Tuesday, July 31, 2018

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

Cancer/Tumor Markers

A-001

Lot-to-Lot Consistency of Abbott Tumor Marker Assays

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Background: Serological tumor marker assays are a valuable tool, aiding clinicians in the prognosis and management of cancer patients. For patients being monitored with tumor marker assays, changes in values can have significant implications for therapy or intervention. It is therefore imperative that the analytical performance of these assays remain consistent from one lot of reagent to the next to impart confidence that fluctuations in values are due to changes in the patients' tumor status and not to reagent lot-to-lot variability. Objective: The goal of this study was to evaluate the lot-to-lot performance of eight tumor marker assays (CA19-9, CA15-3, CEA, AFP, Total PSA, Free PSA, CA125 and HE4) over an extended period of time. Methods: Quality controls (QC) with values across the measurement range and human serum panels targeted at or near medical decision points were tested on each new reagent lot manufactured over a nine- to 33-month period. Assays were run on the Abbott Architect. For CA19-9, CA15-3, CA125 and HE4, QC was tested in replicates of two (HE4), five (CA15-3 and CA125), or 10 (CA19-9) and panels were tested in singlicate in one run on one instrument at time of manufacture. For CEA, Total PSA, and Free PSA, QC and panels were tested in replicates of 15 in two runs on one instrument. AFP QC was tested in replicates of 15 in two runs, with each run on a different instrument. For all assays, multiple calibrator lots, instruments and, in some cases, panel lots were used over the duration of analysis. Imprecision (percent coefficient of variation (%CV)) was calculated across all reagent lots on each control and panel level mean for each assay. Results: The imprecision for each control value for all assays was less than or equal to 4.7%CV. Human serum panels at or near important medical decision points had the following imprecision (CV): HE4, ≤6.8%; CA15-3, ≤4.8%; CA125 ≤4.1%, CA19-9, ≤5.2%, Total PSA, ≤4.7%; Free PSA, ≤4.0%; and CEA ≤2.8%. Conclusions: Each of the eight tumor marker assays evaluated showed consistent lot-to-lot performance on all controls and human serum panels near important medical decision points. Reliable laboratory results give physicians confidence that changes in tumor marker values are reflective of a change in patient status and will lead to more informed treatment decisions.

A-002

Serum Free Light Chain Stability at -20°C, +4°C and +22°C

M. D. Coley, C. T. Wilson, K. L. Sharp, S. J. Harding. *The Binding Site, Birmingham, United Kingdom*

Introduction

Serum free light chain (FLC) assays aid in the diagnosis and monitoring of plasma cell dyscrasias and associated conditions. Freelite® assay reagents, calibrators and controls are subjected to extensive testing during product development to ensure that they are robust to changes in temperature encountered during shipping and storage. However, this stability cannot be assumed of FLCs in patient serum samples. In this study the stability of κ and λ FLCs in serum at -20°C, +4°C and +22°C was assessed using Binding Site Optilite® Freelite assays.

Method

Ten healthy adult donors were selected at random from a pool of consenting donors. Whole blood was obtained from these donors by venepuncture into BD Vacutainer™ SST™ serum separator tubes and centrifuged according to the manufacturer's instructions. The day of venepuncture was designated as 'day 0'. Vials containing 0.35µL of serum from each donor were separated into three groups and stored at either -20°C, +4°C or +22°C until testing; one vial from each donor was set aside for day 0 testing. κ and λ FLCs were measured on the Optilite analyser (The Binding Site Group Ltd., Birmingham, UK) using Freelite kappa and lambda assays (The Binding Site Group Ltd., Birmingham, UK). Assays were calibrated and validated as instructed in the product inserts. Each sample at each storage temperature was tested in triplicate at days 1, 2, 3, 4, 7, 10, 14, 22 and 28. On each day the results were re-validated with an unopened vial of reagent and compared to day 0 results with the Kruskall-Wallace statistical test with Bonferroni correction using the Analyse-it™ software package. A statistically significant difference (p-value <0.05) indicated sample instability.

Results

When results were compared to day 0, serum κ FLCs stored at -20°C and +4°C produced a p-value of >0.05 at each time-point. On day 7, at +22°C, there was a -24.8% change in the median sample result compared to day 0 (p-value <0.05), and a p-value of <0.05 was produced at each time-point thereafter. Serum λ FLCs produced a p-value of 1.0 for each temperature and time-point tested.

Conclusions

We conclude that κ FLCs are stable in serum for up to 28 days at -20°C and +4°C. However, κ FLC stability was only observed for up to 4 days at 22°C. λ FLCs are stable for up to 28 days at -20°C, +4°C or +22°C. These findings validate the directions given in the product insert, which states that "Samples may be stored at 2-8°C for up to 21 days, but for prolonged storage they should be kept frozen at -20°C or below". Knowledge of the stability of free light chains in serum is important so that laboratories are aware of the appropriate patient sample transport logistics to ensure sample integrity is maintained.

A-003

Evaluation of Des-gamma-Carboxiprothrombin (PIVKA II) as complement of serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in Mexican people

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Background: The 80% of hepatocellular cancer (HCC) is detected in the advanced stage in Mexico. Serum alpha-fetoprotein is preferred biomarker in HCC, nevertheless has a low sensitivity, specificity and positive predictive value. Some studies suggest the Des-gamma-Carboxiprothrombin (DCP) is a potential marker of HCC. Study aims to evaluate the predictive value of DCP test as complement of serum alpha-fetoprotein (AFP) for diagnosis of hepatocellular carcinoma in Mexican people. **Methods:** We conducted a retrospective study from November 2017 to January 2018. 56 patients with AFP> 10 ng/mL were included to measured DCP (PIVKA II Abbott ARCHITECT STAT assay). Demographic and clinical information were collected from the clinical file. The results of hepatic elastography and anatomic pathology were used to determine liver pathology (cirrhosis and HCC) or non-hepatic pathology. Sensitivity, specificity and positive predictive value (PPV) were calculated based on these results. For the reference intervals we used EP Evaluator and the cutoff point was established in 40 mAU/mL. **Results:** 14 patients had DCP <40 mAU/mL, none of them had HCC, one patient has cirrhosis and 13 other non-hepatic tumors. 62 patients had DCP >40 mAU/mL, of which 20 had HCC, 8 patients have cirrhosis and 14 other non-hepatic tumors. The sensitivity was 96.9%, the specificity 48.1% and the PPV 66.6%, which is indicative that if a patient has positive AFP and positive DCP has a 66.6% probability of having HCC. **Conclusion:** Our study support DCP determination as complement of serum alpha-fetoprotein as an important marker of probability of hepatocellular carcinoma in Mexican people and points the way for further cohort prospective clinical studies to explore it´s value in monitoring response to therapy and patient´s follow-up.<!--EndFragment-->

A-004

Hereditary Cancer Multi-gene Panel screening among Brazilian patients

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Background: Genetic panels have become powerful and accessible tests for screening germline variants, providing a source for diagnosis and medical guidance. Although there are several types of cancers already linked to hereditary predisposition with well-known genes as key players, such as *BRCA* genes and breast cancer, other secondary genes are also important for disease development and are not usually analyzed at first screening. Different hereditary cancer panels are currently commercially available, recommended for patients with familiar history of cancer. **Objectives:** Screen the prevalence of variants in medical reports in a hereditary cancer-related multi-gene panel among Brazilian patients through next generation sequencing (NGS). **Methodology:** We used an Ampliseq custom 40 genes panel to investigate variants in 49 pa-

tients with familiar history of cancer along the years of 2016 and 2017. Genomic DNA libraries were prepared from blood samples, following the manufacturer's instructions. Sequencing was performed on the Ion Torrent PGM™ or S5™ platforms. The sequence data were processed using standard Ion Torrent Suite™ Software. Reads were aligned to the human genome reference (hg19/GRCh37) with TMAP and variant calling were performed by Ion Reporter™ Software. Variants in the entire coding regions and 10 pb of exon boundaries with MAF <0.02 were filtered. Variant classification was performed according to ACMG-AMP guideline. Pathogenic, probably pathogenic and variants with unknown significance (VUS) were reported. **Results and Discussion:** There was a 4.4X increase in the number of tests from 2016 to 2017. Although cancer diagnoses are more common in men than in women, males and females represented respectively 22% and 78% of the patients. This may be due to more usual cancer screening in women, who seeks more contact with health professionals throughout their lives. The average age of males was higher (52.3 ± 13.5 years) than females (46.2 ± 11.5 years). Since men are usually less willing to talk about their health concerns, this may lead to a late screening and diagnosis. 33 patients (67%) presented at least one reported variant. A total of 62 distinct variants (56 SNPs and 6 INDELs) were found in 29 genes (*APC, ATM, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FANCC, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD51C, RECQL, RET, SMAD4* and *TP53*). 53 variants were classified as VUS and nine as pathogenic or probably pathogenic. From the VUS, 15 are novel variants found in 11 patients. *APC* was the most mutated gene with seven VUS in six patients. **Conclusions:** This study shows the importance of disease screening with multi-gene panels for patients with familiar history of cancer, as relevant variants could be frequently detected. Surveys of the reported variants may contribute to define their importance as prevention and diagnostic tools and guide future genetic counseling campaigns aiming early cancer detection. The annually increasing amount of samples will also significantly contribute to our knowledge about previous and new potentially pathogenic variants among the Brazilian population.

A-005

A 83-01 inhibits the epithelial-mesenchymal transition and increased invasion induced by platelets in ovarian cancer cells

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Background: Ovarian cancer is the leading cause of death among all gynecological malignancies. The high mortality is partly resulted from metastasis, mainly referred to peritoneal dissemination and hematogenous metastasis for ovarian cancer. It has been demonstrated that platelets play an important role in promoting metastasis in ovarian cancer. However, antiplatelet agents could not inhibit platelet-induced epithelial-mesenchymal transition (EMT) gene expression alterations. Therefore, there is an urgent need for more reliable drugs, which can abolish the effects of platelets on ovarian cancer cells. Many studies have shown that platelets enhance the metastatic activity of tumor cells through TGF-β/Smad pathway. However, whether A 83-01 (a TGF-β inhibitor) could inhibit the platelet-induced EMT and invasion in ovarian cancer cells remains unknown. **Objective:** In the current study, we sought to examine the possible role of A 83-01 in inhibiting platelet-related prometastasis in ovarian cancer cells. **Methods:** SK-OV-3 and OVCAR-3 cells were seeded in 6-well cell culture plates with appropriate culture medium. After adherence, cells in each well were treated with platelets, platelets + A 83-01 or A 83-01 alone, respectively. Cells without any treatments were used as controls. The EMT alterations in ovarian cancer cells at molecular level were assessed by EMT-related markers using quantitative real-time PCR (qPCR) and Western blot. Transwell assays were used to analyze the invasive capacity of the cell lines. **Results:** Analysis of mesenchymal markers and transcription factors involved in EMT revealed that the mRNA expression of snail, vimentin, N-cadherin, fibronectin (Fn1) and matrix metalloproteinase-2 (MMP2) was significantly up-regulated in both cell lines. Additionally, there was a significant down-regulated in the mRNA expression of epithelial marker E-cadherin in SK-OV-3. Western blot analysis revealed the reduced E-cadherin protein levels and increased N-cadherin protein levels in platelet-treated SK-OV-3 and OVCAR-3 cells. The protein levels of MMP2 were also increased in comparison with controls. Co-culture with platelets markedly increased the invasive properties of SK-OV-3 and OVCAR-3 cells. Specifically, platelets induced a 3.2-fold ($p < 0.0001$) and 3.1-fold ($p = 0.0004$) increase in SK-OV-3 and OVCAR-3 cells invasion, respectively. Upon addition A 83-01 to media from platelets-treated ovarian cancer cells, the EMT-like alterations were inhibited at the transcriptome level. A 83-01 treatment also restored the expression of E-cadherin and repressed that of N-cadherin and MMP2 in both cell lines at protein level. Concordantly, exposure of platelets-treated SK-OV-3 and OVCAR-3 cells to A 83-01 resulted in a 2.6- and 2.3-fold significant reduction in their invasion activity compared to those incubated with platelets alone, respectively. **Conclusion:** A 83-01 inhibits the EMT and increased invasion induced by platelets in ovarian cancer cells. The findings of the present study suggest that A 83-01 may be useful for establishment of therapies tailored to inhibiting ovarian cancer metastasis.

