarys and comparison methods were well correlated, with minimal bias. Linear regression analysis yield the following: y (HbA_{1c} Capillarys center 1)= 1.033 × (HbA_{1c} Tosoh G8)-0.34, r=0.997, S_{yx}=0.16; y (HbA_{1c} Capillarys center 2)= 1.084 × (HbA_{1c} Bio-Rad D10)-0.67, r=0.995, S_{yx}=0.22. Results of the Capillarys were comparable between centers with mean bias ≤ 0.1% HbA_{1c} (y (HbA_{1c} Capillarys center 1) = 0.982(HbA_{1c} Capillarys center 2) + 0.05, r=0.996, Syx=0.18). Ninety-five % (center 1) and 97 % (center 2) of single Capillarys results were within ±6% of the SRL9 mean (NGSP manufacturer certification criteria: ≥ 92.5% of results within ±6%).

Conclusion: The analytical performance of Capillarys HbA_{1c} is within NACB and NGSP recommendations, has minimal interference, and is suitable for clinical application.

B-446

Homogeneous high-sensitivity CRP assay on MagArray biosensors

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Background: MagArray platform is based on the detection of magnetic particles as labels in bioassays. In contrast to systems based on optical signals, magnetic signals are not affected by the common optical interference in complex matrices. In addition, since the biosensors are designed to detect magnetic particles only when particles are bound or captured to the sensor surface, this proximity detection mechanism allows the possibility of homogeneous immunoassays. We report here that we have developed a one-pot homogeneous assay for CRP with high sensitivity. We wish to demonstrate MagArray platform is well suited for homogeneous assays that require both simplicity and sensitivity.

Methods: Antibody pairs for CRPassay were screened and selected on MagArray platform, and the detection antibody is conjugated to magnetic particles for both capping CRP and generating signals. The assay consists of two simple steps of mixing the magnetic particles with serum sample and addition of the mixture to magnetic sensors. Signals can be read in as short as 2 min for a result, and more accurate results can be obtained after 5 min. The whole process requires no shaking and rinsing or other separation steps. Standard curves of CRP in both pure buffer and sera were established and compared.

Results: The detection sensitivity of CRP in serum on MagArray platform is less than 1 mg/L for a homogeneous assay. The CVs for lower concentrations are less than 10% and less than 5% for medium and high concentrations. No prozone effect was observed for CRP concentrations of up to 200 mg/L. Our preliminary tests showed no interference effects (<= 10%) from lipids, HAMA and Rheumatoid Factor.

Conclusion: MagArray platform provides a unique opportunity of detecting proteins in a simple and homogeneous fashion. Since this assay only involves mixing and adding reagents to the biosensors, the complexity of the assay format is greatly reduced. The detection of magnetic signals from magnetic particles in proximity is a key to applying MagArray platform for a homogeneous immunoassay.

B-447

Modifying tosyl-activated magnetic bead coating protocols to improve biomolecule binding efficiency

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Background Tosyl-activated magnetic beads are widely used as a solid phase in immunoassays and biomagnetic separations. With the right conjugation protocol any biomolecule whether it be antibody, protein, peptide or glycoprotein containing NH2- or SH- groups can be covalently coupled to the surface of tosyl-beads. Optimising the coupling protocol is key to maximizing the binding efficiency of biomolecules to beads. Using immunoaffinity capillary electrophoresis (IACE) with UV-detection, bovine β-lactoglobulin B levels bound to magnetic beads (MBs) following different antibody coupling protocols were measured. Results were further verified using a bicinchoninic acid (BCA) protein test (BCA kit, Pierce, USA) to estimate the amount of antibody bound to the MBs. A coupling protocol has been developed which shows higher binding efficiecy for the same concentration of beads when compared with a published protocol (herein referred to as Protocols 'E' and 'A' respectively). Moreover, with Protocol 'E' similar levels of antibody loading can be achieved by incubating at 37ºC for 6h rather than 12h at room temperature.

Method Protocol A: 2µl of rinsed tosyl-activated MBs were mixed with 8 µl of coating buffer (100mM sodium borate, pH9.5), 8 µl of 3M ammonium sulphate and 8 µl of antibodies (5mg/ml). The mixture was incubated for 24h at 37ºC under continuous stirring to avoid sedimentation. After incubation, beads were rinsed with 10mM PBS and stored in PBS containing 0.025% Tween-20 and 0.02% sodium azide.

Protocol E: 4µl of rinsed tosyl-activated MBs were mixed with 280µl of coating buffer, 166 µl of 3M ammonium sulphate and 54µl of antibodies (1.48mg/ml). The mixture was incubated overnight (~12h) at room temperature under continuous stirring to avoid sedimentation. Beads were subsequently incubated with a blocking buffer for 1h at room temperature under continuous stirring. Finally, beads were rinsed with washing buffer and stored in PBS containing 0.025% Tween-20 and 0.02% sodium azide.