A-006

Insights into Analytical Validation and Clinical Utilization of Alternating Current Electrokinetics (ACE)-Based Assays for the Quantification of Cell-Free DNA

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The isolation and quantification of analytes in complex biological fluids continue to be challenging for researchers and clinicians studying the role of macromolecules in diseases such as cancer. We have applied a novel technology based upon Alternating Current Electrokinetics (ACE) to enable direct isolation and on-chip analysis of nanoparticles and macromolecules such as high molecular weight cell-free DNA (hmw cfDNA). The utilization of a simple and rapid workflow which does not require extensive purification and processing steps constitutes one of the primary advantages of this application of ACE technology. For our ACE-based assay that quantifies hmw cfDNA, blood is first processed to plasma via centrifugation. A fluorescent detection reagent is added to the plasma sample, which is then pipetted into a single-use cartridge containing a microelectrode array. The cartridge is inserted into a dedicated instrument which provides the appropriate mechanical, microfluidic, electrical, thermal, and optical control to enable capture and visualization of hmw cfDNA, and the resultant images are quantified via purpose-built software. We demonstrate the feasibility of assays based upon ACE technology to quantify DNA in both defined buffer systems and complex biological fluids such as plasma, with concentrations being determined via reference to calibrators with known concentrations of DNA. Assay parameters are still being optimized for specific assay formats, but prototype assays show a Limit of Detection (LoD) of <10 ng/mL and excellent linearity over a reportable range of at least two orders of magnitude. These analytical parameters enable the quantification of hmw cfDNA in the vast majority of clinical samples without dilution, and in one analysis of 177 plasma samples derived from cancer patients, at least 91% (162 samples) had hmw cfDNA concentrations within the reportable range. It has been known for some time that hmw cfDNA is present at elevated levels in the blood of cancer patients. Since many of these studies employed quantitative PCR (qPCR) as the methodology to determine cfDNA concentrations, we have conducted experiments to compare quantitative measurements of cfDNA by ACE-based and qPCR assays in a set of plasma samples processed from the blood of cancer patients and healthy normal donors. For one representative set of assays from this series of experiments, regression analysis comparing ACE-based and qPCR assays on 19 plasma samples from Non-Small Cell Lung Cancer (NSCLC) patients receiving systemic treatments resulted in an excellent degree of fit between the two assays ($R^2>0.9$). Initial results with another set of samples demonstrate that changes in hmw cfDNA levels over time are correlated to disease progression in a cohort of NSCLC patients. We are pursuing the use of ACE-based assays for monitoring cancer progression and also for early-stage cancer detection, and further development of the technology and assays are enabling its application to the isolation and quantification of other disease-relevant analytes.

A-007

Towards development of an exosomal protein biomarker signature to monitor cancer progression in uveal melanoma

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Uveal melanoma (UM) is a particularly rare type of non-cutaneous melanoma that arises from melanocytes in the uveal tract, forming a malignant tumor of the eye. Metastatic disease frequently involves the liver and is associated with a high rate of mortality, typically within 15 months. There is a significant deficit in our ability to diagnose early and effectively treat UM, which accounts for the poor outcomes. By advancing diagnostics to detect UM in a pre-metastatic state, we may be able to improve outcomes. Exosomes are membrane bound vesicles formed in multivesicular endosomes that carry proteins, genetic material, among other molecules, out of the cell through cellular secretion. Melanoma cell-derived exosomes appear to be intricately involved in initiation, growth, pre-metastatic niche formation, and subsequent metastasis of melanoma. Thus, we hypothesize that these molecules can be used as biomarkers to diagnose disease earlier. In addition, these vesicles are readily found in clinical samples and provide

a specific protein signature allowing for the development of a diagnostic assay. UM in particular is poised to benefit most from improved detection because of the difficulty of pre-metastatic detection and severely negative outcomes associated with late diagnosis. Despite the growing relevance of circulating exosomes as cancer biomarkers within the clinical community, their use has been limited largely due to a lack of efficient, straightforward isolation procedures that can be used in a clinical laboratory setting. We report here the development of a simple, quick, and inexpensive procedure for isolating urine exosomes. Our method requires no specialized equipment and we have established a standard operating procedure that can readily be adapted to clinical laboratories. Briefly, the first spin removes dead cells and cellular debris from the sample. A second higher speed spin removes larger extracellular vesicles, along with Tamm-Horsfall protein filaments. This pellet is subsequently chemically treated to remove the Tamm-Horsfall protein, thereby freeing the entrapped exosomes, and spun a third time to concentrate the exosomes. Successful isolation was confirmed by western blot analysis using six previously identified protein exosome markers: CD9, CD81, ALIX, HSP90b1, HSP1a1, and ACTN4. Comparison between native and processed urine revealed a robust enrichment of exosomes in the processed sample. We have also used an adaptation of this procedure with cell culture samples and have detected key signaling molecules, including mature integrin b1 and tyrosine kinase receptors, within UM exosomes. The presence of these molecules in exosomes yields insight into signal transduction pathways activated in the cancerous cells from which the vesicles are derived, and can be used to inform clinical decision making. This biomarker signature, comprised of exosomal proteins and signaling molecules, is a harbinger of aberrant processes in cancer progression. Detection of this biomarker signature in noninvasively obtained clinical samples, such as urine, can be used to monitor disease progression in real-time, thereby leading to more effective care for UM patients.

A-008

Novel Prognostic Scores Based on Plasma Prothrombin & Fibrinogen Levels in Patients with AFP-negative Hepatocellular Carcinoma

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Background: Non-invasive tools for the prognosis of and alpha-fetoprotein(AFP) negative hepatocellular carcinoma(HCC)are urgently needed. The present study proposed a prognostic system based on preoperative plasma prothrombin time(PT) and fibrinogen(Fbg) (PT/Fbg system). **Methods:** With respect to AFP-negative HCC, we compared the prognostic value in PT/Fbg system, Glasgow prognostic score (GPS) and aminotransferase (ALT)/aspartate aminotransferase (AST) ratio(LSR). The present study retrospectively analysed patient characteristics, clinicopathological factors, and the level of pretreatment biomarkers (PT, Fbg, albumin, C-reactive protein, ALT and AST) in 628 patients with CRC. **Results**: Patients with increased PT and Fbg levels were allocated a score of 2, patients with only one of these abnormalities were assigned score 1, and patients with neither of these abnormalities were allocated a score of 0. The discriminatory ability of the PT/Fbg system, GPS and LSR were assessed using an ROC curve.The optimal cut-off values of biomarkers were determined using ROC analysis, and albumin and C-reactive protein were estimated as described previously. The following distributions of the PT/Fbg system scores were observed: 187(29.78%) patients had a score of 0, 305(30.65%) had a score of1, and 134(22.69%) patients had a preoperative score of 2. The prognostic significance of the PT/Fbg system was determined using univariate and multivariate cox hazard analyses in AFP-negative HCC. Multivariate analysis revealed that patients with a higher PT/Fbg systemexhibited worse OS than patients with a lower PT/Fbg system. Furthermore, the PT/Fbg system was associated with treatment and exhibited a higher AUC value(0.684). **Conclusions**: These results suggest that the overall survival (OS) was shorter in AFP-negative HCC patients with a high PT/Fbg system. Therefore, our study proposes preoperative evaluation of the plasma PT/Fbg system to predict the OS of AFP-negative HCC patients.

A-009

Point of Care Dipstick Assessment of Ascitic Fluid: Comparism With Serum Ascites Albumin Gradient (SAAG) in Differentiation of Ascitic Fluid Into Exudate and Transudate.

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Background: Excessive ascitic fluid accumulation in the peritoneal cavity is seen as a clinical problem in all parts of the world by many doctors. This study attempts to differentiate exudate and transudate ascitic fluid by use of point of care dipstick. ***Material/method:*** A total of 67 patients with ascites were recruited for this study. Using serum ascites albumin gradient (SAAG) as standard way of classifying ascitic fluid into exudate and transudate, dipstick protein levels were assessed to classify the ascitic fluid into exudate and transudate. Ascitic fluid was obtained by abdominal paracentesis at the same time of collecting venous blood from the patients. SAAG was calculated based on albumin values of venous blood and ascitic fluid (SAAG less than 11g/L represented exudate while SAAG greater than 11g/L represented transudate. Values obtained were classified as high protein dipstick and low protein dipstick. ***RESULTS:*** Of the 67 adult population in this study,38 of them(56.7% were female, while 29(43.2%) were males($p<.005$).The age range was 18-65years with mean age of 47.11±11.21 for both sexes(male:50.8±9.4, female:44.29±11.78, p value of .0168). Mean body mass index(BMI) was 24.19±2.9.A total of 41(61.2%) had high ascitic fluid protein based on dipstick(500mg/dL),while 26 had low ascitic fluid protein(30mg/dL).SAAG was the gold standard used to differentiate exudates from transudate. In comparing dipstick protein(high or low)to correlate with low SAAG(exudates) and high SAAG(transudate) yielded a sensitivity,specificity,PPV,NPV and accuracy of 63.4%,42.3%,63.4%,42.3% and 70.3% respectively. ***CONCLUSION****:* SAAG is a better indicator of classifying ascitic fluid into exudate or transudate than urine dipstick. ***Keywords:*** *Dipstick, point of care, ascites, exudate, transudate, serum ascites albumin gradient.*

A-010

ORAL SUPPLEMENTS ASSOCIATION WITH DIETARY BENZO{A}PYRENE IN PROSTATE CANCER IN IBADAN,NIGERIA.

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Introduction
Prostate cancer has been linked to dietary factors. The presence of Benzo{a} pyrene; a prototype of Poly Aromatic Hydrocarbon (PAH) in foods is linked with various methods of food preparation. The relationship between dietary factors and the level of the toxicant- PAH in men with prostate cancer in Ibadan therefore needs to be established.
Methods
The study included thirty (30) prostate cancer patients aged 55-85 and thirty apparently healthy controls. Prostate Specific Antigen (PSA), Benzo {a} pyrene (BaP) and Total antioxidant Status (TAS) were analyzed using standard methods. BaP was analyzed using high performance liquid chromatographic technique (HPLC). Data obtained were analyzed statistically as appropriate and $p < 0.05$ was considered significant.
Results
The mean value of PSA was predictably significantly higher in Prostate cancer patients compared to the controls while, surprisingly there was a significant decrease in the level of BaP in men with prostate cancer compared with the controls. Correlation analysis however showed a significant positive correlation between smoked fish intake and BaP in the patients. TAS was significantly higher in the controls than in prostate cancer patients. Increased consumption of smoked foods, reduced TAS and resultant oxidative stress may contribute to the aetio-pathogenesis of prostate cancer. Antioxidant supplementation may enhance the total antioxidant status and may be of help in the management of prostate cancer.
Keywords: Prostate cancer; BaP, TAS, PSA

A-011

Establishment and performance evaluation of serum HE4 detected by chemiluminescence.

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Background: We intend to find a CLIA (Chemiluminescence immunoassay) method with good repeatbility, high sensitivity, wide linearity range to detect the serum HE4 rapidly, and then set up the performance. **Methods:** To establish adouble antibody sandwich CLIA method to detect the serum HE4. and evaluate the analytical performance including sensitivity, precision, accuracy, linearity, specificity under the optimized condition; we alsocompare the analytical performance and correlation with the commerical HE4 EIA test kit; Test clinical samples, then calculate the sensitiv-

ity, specificity and area under ROC (Receiver operating characteristic curve). **Results:**The precision of with-in lot and with-out lot are less than 4.0% and 5.0% respectively; Serum recovery is within 90.0% -110.0%; LoB is 5.0pmol/L;functional sensitivity is 15.0 pmol/L; measure range is 15.0~1500.0pmol/L; report range is 15.0~7443.0pmo/L; the R^2 is bigger than 0.95 comparing with commercial EIA test kit according to EP9A2; the diagnostic sensitivity is 79.5%, specificity is 92.0%, AUC(area under curve) is 0.908 (95% CI 0.865~0.951). **Conclusion:** we have established a sensitive and rapid CLIA method with highrepeatability and wide measurerange; it has good correlation with commercial EIA HE test kit, and there is no statistics difference on clinical diagnosis.

A-012

Profiling Of 5-hydroxymethylcytosine From Liquid Biopsy Samples: A Novel Approach For Early Diagnosis And Monitoring Of Cancer

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Background: The epigenetic modification 5-hydroxymethylcytosine (5hmC) plays a pivotal role in gene expression and has been associated with many diseases, including cancer. Unlike the relatively static genomic sequence, epigenetic DNA modifications are highly dynamic and change in response to both environment and disease status. Further, DNA modifications have been shown to occur early in disease development and exhibit tissue-specific signatures — making them exceptionally well-suited to the diagnosis and monitoring of disease using cell-free DNA (cfDNA). This study aims to assess the utility of a new, highly-sensitive 5hmC discovery platform to identify cancer-specific signatures from minimally invasive, liquid biopsy (LQB) samples. **Methods:** We have developed a novel 5hmC-pulldown technique (HMCP) that allows genome-wide profiling 5hmC distribution using low ng quantities of input DNA such as are typically recovered in cfDNA samples. Starting with 1ml of plasma collected from a cohort of healthy individuals and patients with different stages of cancer, we isolated cfDNA and processed these samples through the HMCP workflow prior to NGS analysis using specialised informatics algorithms. **Results:** We successfully identified 5hmC features that could differentiate colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) cases from controls and early from late-stage disease. These results indicate that this novel and sensitive method of 5hmC profiling holds the potential to identify novel disease- and tissue-specific biomarkers for the development of minimally invasive diagnostic and prognostic assays. **Conclusion:** 5hmC is a dynamic epigenetic mark that can provide valuable information for disease diagnosis and monitoring. The HMCP workflow developed by Cambridge Epigenetix is a robust, reliable, affordable and highly sensitive method to reveal the true hmC profile of DNA from different clinical conditions. By analysing cfDNA-derived hmC profiles from cancer patients and healthy individuals we have demonstrated that this could form the basis of a useful prognostic marker, especially for early stage cancer diagnosis.

A-013

Lack of Harmonization in Diverse Analytical Methods for Measuring Tumor Markers; CEA and CA19-9

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Background: Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are widely used tumor markers for cancer screening and therapeutic monitoring. However, different results obtained for the same sample by using diverse analytical methods that are not harmonized can lead misinterpretation of the results and inappropriate clinical decision making. In this study, we performed a method comparability test for CEA and CA 19-9 near the claimed cutoff values by using widely used analyzers. **Methods:** Total of 103 residual samples for the CEA test and 101 samples for the CA19-9 test near-cutoff values, were collected from patients who have visited Seoul national university hospital (SNUH) and undergone health checkup or therapeutic monitoring from May 2016 to Jul 2016. Samples were stored at -70℃ and measured by four immunoassay analyzers, Architect i2000 (Abbott Laboratories, Abbott Park, IL, USA), Moduler E170 (Roche Diagnostics, Mannheim, Germany), Unicel DxI 800 (Beckman Coulter, Fullerton, CA, USA), ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), and immunoradiometric assay (IRMA). The results of each sample were collected and analyzed via method comparability test based on the CLSI EP9-A2 guideline, using Microsoft Excel (Microsoft, Redmond, WA, USA), IBM SPSS (SPSS Inc., Chicago, IL, USA), and EP evaluator (Data Innovations, South Burlington, VT, USA). Correlation between positive test results and clinical progress identified by medical record review was also analyzed using chi-square test. **Results:** Method comparison tests, comparing Architect the main instrument used in SNUH with other analyzers, were poorly correlated; results of quantitative measurement for CEA and CA19-9 were not clinically equivalent. We observed concordant qualitative test results in CEA measurement determined by the claimed cut-off values, between Architect and other analyzers (Moduler E170, $p < 0.001$; Unicel DxI 800, $p < 0.001$; ADVIA Centaur XP, $p = 0.001$) except IRMA ($p = 0.232$), and concordant test results in CA19-9 measurement were observed for all assays including IRMA ($p < 0.001$). However, we observed considerable disagreement, from 14 to 76 % for CEA, and from 13 to 35 % for CA19-9. There was no significant correlation between clinical disease progression in patients with cancer and the positive test results for CEA obtained from each assay (Architect, $p = 0.868$; Moduler E170, $p = 0.873$; Unicel DxI, $p = 0.204$; ADVIA Centaur XP, $p = 0.817$). Conversely, positive test results for CA19-9, obtained by using Moduler E170 and Unicel DxI showed statistically significant correlation with progressive disease ($p = 0.012$; $p = 0.028$, respectively). **Conclusion:** Considerable differences between analyzers in CEA and CA 19-9 assays were observed near-cutoff values; this discrepancy can lead to confusion in clinical decision making. The difference was greatest between the chemiluminescence immunoassay and immunoradiometric assay. Careful interpretation of test results with near-cutoff values and harmonization of different immunoassays, need to be mandatory.

A-014

Multicenter Comparison of Automated Immunoassay Analyzers for Prostate Specific Antigen

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Objectives: A multicenter study were performed to compare results of prostate specific antigen (PSA) using four different automated immunoassays (Immulite 2000 XPi, Unicel DxI800 Access, ADVIA Centaur XPT, and ARCHITECT i2000SR). **Methods:** One hundred and twenty serum samples collected on patients with PSA values between 0.00 and 104.37 ng/mL were assayed by the four reagents and analyzers. Wilcoxon test, Spearman's correlation, and Bland-Altman plot analysis were performed. **Results:** Among the 4 automated immunoassays, Immulite 2000 XPi showed the lowest median and mean value of PSA. The Unicel DXI 800 revealed the highest median and mean value. The significant differences in the results of PSA were observed in the comparison of each 4 instruments. However, the range of the correlation coefficients (r) with 4 instruments was between 0.991 to 0.995. Compared to Immulite 2000 XPi Immunoassay, the mean bias was less than + 0.816 ng/mL in other 3 instruments. Compared to Unicel, the mean bias was -0.767, -0.687, -0.816 ng/mL (vs ADVIA Centaur, Architect, and Immulite, respectively). In case of Architect, the mean bias was less than ± 0.687 ng/mL. The comparison of ADVIA Centaur and other 3 instruments, the mean bias was less than ± 0.767 ng/mL. The total agreements at the clinically relevant cut-off were optimal: between 95-99% at 4.0 ng/mL. **Conclusions:** The results of the automated four immunoassay analyzers comparison studies for PSA were acceptable. We evaluated this study by comparing 4 automated analyzers. Although Immulite 2000 XPi displayed a tendency of lower results under especially the clinical relevant cut off compared to other 3 analyzers, it yields high correlation coefficients and agreement at cut off. The results of the automated four 4 immunoassay analyzers comparison studies for PSA were acceptable and applicable at the clinically relevant cut-off.

Table 1. Comparison of PSA results obtained with 4 automated immunoassays

	Wilcoxon test (p)	Spearman's correlation (r)	Mean bias and 95% CI (ng/ml)	Agreement[a]
Immulute 2000 Xpi vs UniCel DXI 800	<0.0001	0.992 (p<0.0001)	0.816 (0.037 to 1.596)	95% (K, 0.92)
Immulute 2000 Xpi vs ADVIA Centaur XPT	0.0253	0.991 (p<0.0001)	0.049 (-0.366 to 0.464)	99% (K, 0.96)
Unicel DXI 800 vs ADVIA Centaur XPT	0.0004	0.995 (p<0.0001)	-0.767 (-1.395 to -0.140)	95% (K, 0.92)
Unicel DXI 800 vs ARICHITECT I2000SR	<0.0001	0.995 (p<0.0001)	-0.687 (-1.533 to 0.159)	98% (K, 0.96)
ARICHITECT I2000SR vs ADVIA Centaur XPT	0.0073	0.994 (p<0.0001)	-0.080 (-0.418 to 0.259)	98% (K, 0.96)
ARICHITECT I2000SR vs Immulute 2000 Xpi	0.0259	0.993 (p<0.0001)	-0.129 (-0.508 to 0.250)	98% (K, 0.96)

A-015

Development of a Versatile Platform for Routine Flourescence in-situ Hybridization

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Background: Fluorescence microscopy is a highly sensitive and valuable method in clinical diagnostics. Genetic aberrations in cancer biopsies are routinely observed with fluorescence in-situ hybridization (FISH). The increasing number of tumor patients, the high diversity of probes and the diagnostic effort for the correct interpretation of FISH signals, lead to the need for the development of an automated reading and evaluation platform. In fact, challenging parts are the heterogeneity and complexity of the tumor derived section. Thus, pre-filtering of tumor derived areas of interest, the detailed focus of these parts with subsequent probe detection and evaluation is required. **Methods and Results:** ***Simultaneous real-time device control*** The platform is based on a motorized microscope with changeable magnification and fluorescence filters, a LED illumination with multiple wavelengths, a precise moveable X-Y stage and a high-resolution greyscale camera[1]. All devices are controlled simultaneously by generating the appropriate driver software developed in house[2]. ***Pre-filtering*** Stitching strategy acquires images of DAPI (4,6-Diamidino-2-penylindole) stained specimens with low magnification (100-200 fold) getting a tissue overview within 30 min. Specific algorithms like the entropy filter remove background and artefacts within 1 min and search dense cell areas that are interesting for the detailed FISH probe analysis. Letter requires higher magnification (400-600 fold). Within these images, single cells are detected and separated with an adjustable watershed transformation algorithm. ***FISH signal analysis*** Detection and interpretation algorithms are adapted to the specific probes (ZytoVision GmbH), which focus the break-apart and translocation of genes and further events like the gain or loss of sequences. Further, recording sharp images of relevant nuclei in a five stack z-layer image gallery with 500 nm distances combined with a maximum intensity projection is necessary for diagnostic documentation. ***End-user evaluation*** Results are demonstrated within a user friendly graphical interface, performed as a diagnostic learning tool. Further, a long term archiving supports the routine workflow. **Conclusion:** The platform is an early stage of development and will be optimized for image processing and the self-learning program by using adapted algorithms. Further, routine diagnostics requires further optimization of scan time and image processing algorithm for multiple cancer sections. **References:** (1) A. Willitzki *et al.* (2012). Clin. Dev. Immunol. 284740. doi:10.1155/2012/284740.; (2) S. Rödiger *et al.* (2013). Adv. Biochem. Eng. Biotechnol. 133 35-74. doi:10.1007/10_2011_132.

A-016

Incidence of Antigen Excess Utilising the Freelite® Assays on the Optilite® Analyser

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Introduction

Serum immunoglobulin free light chain (FLC) concentrations can range from <1mg/L up to >10,000mg/L and therefore, any immunoassay measuring serum FLCs has a risk of underestimation due to antigen excess. The Optilite analyser is able to detect samples in antigen excess through a suite of methods which include reaction kinetic monitoring and the addition of patient sample post-reaction. For both κ and λ Freelite assays, reaction kinetics are monitored over three separate windows; a change in rate of reaction between the windows is indicative of potential antigen excess. Samples with such kinetics are flagged and automatically re-run at a higher dilution to preclude a falsely low result. Here we analyse data collected from four evaluation laboratories between January 2015 and September 2016 to determine the efficacy of the antigen excess check for the Optilite Freelite assays.
Method
We reviewed κ and λ FLC results generated using Freelite assays (The Binding Site Group Ltd., Birmingham, UK) on an Optilite analyser (The Binding Site Group Ltd., Birmingham, UK) at four evaluation laboratories. 10495 individual sample results were analysed using κ Freelite and 10228 using λ Freelite. Results were categorised into the dilution at which they were obtained and the frequency of antigen excess flags at each dilution was reported. The robustness of the antigen excess check was established by comparing each flagged sample result to the result obtained at the next highest dilution. If a higher result was produced at the next dilution, the original antigen excess flag was determined to be correct. Conversely, if a lower result was obtained, the antigen excess flag was deemed incorrect.
Results
Overall, 11% (971/8573) of samples were flagged for antigen excess using the κ Freelite assay at the standard sample dilution of 1/10, 10% (156/1518) at the reflex high dilution of 1/100 and 7% (18/266) at the further reflex high dilution of 1/1000. For the λ Freelite assay, 6% (512/8536) of samples were flagged for antigen excess at the standard sample dilution of 1/8, 11% (87/795) at the reflex high sample dilution of 1/80 and 5% (7/134) at the further reflex high dilution of 1/800. The frequency of correct antigen excess flags was ≥95.6% for κ Freelite and ≥98.9% for λ Freelite at each sample dilution. Since March 2016 there have been no examples of undetected antigen excess reported.
Conclusions
Of over 10,000 sample results, 98% (1121/1145) of samples were flagged correctly for antigen excess using κ Freelite assays whilst 99% (601/606) of samples were flagged correctly for antigen excess using λ Freelite assays. This validates the ability of the analyser to accurately flag and re-dilute samples in antigen excess and demonstrates that the occurrence of undetected antigen excess with these assays is negligible.

A-017

Laboratory performance of serum free light chain immunoassays in baseline and post-treatment samples of light chain multiple myeloma patients

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Background: Serum free light chain (sFLC) measurements are routinely used in the diagnosis and management of patients with monoclonal gammopathies. Assessment of sFLCs is particularly informative in light chain multiple myeloma (LCMM) patients; an abnormal sFLC ratio is expected in all cases at diagnosis and monoclonal (involved; iFLC) levels provide a sensitive marker of response. This patient group can present a particular challenge for FLC measurements since high iFLC levels are often present at diagnosis, requiring the analyser to perform additional sample re-dilutions until a final result is reported. However, novel treatments for MM can lead to rapid reductions in the concentration of monoclonal FLCs in serum. Here we compare the number of Freelite® re-dilutions required following initial treatment of LCMM patients compared to baseline samples, within the context of a clinical trial. **Methods:** Serum samples from 113 LCMM patients enrolled on to the IFM 2009-01 study were assessed for κ and λ FLC concentrations using Freelite immunoassays (The Binding Site Group Ltd, UK) on the BN™II nephelometer (Siemens, Germany). Samples were analysed at baseline, and following 1 and 3 cycles of induction therapy with bortezomib, lenalidomide and dexamethasone. Sample re-dilutions needed for achieving a final iFLC result was compared at each time-point. **Results:** At baseline, all 113 patients had an abnormal κ/λ FLC ratio (72 κ and 41 λ). The mean iFLC was 3798.2, 613.8 and 204.0 mg/L for κ FLC, and 3402.1, 395.7 and 125.0 mg/L for λ FLC at baseline, cycle 1 and cycle 3, respectively. For κ iFLC measurements, 11% of samples gave a final result on the standard dilution at baseline, compared to 31% after cycle 1 and 54% after cycle 3. For λ iFLC measurements, 5% of samples gave a final result on the standard dilution at baseline, compared to 54% after cycle 1 and 63% after cycle 3. The average number of re-dilutions required at baseline to achieve a final result per patient was 1.9 for both κ and λ iFLC.