Results Under the same conditions of IACE analysis, electropherograms showed larger peaks of antigen β-lactoglobulin B (5µg/ml) in the case of MBs coated using Protocol 'E' (area of 0.328 (±0.009) units) compared with Protocol 'A' (area of 0.155 (±0.006) units). Using the BCA test to quantify the amount of bound anti-β-lactoglobulin B antibody, it was demonstrated that 42µg of antibody was bound per 1mg of MBs using Protocol 'E' compared with 24µg for Protocol 'A'. A further enhancement of Protocol 'E' was made to decrease the antibody incubation time with the MBs. Comparative peaks were observed from the IACE electropherograms for β-lactoglobulin B levels (50µg/ml) following 12h incubation at room temperature and 6h incubation at 37ºC (areas of 2.28 (±0.06) units and 2.14 (±0.05) units respectively).

Conclusion From the IACE detection of β-lactoglobulin B levels and BCA analysis of bound anti-β-lactoglobulin B antibodies, it can be concluded that Protocol 'E' provides a higher antibody coating efficiency of MBs than Protocol 'A'. Moreover, the coating efficiency is retained at 37ºC allowing for an overall shortening of experimental time.

B-448

Performance Evaluation of a Protein-Free Reagent to Design Out Common Interferences in Diagnostic Assays

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Background: Protein stabilizers, especially those containing bovine serum albumin (BSA), can lead to increased non-specific binding due to protein interference. Additional BSA-related issues include: anti-BSA antibodies, heterophilic false positives, cross reactivity, reliable sourcing, lot-to-lot variability. The ability to stabilize proteins in-solution, without the use of BSA or alternative proteins, would be an invaluable way to eliminate these protein-related interferences and issues. **Objectives**: To demonstrate the ability to stabilize proteins in solution using a synthetic protein stabilizing technology versus commercially available protein-containing stabilizers.

Methods: We tested 4 stabilizers: A. commercial BSA stabilizer, B. protein-free stabilizer, C. commercial alternative protein stabilizer (non-BSA), D. 1%BSA in PBS (negative control). All stabilizers were evaluated in terms of non-specific binding and retained activity of the target antibody. Data are derived from stability studies utilizing anti-rabbit antibodies from different host species. Anti-rabbit IgG-HRP conjugated antibodies (hosts of origin including: guinea pig, sheep, chicken and mouse) were diluted into the different stabilizing buffers (22 - 120 ng/mL) and equally divided for storage at 4°C and 37°C. The test solutions of each stabilizer were evaluated in an ELISA. At each time point a percent retained activity was determined by comparing the activity of the aged conjugate (37°C) to that of the control conjugate (4°C). Results: After 127 days at 37°C the A. commercial BSA stabilizer, B. protein-free stabilizer, and C. commercial alternative protein stabilizer (non-BSA) demonstrated averaged retained activities of 79, 77 and 69% respectively across all species. Based on Arrhenius projections, the stability of the conjugated antibody is equivalent to 3.4 years at 4°C. **Conclusion:**These data represent an effective protein-free approach to reduce non-specific binding and still perform equal to or better than protein-containing stabilizing reagents. When protein-related interferences arise, the protein-free stabilizer provides a valuable alternative for assay developers.

% Retained Activity after 127 days					
Stabilizer	Guinea Pig	Sheep	Chicken	Mouse	Mean
A	74.9%	77.4%	72.9%	90.9%	79.0%
B	68.7%	75.9%	73.8%	89.5%	77.0%
C	60.1%	66.3%	66.1%	85.4%	69.5%
D	0	0	0	0	0
% Retained Activity = (Abs OD of 37C sample / Abs OD of 4C Sample) x 100					
* Data points were averaged from 4 ELISA wells (n = 4)					
** %CV's for each data point were less than 10%					

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A Novel Method for the Measurement of Serum Viscosity: Evaluation of the Rheosense microVISC™ Viscometer

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Background: Measurement of serum viscosity is used to evaluate Hyperviscosity Syndrome (HVS), which is associated with plasma cell dyscrasias, myeloma, connective tissue diseases, and other inflammatory conditions. Rapid treatment of HVS is critical to ensure effective reduction of risk from serious complications. Increased viscosity, caused by the excessive intravascular paraproteins, leads to impaired transit of blood through the microcirculatory system. The vascular stasis and resultant hypoperfusion can cause severe complications which include cardiopulmonary symptoms such as shortness of breath, hypoxemia, acute respiratory failure, and hypotension; neurological effects such as confusion / mental status changes; ocular damage including dilation of the retinal veins and retinal hemorrhages; bleeding from the mucous membranes; and renal failure. Measurement of serum viscosity is essential for an accurate diagnosis, but traditional methods are labor intensive and not amenable to STAT analysis. We have evaluated a new instrument, the Rheosense microVISC™, for the rapid analysis of serum viscosity.