After 1 treatment cycle the average number of re-dilutions required for achieving a final result was 0.8 for κ iFLC and 0.7 for λ iFLC. The average number of re-dilutions required at cycle 3 was lower than at cycle 1 for both κ (0.5) and λ (0.4). **Conclusion:**Novel myeloma drugs lead to rapid reductions of monoclonal sFLC levels in LCMM patients, impacting the number of sample re-dilutions required by Freelite immunoassays for reporting a final result on the BNII instrument. Laboratories should be reassured by the reduction in the need for re-dilutions in individual MM patients after initiating treatment.

A-018

Development and Testing of Reference Materials for NGS based Somatic Variant Detection and Fusion Detection in Myeloid Cancers

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Background: Myeloid malignancies are clonal diseases of hematopoietic progenitor cells which can lead to accumulation of immature blast cells in the bone marrow and peripheral blood. Understanding the molecular changes that lead to the clonal proliferation can aid in both determining prognosis and therapy selection. The large range of genetic aberrations and genes involved make Next Generation Sequencing (NGS) tests a cost effective and sensitive way to determine these molecular changes. There are an increasing number of NGS-based oncology tests available for somatic mutation detection in patients with hematological malignancies. However, reference materials that contain mutations relevant to blood cancers are lacking. **Methods:** We designed a reference material in purified RNA format that contains nine RNA fusions important in myeloid cancers including two different ETV6-ABL1 transcripts, as well as BCR-ABL1, MYST3-CREBBP, RUNX1-RUNX1T1, and PML-RARα among others. We designed a second reference material in purified DNA format to include 23 somatic variants included two FLT3 internal tandem duplications. The biosynthetic RNA or DNA constructs were mixed with either total RNA or purified genomic DNA from GM24385 reference human cell line. Digital PCR was used to quantify the variant sequences to determine the abundance of the fusion RNAs and the allele frequencies of the somatic mutations. Testing was then performed on a variety of targeted NGS assays to show compatibility. **Results:** Testing of the Seraseq™ Myeloid RNA Mix by Digital PCR confirmed that each of the nine fusions in the reference samples are present at approximately 100 fusion copies per nanogram of total RNA. FusionPlex Myeloid Kit for Illumina (ArcherDx) as well as Oncomine Myeloid Panel (Thermo Fisher) showed positive detection for all fusions present in the reference samples. Seraseq™ Myeloid DNA Mix was also tested by digital PCR and results confirmed the variant allele frequencies were on target at the 5%, 10% or 15% VAF. Effective dPCR allele specific assays could not be obtained for CEBPA mutations. However, NGS testing of the reference samples by VariantPlex Core Myeloid Kit for Illumina (ArcherDx) and Oncomine Myeloid Panel (Thermo Fisher) confirmed the presence of these mutations at the expected levels, as well as all of the other variants in the DNA reference samples. **Conclusion:** SeraCare has developed highly multiplexed DNA and RNA-based reference materials for evaluating Myelogenous disorders that allow monitoring of a broad range of somatic mutations and gene fusions. These reference materials aid optimization and verification of detection limits for NGS-based Myeloid disease assay testing, and provide laboratories greater assurance in their ability to correctly detect and quantify various types of genetic events in diseased patient samples.

A-019

Potential Serum Metabolic Markers in Thyroid Carcinoma Diagnosis

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Background: Distinguishing between the benign and malignant types of thyroid nodules is still challenging in clinical laboratories. Since these two types of tumors require totally different interventions in clinic, it is necessary to establish new methods for accurate diagnosis of thyroid cancer. Methods: We use Nuclear magnetic resonance spectroscopy (mostly ^{1}H-NMR) -based metabolomics approach to analyze the metabonome of human serum from 33 thyroid cancer (TC) patients including 20 Papillary thyroid cancer (PTC) patients and 13 Medullary thyroid cancer (MTC) patients, 17 nodular goiter (NG) patients and 14 healthy controls (HC) from the Peking Union Medical College Hospital (PUMCH) between 2013 August and 2014 December. The unique feature of this study was the combination of metabolomics and classification tree analysis in order to improve the discrimination with the least error. Results: Compared with NG group and HC group, TC group has increased glucose, creatine, betaine, sucrose, L-serine, L-valine, phenylalanine and methionine levels and decreased concentration of fructose, tyrosine, aspartate, L-lactic acid, glycine, glutamic acid, guanosine, β-alanine, histidine, L-glutamine, L-alanine and the receiver operating curve (ROC) shows good sensitivity and specificity in classifying cases. Betaine, D-glucose, L-alaine and Glucose stand out with the area under roc curve (AUC) greater than 0.850. Among those four chemicals, D-glucose shows an excellent performance in sensitivity and specificity (both 0.9) and the AUC is 0.888. Further analysis shows relative high serum Lactate, L-proline and sucrose levels and lower levels of aspartate, betaine, D-glucose, L-alanine and maltose in PTC group when compared to MTC group. Conclusion: Our study illustrates that the NMR-based metabolomics approach was shown to investigate the possible serum biomarkers for thyroid cancers. We are aware that metabolomics profile of serum sample extract may be altered and released from the original cancer cells. Serum can also be used to construct a predictive model with high sensitivity and specificity for thyroid cancer as less invasive but more acceptable sample type.

A-020

Development and Validation of ColoScape - A New Colorectal Cancer Mutation Detection Assay

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Background: Colorectal cancer is a highly preventable disease. Herein we report the development and validation of ColoScape, a novel multigene mutation biomarker real-time PCR based assay for qualitative detection of colorectal cancer associated biomarkers in the following genes: *APC (Exon 15), KRAS (Exon 2), BRAF (Exon 15) and CTNNB1 (Exon 3)*. **Methods:** The high sensitivity of ColoScape is achieved by xenonucleic acid (XNA) probe technology. XNA probes are backbone modified oligomers with natural nucleoside bases (A,T,C and G) that hybridize by Watson-Crick base pairing to natural DNA and RNA with higher binding affinity. XNA probes bind to the selected wild-type sequences at the respective genetic loci in the target genes. For selected mutation sites, primers and FAM-labeled TaqMan probes were designed and tested with the selected XNA oligomers. An internal PCR control selected in the Human b-Actin (ACTB) gene was employed utilizing a HEX-labeled TaqMan probe. Performance parameters were established on colorectal cancer patients DNA extracted from FFPE and reference DNA materials. **Results:** At least 0.5% mutation in wild-type background can be detected by Coloscape for target mutations in APC (exon 15), CTNNB1(exon 3), KRAS (exon 2) and BRAF V600 (exon15) with APC c1450 and CTNNB1 assays showing sensitivity of < 0.1% mutation in 5-10 ng of WT DNA/well. No cross reactivity was observed with wild-type up to 320ng purified gDNA and up to 20ng FFPE DNA per reaction demonstrating high specificity of the ColoScape. Intra-assay, inter-assay, lot-to-lot and operator variation comparison showed CV% between 3% and 8%. Excluding pre-cancer samples, the assay clinical specificity and sensitivity were 95% and 100%, respectively. Pre-cancer detection sensitivity was 60% (6 out of 10 FFPE samples) and 62.5% for stool samples. For tested FFPE clinical samples, the assay specificity and sensitivity were 95% and 91% respectively while the assay clinical specificity and sensitivity were both 100% for plasma samples. **Conclusions:** The ColoScape Colorectal Cancer Mutation Detection assay is a sensitive tool intended to facilitate research in colon cancer development, early detection, disease monitoring and therapeutic interventions.

A-021

EGFR mutation detected in cfDNA from cerebrospinal fluid permit the diagnosis of leptomeningeal metastases in a patient with lung adenocarcinoma

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Background: EGFR activation mutations predict susceptibility to tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC). Although patients render

positive response to TKIs initially, resistance could eventually develop due to the secondary mutation T790M. Multiple resistant subclones can arise following treatment of NSCLC in patients with EGFR-targeted therapies. Therefore, the degree of heterogeneous distribution of these mutations regarding the metastasis localization may be of importance in order to a better tailored treatment. Leptomeningeal metastasis is an uncommon complication in patients with solid tumors and appears to be more frequent in lung cancer patients harboring EGFR mutations. Liquid biopsies using plasma-derived cell free DNA (cfDNA) are non-invasive tests that allow a better selection and monitoring of the patients with NSCLC. cfDNA can be found in serum, plasma or urine. However, there exists little evidence of cfDNA detection in other body fluids, where the methodology is not optimized yet. **Case report:** A 63-year-old man was admitted to the hospital for an episode of oppressive frontal headache that lasted ten days, not responding to treatment. 15 months before, he had been diagnosed with stage IV pulmonary adenocarcinoma by tissue biopsy, showing EGFR mutated with deletion in exon 19. Since the beginning of this diagnosis afatinib was administered. A new tissue biopsy assay, performed two months before the headache event, showed the additional acquisition of the T790M resistance mutation in EGFR, with a change in the pharmacological therapy to osimertinib. MRI performed on admission showed neither space occupying lesions nor suggestive signs of metastatic. Small lesions with a pattern suggestive of involvement of small vessels were described and a CSF study was requested. CSF biochemical results were: Proteins, 54.10 mg/dL (15.00-45.00 mg/dL); glucose, 57.60 mg/dL (plasma glucose, 73.20 mg/dL); Chlorine, 118.20 mEq/L (120-130 mEq/L). The CSF was transparent in appearance and the cellular recount was 28 cells/mm3. The cells were stained using a rapid panoptic and observed at 1000x magnification. They were of bigger size than leukocytes and showed an atypical appearance, with large and rounded nucleus. They were reported as possible tumor-derived cells, suggesting leptomeningeal carcinomatosis secondary to pulmonary adenocarcinoma. Subsequently, a cell free DNA (cfDNA) test for EGFR mutations by Real Time qPCR was performed on the CSF supernatant. It resulted in the detection of EGFR deletion of exon 19. We did not detect the T790M resistance mutation in the CSF sample, althought this mutation did was demonstrated in conjunction with the deletion of exon 19 in a subsequent analysis of cfDNA in plasma. **Conclusions:** The microscopic analysis of CSF performed in the emergency laboratory oriented the clinical diagnosis towards leptomeningeal dissemination of adenocarcinoma cells. The cfDNA study of EGFR was adapted for the detection of EGFR mutated in CSF, where a positive result confirmed the malignant origin of the cells. The presence of EGFR exon 19 deletion and the absence of the T790M resistance mutation in CSF, show the existence of tumor heterogeneity, revealing in this patient possible differences in the metastatic spread or a differential site-dependent pharmacological response of both clones of cells.

A-022

ASSOCIATION BETWEEN HE4 AND COLORECTAL CANCER

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Background: Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. Serum levels of HE4 have been investigated in patients with ovarian and endometrial cancer but only a few have described the role of HE4 in colorectal cancer. **Methods:** One hundred and twelve healthy individuals (47 men and 65 women) were recruited. Eighty seven patients with a diagnosis of colorectal cancer were selected (50 men and 37 women). No other pathology was found in this group. Ca19.9 and CEA were performed using ADVIA Centaur XP System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®). Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney. Student's t-test was made for Age comparison and Chi-squared for gender comparison. **Results:** HE4 median in case group was 85.67 (47.23) pmol/L. HE4 median in control group was 52.19 (19.51) pmol/L. Ca19,9 median in case group was 18.00 (73.50) U/ml and 9.23(11.00) U/mL in control group. CEA median in case group was 2.80(7.00) ng/mL and 0.76(0.93) ng/mL in control group. We found a statistically significant differences in HE4 adjusting by age ($p<0.001$), Ca19.9 and CEA showed an adjusted by age significant of $p=0.012$ and $p=0.005$ respectively. HE4 sensitivity and specificity was calculated with a result of 82.8% and 82.3% respectively with a cutoff point of 63.30 pmol/L. **Conclusion:** The present study suggests a positive association between HE4 levels and colorectal cancer which is stronger than Ca19.9 and CEA. Further studies are needed with a longitudinal design to investigate the value of HE4 as a biomarker in this pathology.

A-023

Role of β human chorionic gonadotropin, α-fetoprotein and lactate dehydrogenase for testicular germ cell tumors in a sample in the city of Sao Paulo.

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Background: Testicular tumor is the most common cancer in men and can occur at any age. Among the most common types of germ cell tumors (GCT) we have: Seminomatous Tumor that is a type of testicular cancer originating in the germinal epithelium of the seminiferous tubules. The Nonseminomatous, where we find the Embryonic carcinomas that are highly malignant, with rapid growth and with early metastases, while the Choriocarcinomas are the rarest and most aggressive. Finally, we have the Teratomas that are formed by one or more tissues embryonic. Most non-seminomatous tumors are mixed with at least two types. The objective of this study was to study the behavior of the tumor markers of GCT before the pathological anatomy tests of testicular biopsy.METHODS-This was a retrospective, observational study in which 4943 β-HCG positive for the period from 2015 to 2017 were analyzed in male patients from a laboratory in Sao Paulo. Of these, we performed 328 positive b-HCG tests with AFP and DLH for GCT. We have focused on 30 tests with testicular biopsies performed together with the serum tumor markers in the same laboratory. For the dosages of β-HCG and AFP, we use the equipment ARCHITECT i2000SR Abbot® and UNICEL DXI Beckman&Counter . DHL through the equipment AU 5842 Beckman&Counter. Pathological anatomy is analyzed by optical microscopy. Immunohistochemistry through the Autostainer Link 48 Agilent and optical microscopy. RESULTS- The findings of the 30 biopsies and β-HCG positive for testicular cancer were shown in Table 1. CONCLUSION-Serum tumor markers studied for GCT showed great relevance for identification of tumor origins, being essential for stratification of testicular neoplasm, including the classic treatment of orchiectomy (removal of the testis).The classic seminoma, a germ cell tumor with higher incidence, showed a significant alteration in 50% of the cases of β-HCG dosages and there was no cases of AFP changes.

Table 1: Differences of testicular cancer and the relation regarding the alteration of tumor markers β-HCG, AFP and DHLin n=30 cases:

		βHCG+	DHL+	AFP+
N=30	n	%	%	%
Embryonic Carcinoma	8	100	100	71.4
Choriocarcinoma	1	100	/	100
Teratoma	1	100	100	100
Non-Seminomatous	1	100	100	100
Classic seminoma	10	100	77.7	0
Mixed GCT	9	100	80	87.5

A-024

Expression of Tripartite motif family protein72 (TRIM72) in tumor tissue and serum of patients with colon cancer and its clinical significance

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Background: Colon cancer is one of the most common malignancies worldwide, which causes a major population of cancer-related deaths in the world. The tripartite motif family protein 72 (TRIM72), also known as MG53, is involved in the insulin resistance and metabolic syndrome which are the risk factor of colon cancer. However, the expression of TRIM72 in the colon cancer tissues and its serum level in colon cancer patients still remain unknown. **Methods:** We investigated the expression patterns of TRIM72 in colon cancer tissues and normal tissues by immunohistochemical staining. The serum level of TRIM72 was also measured using ELISA kit. The receiver operating characteristic curve (ROC) curves were applied to evaluate the diagnostic value of TRIM72 level for colon cancer patients. **Results:** The results showed that the expression level of TRIM72 in colon cancer tissues and its serum level in colon cancer patients were significantly decreased compared with normal controls. Additionally, low level of TRIM72 expression was associated with advanced clinical stage, the lymph node and distant metastasis in colon cancer patients. Moreover, the ROC analytic results showed that TRIM72 has a better diagnostic value (AUC = 0.829) than the traditional tumor biomarker Carcinoembryonic Antigen (CEA) (AUC = 0.707) or Carbohydrate Antigen 19-9 (CA199) (AUC = 0.750); and the combination of TRIM72 with CEA and CA199 showed the best diagnostic value for colon cancer (AUC = 0.928).

Conclusion: These results suggested that TRIM72 may have the potential to be a new biomarker for the diagnosis of colon cancer.

A-025

Plasma D-dimer level improves prediction of distant organ metastasis in colorectal cancer

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide, and its mortality rate continues to increase yearly in less developed regions. Distant organ metastasis is mainly responsible for the high rate of death associated with CRC. Currently, the diagnosis of distant organ metastasis of CRC mainly relies on pathological examination, imaging techniques and serum tumor biomarkers, but each has its own limitations. D-dimer is a degradation product of cross-linked fibrin, and plasma D-dimer levels have been demonstrated to be correlated with disease stage and prognosis in CRC patients. However, few studies have focused on the predictive value of D-dimer for distant organ metastasis in patients with CRC. **Objective:** The aim of the study was to evaluate the diagnostic performance of D-dimer level in predicting distant organ metastasis in CRC. The results were then compared with those of serum serum carcinoembryonic antige (CEA) levels, to determine whether D-dimer could improve the predictive value of CEA for distant organ metastasis. **Methods:** 106 CRC patients with distant organ metastasis and 86 ones without distant organ metastasis were included in the study. All patients were histologically diagnosed either by endoscopic or surgical specimen examination. The identification of metastatic lesion(s) was based on the results of imaging examination, image-guided biopsy or exploratory laparotomy. Plasma D-dimer and CEA values were measured in these CRC patients. The diagnostic performance of D-dimer levels in predicting distant organ metastasis was examined by receiver operator characteristic (ROC) curves, and then was compared with the performance of CEA. **Results:** The median of D-dimer values in patients with distant organ metastasis was higher than the level for patients without distant organ metastasis (0.72 mg/L FEU versus 0.27 mg/L FEU, p<0.0001). The result was not affected by patient age, smoking history or previous treatment. D-dimer levels significantly correlated with CEA levels in patients with CRC, but D-dimer had a larger area under ROC curves (0.80) compared to CEA (0.75). Combined with D-dimer assay, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the CEA assay for predicting distant organ metastasis in CRC can be increased to 76.4%, 80.2%, 82.7%, 73.4% and 78.1%, respectively. **Conclusion:** Plasma D-dimer values can improve prediction for distant organ metastasis in CRC. Both D-dimer level and CEA level elevation are clinical indications for detailed imaging or pathological examination for distant organ metastasis in at risk patients.

A-026

Serum carboxylated osteocalcin test as a surrogate marker of bone metastasis in non-small cell lung cancer patients: impact of radiation/chemotherapy on confirmed bone metastasis

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Background: Early detection and active monitoring of bone metastasis (BMs) is important in lung cancer patients. In this retrospective study, we investigated the feasibility of using carboxylated osteocalcin (Gla-OC) as a surrogate marker of BMs and the impact of radiation/chemotherapy on the Gla-OC levels in patients with advanced lung cancer and confirmed BMs. **Methods:** Totally 283 patients with non-small cell lung cancer (NSCLC) were included in this study. Serum levels of Gla-OC was measured and compared between patients with BMs and those without, and patients with/without radiation/chemotherapy on confirmed BMs and lung cancer. Statistical analysis was performed using the SPSS software package (SPSS, Munich, Germany). **Results:** Among 283 advanced lung cancer patients with average age (59.1) including 170 male (60.1%) and 113 female (39.9%), Gla-OC in the 99 BMs patients was higher [Median (P25, P75):10.14 (7.02, 16.98) vs.9.75 (7.39, 12.43), P<0.01] than the group of 184 patients without BMs. In a subgroup of patients with lung squamous cell carcinoma (LSCC) without exposure to radiation/chemotherapy, Gla-OC was higher in the BMs group [13.40 (7.74,18.80) vs. 9.52 (6.55,11.76), P<0.05] than control group. Moreover, Gla-OC was lower [7.00 (6.07,11.26), n=9, P<0.05] in BMs LSCC patients exposed to radiation/chemotherapy with a poor detection sensitivity at 1.11%. In patients with lung adenocarcinoma (LAC), Gla-OC was higher in BMs group [12.13 (7.77,17.60) vs. 9.75(7.35,12.15), P<0.01] than the control group. Additionally, Gla-OC was lower [7.46(5.83,11.57), n=27, P<0.001] in BMs LCA patients exposed to radiation/chemotherapy. **Conclusion:** Carboxylated osteocalcin may be a viable surrogate marker of BMs in NSCLC patients who are not exposed to radiation/chemotherapy. Although Gla-OC in patients with radiation/chemotherapy exhibits poor sensitivity for BMs, it might be an indication of effective radiation/chemotherapy. More prospective studies are warranted to determine the Gla-OC in the evaluation of BMs and the impact of radiation/chemotherapy.

	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
All NSCLC	33	20	164	66	33.3%	89.1%	62.3%	71.3%	69.6%
Lung squamous cell carcinoma									
without radiation/chemotherapy*	10	3	20	10	50.0%	87.0%	76.9%	66.7%	69.8%
with radiation/chemotherapy**	1	2	16	8	1.1%	88.9%	33.3%	66.7%	63.0%
Lung adnocarcinoma									
without radiation/chemotherapy*	17	10	96	26	39.5%	90.1%	63.0%	78.7%	75.8%
with radiation/chemotherapy***	5	5	32	22	18.5%	86.5%	50.0%	59.3%	57.8%
*p<0.05 between BMs and control group; **P<0.01 between groups of BMs patients with and without radiation/chemotherapy; *** P<0.001 between groups of BMs patients with and without radiation/chemotherapy;									

A-027

TP53 mutations correlate immunohistochemical staining pattern of p53 and codon 72 polymorphism in mature T-cell and NK-cell lymphomas

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Background: : Mature T-cell and NK-cell lymphomas consist of a heterogeneous group of neoplasms with cytogenetic and molecular diversities. *TP53* mutation is known to be involved in the event of tumorigenesis and present in a variety of cancer subtypes. However, *TP53* mutation in T-cell and NK-cell lymphomas has less been reported. The aim of the study was to identify *TP53* mutation in different entities of mature T-cell and NK-cell lymphomas and further correlate with p53 expression. **Methods :** Fifty-seven cases of T-cell and NK-cell lymphomas obtained between 2006 and 2017 from the archives of the Department of Pathology, Chang Gung Memorial Hospital at Kaohsiung, Taiwan were collected for next-generation sequencing (NGS) and p53 immunohistochemical study. **Results:** By NGS, all samples showed *TP53* mutation with diverse mutation patterns and sites. All cases (100%) had missense variant of *TP53* mutation followed by stop gained variant (86.0%). Ten cases had mutation burden > 5% VAF, predominantly in extranodal nasal-type natural killer/T-cell lymphoma (NKTCL, 21.7%) and intestinal T-Cell Lymphoma (ITCL, 60%). No angioimmunoblastic T-cell lymphoma patients had *TP53* mutation with high mutation burden. Overexpression of p53 was observed in 11 (19.6%) of 56 tumors with variable extent. The percentage of tumor cells with strong p53 staining was positively correlated with *TP53* mutation VAF (R^2 = 0.95, P < 0.001). Furthermore, six (37.5%) of 16 cases with 72P homozygous genotype showed higher frequency of *TP53* mutation VAF >

5% as compared with 72R homozygotes (2/16, 12.5%) and heterozygotes (2/25, 8%) ($P = 0.04$). **Conclusions :** We demonstrated frequencies of *TP53* mutations in mature T-cell and NK-cell lymphomas with a higher mutation VAF identified in NKTCL and ITCL, and the p53 expression was positively correlated with *TP53* mutation VAF.

A-028

Methylation of NFBP1 as a novel marker for the detection ofplasma cell-free DNAinbreast cancerpatients

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Background: Circulating cell-free DNA (cfDNA) has been considered as a potential biomarker for non-invasive cancer detection, which is worthy of further validation and potentially benefits a broad range of applications in clinical oncology practice. Human Neuroblastoma Break Point Family, member 1 (NBPF1) are originally identified in a neuroblastoma (NB) patient .The present study was the first to determine the presence of NBPF1-methylated circulating cfDNA in plasma among breast cancer,benign breast disease and healthy control . Methods: Specific gene screening using bisulfite sequencing (BS-seq) among 25 consecutive breast cancer patients without treatment before surgery (stage I-III) and 25 benign breast diseases and 25 healthy female volunteers collected at Peking Union Medical College Hospital from 2016 to 2017. After that the three groups of 10 plasma samples were mixed to verify the methylation status of five sites for candidate gene NBPF1 in circulating cell-free DNA and further two sites of promoter for NBPF1 were detected in 50 breast cancer patients, 33 benign breast diseases and 30 healthy control by using methylation-specific PCR (MSP). Results: Breast cancer patients whose NBPF1 methylation levels are significantly higher than those of benign breast diseases and healthy controls at the same time are chosen as candidate gene. MSP result shows five sites for NBPF1 differ among the three groups in mixed samples. Methylation rate of two sites of the NBPF1 promoter were 63.1% and 66.7% of breast cancer patients (stage I-III) and 57.1% and 50.0% of benign breast disease, and 48.0% and 41.0% of healthy control respectively . One site methylation rate was significantly different among the three groups ($p<0.05$). Conclusions: These results indicate that NBPF1 promoter hypermethylation, which occurs in a significant proportion of breast tumors, and that NBPF1-methylated cfDNA thus may serve as a tumor marker for breast cancer in a large cohort of breast cancer patients. Large samples are still needed to verify the results, we will explore further.

A-029

Next generation sequencing identifies additional actionable markers of primary colon and lung adenocarcinomas in a south Florida veteran population.