Methods: The microVISC™ (Rheosense, Inc.) is a small portable instrument which uses VROC® (Viscometer/Rheometer-on-a-Chip) technology. The VROC® sensor obtains a viscosity reading by measuring the pressure drop as a sample flows through a flow channel. Pressure is measured at positions of increasing distance from the inlet. The slope of the straight line in the plot of the pressure vs. sensor position is proportional to the viscosity. We evaluated this new instrument for use in the clinical laboratory. The performance evaluation in this study included within-in run and between-run precision and linearity, using standards purchased from Rheosense, Inc. Accuracy was determined by correlation of the microVISC™ (Rheosense, Inc.) to a cone and plate viscometer using patient samples. We used serum samples from patients with a normal comprehensive metabolic panel for verifying the reference range. Statistical analyses were performed by EP Evaluator®

Results: The within-run precision for normal and abnormal controls was < 1%. Mean/ standard deviation/ % CV for between-run precision was 1.54 cP/0.05 cP/3.25% and 4.12 cP/0.10 cP/2.43% for normal and abnormal controls respectively. The linear range was verified for 0-6.38 cp. The microVISC correlated well with the cone and plate method: y= 0.836x + 0.064 (r= 0.979). The small negative bias was reflected in a slightly lower reference range of 1.10-1.60 cP for serum samples.

Conclusion: We validated the microVISC instrument for the rapid, accurate, and reproducible measurement of serum viscosity in a clinical laboratory setting. The instrument is small, portable, and easy to use and maintain.

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Direct Single Nucleotide Polymorphism Genotyping from Blood, Plasma or Serum

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Background and objectives: Real-time PCR is a powerful, sensitive and favorable method for large-scale single nucleotide polymorphism (SNP) genotyping. However, prior purification of genomic DNA from blood is necessary since PCR inhibitors and quenching of fluorophores from blood prevent efficient amplification and subsequent detection of PCR products. We have developed a simplified "direct-on-sample" SNP genotyping without prior DNA enrichment/isolation steps to further throughput and reduce time, cost and labor. This methodology can be applied in pharmacogenomic analysis on various platforms e.g., fluorescent-, florescence resonance energy transfer-, or electrochemical voltammetry-based detection.

Methods: Diluents and procedures designed to specifically overcome PCR inhibition and quenching of fluorescence were evaluated by genotyping on 4 SNPs from Factor II, Factor V, MTHFR genes, 3 CYP2C9, VKORC1 gene polymorphisms, and 7 SNPs of PCSK9 gene. Paired DNA and blood, plasma or serum samples are collected and analyzed for concordance using eSensor and ABI7900HT instruments.

Results: The performances of either DNA purified from blood or the same blood without DNA purification were analyzed using GenMark eSensor technology on 4 different variants prevalent in Factor II, Factor V and MTHFR genes (Thrombophilia Risk Panel), and 3 variants from CYP2C9 and VKORC1 genes (Warfarin Sensitivity Panel). Genotyping of 7 SNPs in the PCSK9 gene was conducted by TaqMan/ ABI7900 platform. Overall, genotyping from purified DNA and the corresponding blood showed concordance of 84.8% (N=66), 87.5% (N=24) and 100% (N=12) for PCSK9, Thrombophilia and Warfarin panels, respectively.

To further validate the methodology to perform large-scale high-throughput genotyping directly from blood, plasma or serum, paired DNA/blood (N=50), DNA/ plasma (N=30) or DNA/serum samples (N=20) were analyzed on 7 SNPs of PCSK9 gene using ABI TaqMan real-time PCR machine. High concordance was achieved and resulted in sensitivity/specificity of 100%/90.9% for direct blood, 97.9%/66.7% for direct plasma, and 94.5%/75.0% for direct serum genotyping.

Conclusions: The methodology described is simple and fast that allows accurate gene polymorphism test directly from a drop of blood, plasma or serum. This method can be applied to a broad range of clinical genetic tests with the advantages of immediate sample testing, improving workflow, and lowering workload, cross-contamination, costs and turnaround time.

Abbreviation: PCR, polymerase chain reaction; PCSK9, proprotein convertase subtilisin/kexin type 9; MTHFR, methylenetetrahydrofolate reductase; VKORC1, Vitamin K epoxide reductase complex subunit 1.