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Background: Molecular profile testing of tumor tissues is a growing and vital need in the treatment of patients with colon cancer and non-small cell lung cancer. According to the National Comprehensive Cancer Network (NCCN) guidelines, routine molecular testing is recommended to identify rare driver mutations that may be present in tumor tissues. Proven responses to molecular therapies have greatly increased the improvement of care in colon and lung cancer patients. Currently, NCCN guidelines for colon adenocarcinomas suggest testing for tumor mismatch repair or microsatellite instability status and determination of tumor gene status for RAS (KRAS and NRAS) and BRAF. The current NCCN guidelines for adenocarcinomas in non-small cell lung carcinoma include testing for EGFR, ALK, ROS1, BRAF and PD-L1 expression. Present test methods for both sets of guidelines include FISH, PCR based sequencing, and immunohistochemistry. The objective of the study was to identify additional actionable markers from colon and lung adenocarcinomas, through next generation sequencing, that would not be detected based on current test methodology. **Method:** Eighteen cancer cases were reviewed and selected from male adenocarcinoma patients for next generation sequence testing. Samples were sent to Personal Genome Diagnostics (Baltimore, MD) for Cancer Select 125 testing. Next generation sequence testing was performed by analyzing the coding regions of 125 genes and identifying tumor-specific alterations in categories including: microsatellite instability, sequence mutations, amplifications, and rearrangements. The annotated reports included detailed analysis of mutations detected, FDA-approved therapies (for same and other indications) and current clinical trials. Actionable markers were identified and categorized based on sequence mutations, amplifications and rearrangements. **Results:** Actionable markers detected by next generation sequencing were divided based on colon versus lung primary tumors. Results were accumulated over a six-month testing period. Current molecular markers tested, according to NCCN guidelines, were removed from the data set leaving only the additional actionable markers. Microsatellite instability markers, KRAS, BRAF, and NRAS mutations were removed from the colon adenocarcinoma category. EGFR mutations, BRAF mutations, ALK and ROS1 rearrangements were removed from the lung adenocarcinoma category. The colon primary tumor category (N=14 patients) yielded 12 additional actionable sequence mutations, 9 amplifications and 5 microsatellite markers. The lung primary tumor category (N = 4 patients) yielded 9 additional sequence mutations, 5 amplifications and 1 rearrangement. **Conclusions:** Next generation sequencing of primary colon and lung adenocarcinomas provides accurate and comprehensive data detailing actionable gene alterations within a tumor sample. Tumor molecular alterations are listed in categories such as: consequence of sequence mutation, exon location, mutant fractions, and fold increases for amplifications. Gene alterations are further designated by approved current FDA approved therapy and current clinical trials. Our study identified several additional actionable markers within each sample that would not have been discovered utilizing our current test methods. Based on our findings, we conclude that next generation sequencing is an accurate and powerful test method that should be performed whenever adequate tumor tissue is available. This material is the result of work supported with resources and the use of facilities at the James A. Haley VA Hospital.

A-030

Performance Evaluation of the New Latex FLC kappa and lambda Assays on the Atellica® CH930 Analyzer

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Background: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free light chain (FLC) determination as aid in diagnosis and management of clonal plasma cell disorders. We describe reproducibility, imprecision, method comparison, and limit of quantitation (LoQ) data for the application* of N Latex FLC kappa and lambda assays on the new Atellica® CH 930 Analyzer. **Methods:** Latex-enhanced mouse monoclonal antibody reagents from Siemens Healthineers for FLC kappa (N Latex FLC kappa) and lambda (N Latex FLC lambda) were assayed on the Atellica® CH 930 Analyzer. A precision study was conducted according to CLSI guideline EP05-A3 to estimate repeatability of four sample pools and two controls each for FLC kappa and lambda, in combination with one reagent and one calibrator lot. Each sample was assayed in quadruplicate twice a day for five days. A method comparison study was conducted according to CLSI guideline EP09-A3. The results were correlated with data generated on the BN ProSpec® System. The results were analyzed using Passing-Bablok linear regression analysis. LoQ was determined for both applications with one reagent lot following CLSI guideline EP17-A2. **Results:** The within-run CV for the new FLC kappa application on the Atellica® CH 930 Analyzer ranged from 0.80−2.09% and the total (within-instrument) CV from 1.50−3.78%. For FLC lambda, the within-run CV was 0.81-2.18%, and the total (within-instrument) CV was 1.76-4.75%. Passing-Bablok regression results between the BN ProSpec System and Atellica® CH 930 Analyzer were y = 1.008 x -0.064 mg/L (r = 0.986) for FLC kappa (n = 175) and y = 1.033 x -0.695 mg/L (r = 0.973) for FLC lambda (n = 168). LoQ was determined to be 1.084 mg/L for FLC kappa, showing a total error of 28.01%. LoQ for FLC lambda was 1.558 mg/L, with a total error of 15.76%. **Conclusion:** The new application of N Latex FLC assays on the Atellica® CH 930 Analyzer demonstrated acceptable and consistent imprecision. Method comparison results showed good agreement with an on-market assay. *Under development. Not available for sale.

A-031

Performance evaluation of serum PIVKA-II measurement using HISCL-5000 and method comparison of HISCL-5000, LUMIPULSE G1200 and ARCHITECT i2000

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Backgroud: Protein induced by vitamin K antagonist-II (PIVKA-II) is a useful tumor marker in addition to alpha-fetoprotein for the diagnosis of hepatocellular carcinoma (HCC). In this study, we evaluated the analytical performance of serum PIVKA-II measurement using HISCL-5000 analyzer (Sysmex Corporation, Kobe, Japan), and investigated its clinical usefulness in patients with HCC.

Methods: A total of 502 subjects (347 male and 155 female, median age 59.0 years) were enrolled. Among them, 335 were HCC patients, 45 were patients with non-HCC liver disease including liver cirrhosis, chronic hepatitis, HBV or HCV carrier, hepatic adenoma and intrahepatic cholangiocarcinoma, and 122 were healthy individuals. We evaluated the precision and linearity of PIVKA-II assays by HISCL-5000 analyzer. Method comparison was done among HISCL-5000, LUMIPULSE G1200 (Fujirebio Diagnostics, Fujirebio, Japan) and ARCHITECT i2000 (Abbott Diagnostics, Abbott, USA) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). **Results:** Repeatability (%CV) in low, high level controls and pooled serum was 2.81%, 3.17% and 10.30%, respectively. Within-laboratory precision was 4.33%, 4.24% and 8.86%, respectively. In linearity test, the coefficient of determination (R^2) was 0.9998, ranging from 14 to 54,301 mAU/mL. In comparison, the coefficient of comparison (r) was 0.9644 (between HISCL-5000 and LUMIPULSE G1200), 0.9633 (between HISCL-5000 and ARCHITECT i2000), and 0.9561 (between LUMIPULSE G1200 and ARCHITECT i2000), respectively. Agreements was 93.4%, 97.6% and 94.6%, and the kappa values was 0.855, 0.945 and 0.882 between HISCL-5000 and LUMIPULSE G1200, between HISCL-5000 and ARCHITECT i2000, and between LUMIPULSE G1200 and ARCHITECT i2000, respectively. The cut-off level of PIVKA-II was 40 1 mAU/mL and 98.4% of healthy individuals were below the cut-off value. **Conclusions:** PIVKA-II assay using HISCL-5000 showed acceptable analytical performance including precision, linearity and method comparison. This indicates that HISCL-5000 can be potentially helpful in clinical laboratories. 2

A-032

Low-end Precision Profile for a New PSAII Assay* on the ADVIA Centaur System and Comparison with Commercial PSA Assays *Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

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Background: Total PSA assays are used to aid in the management (monitoring) of patients with prostate cancer. Among various treatments for prostate cancer is radical prostatectomy (RP). Guidelines define biochemical relapse after RP using a PSA threshold of 0.2 ng/mL (Adjuvant and Salvage Radiotherapy after Prostatectomy: ASTRO/AUA Guideline, 2013). Recent publications indicate that lower PSA thresholds after RP detect recurrence sooner (Kang, et al. J Urol. 2015, and Sokoll, et al. J Urol. 2016). The purpose of this study was to investigate low-end precision profiles of commercial PSA assays in comparison with a new ADVIA Centaur® PSAII Assay.* **Methods:** Sample pools were prepared by Siemens Healthineers targeted at 0, 0.005, 0.010, 0.015, 0.025, 0.050, and 0.200 ng/mL of total PSA. Pools were aliquoted and frozen at -70°C. Before testing, samples were thawed at 2–8°C and tested within 2 hours. Pools were run in replicates of five over 5 days on the Roche COBAS Modular Total PSA assay, Beckman ACCESS 2 HYBRITECH and WHO PSA assays, and Abbott ARCHITECT i1000 Total PSA assay at Hamilton Health Sciences Center. For ADVIA Centaur PSAII, pools were run in replicates of five over 5 days internally at Siemens Healthineers. **Results:** Precision was calculated in accordance with CLSI EP15-A3. Results are shown in the table below. Note that not all assays had the same mean for each pool, and differences in observed concentrations affected calculated CVs.

Pool	Abbott ARCHITECT i1000 Total PSA		Roche COBAS Modular Total PSA		ACCESS 2 WHO PSA[a]		ACCESS 2 HYBRITECH PSA[a]		ADVIA Centaur PSAII	
	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV
1	0.001	63.9	0.002	110.3	0.00	NA	0.00	NA	0.000	NA
2	0.004	9.9	0.009	9.0	0.00	NA	0.00	184.5	0.003	31.2
3	0.008	7.5	0.013	7.0	0.01	0.0	0.01	0.0	0.007	17.8
4	0.019	5.3	0.029	3.4	0.02	0.0	0.02	0.0	0.023	5.3
5	0.039	3.6	0.055	3.3	0.02	9.0	0.02	11.6	0.048	4.1
6	0.147	3.0	0.197	1.4	0.17	2.8	0.18	2.8	0.191	2.4

NA: Not applicable. Results are below the lowest amount of signal the assay can detect. a. The ACCESS 2 assays report to two decimal places; thus precision at low concentrations cannot be calculated as accurately as for methods that report to three decimal places. **Conclusion:** The ADVIA Centaur PSAII assay demonstrated similar precision performance at the very low end of the concentration range compared to commercially available total PSA assays from Roche and Abbott.

A-033

Method comparison of CA 125 assay in two analyzers

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Background: Determination of serum CA125 is useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer. CA 125 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. We performed a method comparison study between two different analyzers, Unicell DxI 800 from Beckman Coulter® and Architect isr2000 from Abbott diagnostics®. The aim of this study is to evaluate the clinical concordance between both immunoassays. **Methods:** Measurements were performed in 101 serum samples from real patients. The samples were processed in parallel the same day at both analyzers. Statistical analysis was carried out with the MedCalc software, where the correlation was calculated by the Pearson´s coefficient, the Passing-bablok regression and Bland Altman plots. **Results:** Results from method comparison are resumed in the next data table

Test	Instrument	Study Unit	Component	N	Correlation coefficient Pearson r	Passing-Bablok Slope	Passing-Bablok Intercept	Deviation from linearity
CA-125	**x = CA125 DxI 800 Beckman Coulter y = CA125 isr2000 Abbott diagnostics**	U/mL	Serum	101	**0,9936** CI 95% = 0,9905 - 0,9957	**1,00** CI 95% = 0,96 - 1,04 **1 included**	**2,40** CI 95% = 1,74 - 3,47 **0 not included**	**P = 0,70 No significant deviation from linearity**

Results show a high degree of correlation coefficient and adjustment to linearity; however, there exists a constant bias. It would be necessary to check the clinical concordance of the results, checking if this bias could be ignored under our working standard conditions or we need to use a correction factor. Clinical concordance at the diagnostic according to cut-off (35 U/mL) is 99% (100/101). **Conclusion:** Method comparation results show a good correlation between both methods. Due to the high clinical concordance at the diagnostics, the bias we found in the method comparison could be ignored and the interchangeability of methods is possible. However, changes observed in serial CA 125 assay values when monitoring ovarian cancer patients should be evaluated in conjunction with other clinical methods used for monitoring ovarian cancer patients.

A-034

Lot-to-lot variability of the Binding Site Freelite® assays on the Optilite® and SPAPLUS® analysers

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Introduction: In the absence of a commutable international standard for serum free light chains (FLCs), Binding Site has produced a commutable bulk sample (gold standard) for the internal performance assessment of Freelite assays on multiple instruments. Its role is to ensure accurate and precise measurements that can be replicated across multiple instruments. Here we present the performance of the gold standard and inter-lot variation for 3 randomly selected Freelite assay lots released between June 2016 and October 2017 on the Binding Site Optilite and SPAPLUS analysers. Method: The performance of kappa and lambda Freelite assays on the SPAPLUS and Optilite analysers was assessed prospectively during routine batch manufacture by measuring 68 samples from healthy adult donors, 30 unprocessed and 27 processed panel samples (concentration range: 3-180 mg/L and 6-165 mg/L for κ and λ FLCs, respectively). A commutable gold standard reference material was also analysed. Three randomly selected assay lots released between June 2016 and October 2017 were included in the analysis. Results between lots

on the same platform were compared using Analyse-it® (Passing Bablok, linear regression and Altman Bland analyses) and sigma metrics calculated based upon total allowable error (Braga et al. Biochimica Clinica 2013;37:376-82). Results: Analyse-it results are shown in Table 1. Analytical process performance for three Kappa Freelite lots gave sigma values of 5.2, 5.9 and 9.1 on the Optilite and 7.3, 3.0 and 5.1 on the SPAPLUS. For three Lambda lots, sigma values of 7.0, 10.6 and 6.3 were obtained on the Optilite and 23.7, 7.2 and 18.4 on the SPAPLUS.

Table 1: Passing Bablok, linear fit and Altman-Bland results

Platform	Analysis	Kappa free		Lambda free	
		Lot 1 vs Lot 2	Lot 2 vs Lot 3	Lot 1 vs Lot 2	Lot 2 vs Lot 3
Optilite	n	106	105	118	115
	Passing Bablok slope and intercept	0.95 - 0.17	1.01 + 0.92	0.99 - 1.45	0.93 + 0.46
	Linear fit r value	0.996	0.997	0.996	0.998
	Altman Bland bias	-6.9%	7.6%	-9.1%	-4.4%
SPAPLUS	n	113	110	124	122
	Passing Bablok slope and intercept	1.02 + 1.37	1.02 - 1.43	1.01 - 1.11	1.02 + 0.18
	Linear fit r value	0.998	0.994	0.999	0.993
	Altman Bland bias	9.6%	-5.6%	-5.5%	2.2%

Conclusion: Inter-lot agreement for Optilite and SPAPLUS Freelite assays were within acceptable limits. With the exception of one SPAPLUS Kappa lot, all sigma values were >5. In future, the commutable gold standard internal reference material will be included in Freelite assay manufacture.

A-035

Serum free light chain analysis using the Optilite®analyser: a clinical laboratory perspective

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Background: Serum free light chain (sFLC) assessment has become routine laboratory practice, aiding in the clinical management of patients with monoclonal gammopathies. Monoclonal FLCs are not simple analytes to measure, with serum concentrations ranging from <10 mg/L to >10000 mg/L. Very high values may be attributed to FLC aggregation and, as with all immunoassays, high sFLC concentrations may result in underestimation due to antigen excess (AgXS). The structural diversity of monoclonal FLCs can also cause sample non-linearity. Here we determine the frequency of these issues and evaluate Freelite® assay performance on the Optilite in a routine laboratory setting. **Methods:** κ and λ sFLCs were measured in patient serum tested in our laboratory between 10/11/2016 and 22/12/2017 using Freelite assays on the Optilite (The Binding Site Group Ltd., UK). Overall there were 18418 κ sFLC measurements from 15353 samples and 18453 λ sFLC measurements from 15478 samples. We assessed the proportion of FLC values falling within the following ranges: ≤100 mg/L, >100-1000 mg/L, >1000-10000 mg/L, >10000 mg/L, and the number of samples requiring re-dilutions. The incidence of AgXS was determined by establishing the frequency of 'high activity' flagged samples and comparing the result to the value obtained at the next dilution; a higher result confirmed AgXS. Any observed cases of AgXS which were undetected by the analyser were noted. Non-linearity was evaluated for samples which gave a > value at a given dilution and a lower measurement at the next higher dilution; notable non-linearity was determined if the value was >30% lower. **Results:** Median κ and λ FLC concentrations were 27.94 mg/L (range 0.68-33649.00) and 19.25 mg/L (range 1.47-50638.00), respectively. Only 0.12% of κ and 0.08% of λ Freelite values were >10000 mg/L. Freelite values >30000 mg/L were extremely rare (5/15353 (0.03%) for κ and 13/15478 (0.08%) for λ). Overall, Freelite gave a final result in 83.12% (12761/15353) of κ and 82.63% (12790/15478) of λ samples at the standard dilution (1/10 measuring range (MR): 2.9 - 127 mg/L for κ; and 1/8 MR: 5.2 - 139 mg/L for λ). The next higher dilution provided final results for 13.33% of κ (1/100 MR: 29-1270 mg/L), and 8.70% of λ assays (1/80 MR: 52 - 1390 mg/L). Overall 267/36871 (0.72%) Freelite measurements (180/18418 [1%] κ and 87/18453 [0.5%] λ) were non-linear. Of these, the median percentage difference between the results at a given and next higher dilution was 10.57% (range 0.02%- 50.67%). Notable non-linear results were observed for only 0.06% of κ and 0.07% of λ Freelite measurements. In total, 11.16% of κ and 5.5% of λ measurements were flagged by the Optilite for re-dilution due to potential AgXS; which was confirmed in 94.7% of κ and 98.9% of λ cases. We only observed one case of undetected AgXS (1/30831; 0.003%) during the 1-year study period. **Conclusion:** Very high FLC values are rare and >80% of samples provide a final result for κ and λ Freelite values using standard Optilite dilutions. Notable non-linear FLC results are infrequent (<0.1%) and the Optilite provides robust Freelite AgXS detection.

A-036

Performance evaluation of cancer panels on the Alinity i system

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Background: Abbott Laboratories offers a wide variety of assays to test for many types of cancers including, breast, colon, gastrointestinal, liver, ovarian, pancreatic, testicular and prostate cancer. The Abbott oncology solution can help reduce laboratory operational costs while maintaining the quality standards necessary to have a meaningful positive impact to the quality and cost of health care. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 200 tests per hour The Alinity ci system has an increased reagent load capacity, holding up to 47 IA reagents, onboard QC, clot and bubble detection ability, and a dedicated pretreatment lane to provide consistent and reliable results. **Objective:** To demonstrate the analytical performance of representative assays of the Cancer Panel of the Alinity i system which consists of assays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human serum or plasma. **Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met. **Results:** The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Cancer Panel are shown in the table below.

Assay	Total %CV	LOQ	Method Comparison to ARCH. (Slope/r)	Measuring Interval
Total Prostate Specific Antigen (PSA)	≤ 6.2	0.025 ng/ml	0.99/1.00	0.025 – 100.000 ng/ml
Free Prostate Specific Antigen (PSA)	≤ 7.9	0.021 ng/ml	1.06/1.00	0.021 – 30.000 ng/ml
CYFRA 21-1 (Cytokeratin 19)	≤ 5.2	0.15 ng/ml	0.95/1.00	0.50 – 100.00 ng/ml
PIVKA (Protein induced by Vitamin K absence)	≤ 3.2	7.60 mAU/mL	1.00/1.00	7.60 – 30000.00 mAU/mL
PROGRP (Pro-gastrin-releasing peptide)	≤ 4.7	0.93 pg/mL	0.99/1.00	0.93 – 5000.00 pg/mL

Conclusion: Representative immunoassays utilizing CMIA technology on the Alinity i system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT (ARCH) immunoassays.

A-037

Performance of CA72-4 Assay on Fully-automated Chemiluminescent Immunoassay Analyzer* (*In-development)

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Background: CA72-4 assay is widely used for monitoring of Gastric cancer and Ovarian cancer. CA72-4 antigen is a mucin-like, tumor-associated glycoprotein, also called as TAG72. The antigen is defined by 2 antibodies(CC49 and B72.3) which recognize its glycochain epitopes; Galβ(1-3) sialyl Tn and sialyl Tn antigens. The level in blood is elevated in some cancer patients(Ovarian cancer, Gastric cancer, Colon cancer etc.). **Objective:** To evaluate the quantitative analytical performance of newly developed CA72-4 assay (prototype ARCHITECT CA72-4 as-

say) on the fully-automated chemiluminescent immunoassay analyzer. **Material and Methods**: The measuring intervals of prototype ARCHITECT CA72-4 assay cover between 1.0 U/mL to 300 U/mL. Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison with on-market of Roche Cobas CA72-4 were assessed per CLSI protocols. **Results:** Total imprecision, LoQ, and linearity results are shown for ARCHITECT CA72-4 in the table below. Results versus on-market product demonstrated a slope 0.97 and r = 0.96.

Evaluation Item	Result
Total %CV	2 - 5 %CV
LoQ	0.6 U/mL
Linearity	1.0 – 300U/mL
Hook effect	Hook effect is not observed (to 20,000U/mL)
Cross reactivity	Cross reactivity is not observed. (AFP 2090ng/mL, bHCG 550mIU/mL, CA125 3.85KU/mL, CA15-3 836U/mL, CA19-9 4070U/mL, CEA 550ng/mL, CYFRA21-1 110ng/mL)
Interference	Interference is not observed. (Bilirubin, Hemoglobin, Protein, Triglyceride, Biotin)
Available tube type	Serum, Plasma (Serum plain, SST, EDTA-K3, EDTA-K2, EDTA-Na2, Li-Heparin and Na-Heparin)

Conclusion: The ARCHITECT CA72-4 assay demonstrated good precision, LoQ, Linearity, on-board stability, hook effect and Method comparison with on-market comparator assay.

A-038

Electrophoretic and nephelometric methods for monitoring monoclonal free light chains

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Background: Measuring monoclonal free light chains (FLCs) for monitoring response in multiple myeloma relies on urine protein electrophoresis (UPE) and urine immunofixation (uIFE) methods, and on serum FLC (sFLC) assessment using nephelometric/turbidimetric approaches. By contrast, serum protein electrophoresis (SPE) and immunofixation (sIFE) are of limited utility because sFLC levels are often below the sensitivity of these techniques. The highest concentrations of monoclonal FLCs in serum are typically found in light chain multiple myeloma (LCMM) patients. We have assessed the sensitivity of SPE/sIFE for identifying disease and for monitoring monoclonal serum FLC levels in a population of newly diagnosed LCMM patients enrolled onto the IFM-2009 trial, and compared the results to those by UPE/uIFE and by sFLC nephelometry. **Methods:** We included 101 patients with matched data for all five techniques (SPE, sIFE, UPE, uIFE and sFLC) at diagnosis. SPE, sIFE, UPE and uIFE were performed using standard laboratory procedures (Sebia, France). sFLC concentrations were measured nephelometrically using κ and λ sFLC Freelite® assays (The Binding Site Group Ltd, UK). sFLC measurements were considered abnormal if they were outside the manufacturer's reference ranges: κ sFLC (3.3-19.4 mg/L), λ sFLC (5.7-26.3 mg/L), κ/λ sFLC ratio (0.26-1.65). Minimal residual disease (MRD) was assessed by 7-colour flow cytometry after consolidation therapy (bortezomib, lenalidomide and dexamethasone). **Results:** M-protein was identified in 101(100%) screening samples either by an abnormal κ/λ sFLC ratio or positive uIFE, but only in 83(82%) patients by sIFE. UPE and SPE were positive in 79(78%) and 29(29%) patients, respectively. Median levels of involved FLC (3100 vs. 1525 mg/L; p=0.02), Bence Jones protein (1.95 vs. 0.73 g/24h; p=0.01) and total urine protein (2.89 vs. 0.51 g/24h; p<0.01) were significantly higher in SPE positive vs. negative patients, respectively. All patients had measurable sFLC levels (involved FLC >100 mg/L) at diagnosis, meeting current criteria for monitoring haematologic response, and 63% patients had measurable UPE (>200 mg/24h). By contrast, no patients had measurable disease (>10g/L) by SPE. In 77 patients with matched data at the end of consolidation, the κ/λ sFLC ratio remained abnormal in 41(53%) patients; and 15(20%) continued displaying elevated involved FLC levels. uIFE and UPE were positive in only 8(10%) and 1(1%) patients, respectively, whereas 7(9%) and 1(1%) had positive sIFE and SPE, respectively. The positive SPE and UPE result was from 2 different patients; both were lambda patients and had involved FLC >100 mg/L. In a subset analysis of 59 patients assessed by flow cytometry, 16(27%) were MRD-positive, consistent with the presence of malignant bone marrow plasma cells; 15/16(94%) had an abnormal κ/λ sFLC ratio and 7/16(44%) had elevated involved FLC. By contrast uIFE and UPE were positive in 3/16(18%) and 0/16(0%) patients, and sIFE and SPE in 2/16(12%) and 1/16(6%), respectively. **Conclusion:** FLC measurements using serum Freelite and urine immunofixation demonstrate good diagnostic sensitivity for identifying disease in LCMM; however sFLC assessment shows superior sensitivity for monitoring and better agreement with bone marrow assessment. Serum electrophoresis lacks sensitivity both for screening and monitoring and is therefore an unreliable method for monoclonal FLC assessment.

A-039

Heavy/Light Chain Assay In The Monitoring Of Multiple Myeloma.

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Introduction:
Serum protein (SPE) and immunofixation electrophoresis (IFE) have been extensively validated for the routine use of identifying, characterizing and quantifying monoclonal proteins. However, in particular, accurate quantitation of IgA monoclonal proteins can be difficult when they migrate in to the β fraction, due to co-migration with transferrin and complement components. The heavy/light chain immunoassay (HLC) is an additional tool for measuring intact immunoglobulin monoclonal proteins. Therefore, we aimed to examine the clinical utility of the HLC assay for the monitoring of IgG and IgA multiple myeloma (MM) patients.
Methods:
A total of 177 samples from 30 MM patients (21 IgG and 9 IgA) were analysed retrospectively with median number of 6 follow up samples per patient (range 3 – 13). Serum free light chains (sFLC) and heavy/light chains (HLC) were quantified using Freelite® and Hevylite® immunoassays (The Binding Site group Ltd, UK) run on the SPAPLUS turbidimeter (The Binding Site Group Ltd, UK). Details of M-protein concentration, beta globulin levels, total immunoglobulins levels and disease treatment response were obtained from the laboratory and patient information system. Passing-Bablok regression analysis was performed to compare (i) M-protein quantification with involved HLC (iHLC) and (ii) total immunoglobulin with summated HLC pairs for each immunoglobulin type (e.g. IgGκ+IgGλ). Statistical analysis was performed using MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).
Results:
For 127 IgG MM samples, IgG iHLC levels showed a good correlation with SPE quantification (iHLC y=0.96x+4.9; r=0.917) and summated HLC showed a good correlation with total IgG concentration (summated HLC y=0.94x+5.74; r=0.91). In total, 95/127 (75%) IgG MM follow up samples had an abnormal HLC ratio and 122/127 (96%) had a positive SPE, possibly due, in part, to the effects of IgG FcRN receptors recycling IgG and causing persistence of circulating IgG M-protein, even after tumour cell eradication. Consistent with this, one patient assigned a VGPR by IMWG criteria would be assigned a CR based on HLC measurements. For 50 IgA MM samples, 42/50 (84%) had an abnormal HLC ratio. Conversely, 50/50 (100%) of M-proteins showed β fraction migration and were difficult to accurately quantify by SPE. Therefore, M-protein concentration and iHLC did not correlate as well in IgA MM (y=1.9x-8.4; r=0.8) compared to IgG MM. However, there was good correlation between total IgA and summated IgA HLC (IgAκ+IgAλ y=1.35x-0.33; r=0.95). Of the 8/50 (16%) IgA samples with a normal HLC ratio, 6/8 (75%) were consistent with the disease status being in complete remission. Interestingly, in one IgA MM patient, SPE and IFE were negative but the serum FLC ratio and involved FLC were highly abnormal, possibly consistent with the presence of light chain escape.
Conclusion:
Our data suggests HLC measurements could add value to the current monitoring of multiple myeloma patients. In IgG MM patients, the M-protein level correlated well with HLC values. The HLC assay complements the serum FLC assay and is especially useful for monitoring of IgA MM patients who display M-proteins migrating in the β region on SPE.

A-040

Two novel technology approaches for the quantification of cancer miRNA biomarkers in clinical laboratories.

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Background:
MiRNAs have an immense potential to serve as diagnostic, prognostic and prediction biomarkers in the whole field of oncology. Their utilization as circulating biomarkers in non-invasive diagnostics is very promising as well. However, to our knowledge, no miRNA-based diagnostics is routinely used in clinical laboratories. We believe the reason is simple - currently used methods for miRNA determination and quantification are either low in specificity and sensitivity or they are very expensive and high technology demanding. Moreover, these methods are not easy to use, fast and reproducible. Thus, the need for new technologies is emerging.
Methods:
We are introducing new methods for absolute quantification of miRNA suitable for clinical use. The first method is named miREIA® and it is based on immunoassay format, which is very similar to well-known ELISA. It involves hybridization of target miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/miRNA hybrids are then transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. Next, the solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized by a chromogenic substrate. Another approach in miRNA measurement utilizes the enzyme *Chlorella virus* DNA ligase (SplintR® ligase, NEB). This two-step method involves ligation of two adjacent DNA oligonucleotides hybridized to a miRNA target isolated from patient sample, followed by real-time quantitative PCR (RT-qPCR). The use of two PCR detection chemistries (more cost-effective SYBR green or more specific TaqMan probe) is possible.
Results:
Based on these two principles, we have developed assays for quantification of three onco-miRNAs (hsa-miR-23a-3p, hsa-miR-93-5p, hsa-miR-142-5p). Methods showed strong correlation with the TaqMan qPCR assay, where RNA isolated from whole blood and peripheral blood mononuclear cells was used. Both methods also displayed excellent analytical characteristics and high sensitivity. The calibration range of the miREIA® was 0.04-12.5 amol/µl with sensitivity lower than 0.13 amol/µl. The dynamic range of SplintR® ligase qRT-PCR was 7 logs and sensitivity 1 amol/µl.
Conclusion:
We conclude that the novel assays for miRNA quantification, miREIA® and SplintR® ligase qRT-PCR, meet elementary analytical and performance requirements for clinical laboratory methods and are potentially useful in clinical diagnostics.
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A-041

Total Antioxidant and Thiol Levels in Prostate Cancer Patients

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Background: Thiol groups are important anti-oxidants and essential molecules protecting organism against the harmful effects of reactive oxygen species (ROS). The aim of our study is to evaluate thiol-disulphide homeostasis with a novel recent automated method in patients with localized prostate cancer (PC) before and six months after radical prostatectomy (RP). **Material and Methods:** 18 patients with PC and 17 healthy control subjects were enrolled into the study. Blood samples were collected from the controls subjects and patients before and six months after RP. Thiol-disulphide homeostasis was determined using a recently developed novel method. Prostate-specific antigen (PSA), albumin, total protein, total thiol, native thiol, disulphide and total antioxidant status (TAS) were measured and compared between the groups. **Results:** Native thiol, total thiol and TAS levels were significantly higher in the control group than the patients before RP (p<.001). There was a non-significant increase in the native thiol, total thiol and TAS levels in the patients six months after RP in comparison to the levels before RP (p values 0.3, 0.3 and 0.09, respectively). We found a significant negative correlation between PSA and thiol levels. **Conclusion:** Our study demonstrated that the decreased thiol and TAS levels weakened anti-oxidant defence mechanism in the patients with PC as indicated. Increased oxidative stress in prostate cancer patients may cause metabolic disturbance and have a role in the aetiopathogenesis of prostate cancer.

A-042

A Novel Score Based on serum apolipoprotein A-1 & C-reactive protein is a prognostic biomarker in hepatocellular carcinoma patients

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Background: The aim of this study was to purpose a prognostic system based on preoperative serum apolipoprotein A-1 and C-reactive protein (ApoA-1 and CRP, AC score), and to evaluate the prognostic value in hepatocellular carcinoma(HCC) patients. **Methods:** Continuous 539 cases diagnosed with HCC from 2009 to 2012 in Sun Yat-sen University Cancer Center were analyzed. Characteristics, pretreatment lipids (ApoA-1, apolipoprotein B(Apo-B), high-density lipoprotein cholesterol(HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG)) and CRP levels were reviewed, and determined by univariate and multivariate Cox hazard models. Then the AC score was proposed, which combined the independent risk factors (ApoA-1 and CRP). **Results:** The optimal cut-off points in our study were evaluated by reference ranges. Patients with decreased ApoA-1 level(<1.090g/L), increased CRP level(≥3.00mg/L) had significantly poor overall survival (OS) and disease-free survival (DFS). The AC score was calculated as follows: patients with decreased ApoA-1 and elevated CRP were allocated as Score 3, patients with only one of these abnormalities were allocated as Score 2, and with no abnormalities were allocated as Score 1. Patients with higher AC score showed more progressed disease and poorer prognosis, not only in in the entire cohort (For OS, P<0.001; For DFS, P<0.001) but also in the subgroups stratified by pathological stage (stage I-II and stage III-IV). The discriminatory ability of AC score in HCC was assessed by AUC values, AC score (AUC: 0.676, 95%CI: 0.629-0.723, P<0.001) was higher than that of AFP. In addition, the combination of AFP and AC score (AUC: 0.700, 95%CI: 0.655-0.745, P<0.001) was superior to that of AFP, AC score only. **Conclusions:** The AC score is significantly valuable predictor of OS and DFS, and could more accurately differentiate the prognosis of HCC patients. As this study is a retrospective analysis, and the value of AC score should be validated in large prospective trials.

A-043

Heat shock proteins 90α provides a novel and effective diagnosis therapeutic strategy for Nasopharyngeal carcinoma

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Background: EBV infection is closely related to the occurrence of Nasopharyngeal carcinoma (NPC). Previous studies in our research group found that the positive predictive value of EBV antibody for NPC is not ideal. Heat shock proteins (HSP) are a family of proteins which have been produced by many cells, including tumor cells, which is associated with tumorigenesis and tumor progression. There is an urgent need for non-invasive, high-performance biomarkers in clinical laboratory medicine to aid the diagnosis of NPC. In this study, this is the first time to investigate the role of heat shock protein Hsp90α in the diagnosis and progress of nasopharyngeal carcinoma (NPC). **Methods:** Hsp90α was detected in 196 newly diagnosed NPC patients, 76 corresponding post-treatment NPC patients, 230 VCA-IgA positive normal subjects and 106 healthy donors by ELISA. NPC patients group: Between September 2016 and December 2016, 196 untreated NPC patients pathologically diagnosed at Sun Yat-sen University Cancer Center.Clinical stage (2008 staging system): 8 patients in stage I, 16 patients in stage II, 97 patients in stage III and 75 patients in stage IV. EBV VCA IgA were positive in the NPC patients; All the people in our study have similar age, gender, place of residence, place of origin, and smoking history) **Results:** (1) The level of Hsp90α in plasma of 196 patients with NPC was (212.16 ± 144.32) pg/ml, which was significantly higher than VCA-IgA positive normal subjects(68.12±64.94 pg/ml,P<0.001)and healthy donors(35.87±17.47 pg/ml, P<0.001. The level of Hsp90α in plasma of VCA-IgA positive normal subjects was significantly higher than that in healthy donors (P <0.001). (2) The level of Hsp90α in plasma of patients with NPC in the early stage (I+II), stage III and stage IV was 159.69 ± 117.12

pg / ml, 195.24 ± 126.38 pg / ml and 250.85 ± 164.66 pg / ml, respectively. The level of Hsp90α in plasma of patients with NPC in early stage (I + II) and stage IV, stage III and stage IV were significantly different (P = 0.018, P = 0.029); The level of Hsp90α in plasma in patients with metastasis of NPC and those without metastasis was significantly different (P<0.001). (3) The level of Hsp90α in plasma of patients with NPC before and after treatment were significantly different (212.16 ± 144.32 pg / ml vs. 62.36 ± 34.04 pg / ml, P <0.001). (4) The ROC curves demonstrated that the sensitivity of plasma Hsp90α in distinguishing NPC patients from healthy donors was 74.50% and the specificity was 99.10%(AUC=0.931, 95%CI:0.903-0.958); The sensitivity of plasma Hsp90α in distinguishing NPC patients from VCA-IgA positive normal subjects was 74.50% and the specificity was 81.70% (AUC = 0.831, 95% CI: 0.790-0.871). **Conclusions:** Hsp90α is closely related to the clinical stage, metastasis and therapeutic effect of NPC, so it may serve as a new biomarker for diagnosis and treatment of NPC .

A-044

Identification of oncogenic driver mutations in non-small-cell lung cancer patients

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Introduction: Lung cancer is the leading cause of cancer-related deaths worldwide. Identification of genetic aberrations critical to cancer development and maintenance (oncogenic driver mutations) has transformed care in lung cancer patients. Next generation sequencing (NGS) has served as a powerful tool in identifying genetic mutations. Targeted therapies including small molecule inhibitors and antibodies against these driver mutations are currently serving as personalized therapy and are preferable to standard chemotherapy.**Objective**: We determined the frequency of oncogenic drivers in patients with non-small cell lung cancer in an endemic region and their association with age, sex and actionable therapy **Method**: We retrospectively analyzed patients who underwent targeted NGS using a targeted NGS platform (FoundationOne CDx™) between October 2013 and December 2017. Percentage association between mutation status, age, sex and actionable therapy was performed. **Results**: Of the 88 lung cancer patients tested for driver mutations, 2 (2%) patients had no reported genetic alterations. Patients included lung adenocarcinoma (ADC), 66 (75%); squamous cell carcinoma (SCC), 16 (18%); large cell carcinoma (LCC), 3 (3%); atypical carcinoid (ATC), 1 (1%) and sarcomatoid carcinoma (SAC), 1 (1%). Among 85 patients studied, top driver mutations included *TP53*, 55 (65%); *KRAS*, 26 (31%); *STK11*, 16 (19%), *CDKN2A*,14 (16%); *LRP1B*, 10 (12%); *PIK3CA*, 12 (10%); *NF1*; 9 (11%) and *EGFR*, 8 (9%). Sixteen (19%; 14% ADC, 4% SCC and 1% LCC) patients were found with potentially actionable genetic alterations. Of the 496 total mutations, 256 (52%) were in females and 241 (49%) were in males. Mean number of mutations per patients was 5.6 (95% CI, 4.7-6.5) including ADC 4.9 (95% CI, 4.0-5.9) and SCC, 8.4 (95% CI, 6.2-10.7). **Conclusion**: The genomic landscape of our patient's tumors is consistent with previously reported studies and, importantly, emphasizes that 19% (14% ADC, 4% SCC, 1% LCC) of patients had actionable driver mutation. This suggests that identification of these mutations can identify patients who will benefit from targeted therapy, emphasizing the role of laboratory medicine in enabling personalized onco-therapeutics. Additional driver mutations with potential targeted therapies were also identified, however, randomized trials are required for further evaluation.

A-045

Novel Tissue-specific Autoantigens Associated with Clinical Outcomes in Response to PD-1/L1 Directed Immunotherapy in NSCLC.

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Objective: The impressive clinical outcomes resulting from PD-1/-L1 directed immune-checkpoint inhibition has led to a recent paradigm shift in the treatment of advanced non-small cell lung cancer (NSCLC). While tumoral PD-L1 expression remains the only prognostic biomarker to select patients for this therapeutic strategy, the limited performance of this marker spurns on the search for improved molecular diagnostics. The primary objective of this study is to identify candidate biomarkers to better prognosticate response to PD-1/-L1 directed immunotherapy based on differences in a humoral response to the tumor. Resulting autoantibodies **Method:** Lysates from A549 lung adenocarcinoma cells were resolved via 2-dimensional IEF/SDS-PAGE electrophoresis to address our primary endpoint, with 3 gels total performed in parallel: 1 stained for protein and 2 transferred to nitrocellulose. Individual membranes were immunoprobed with pooled pretreatment sera (n=4/group) derived from patients with advanced NSCLC receiving PD-1/-L1 directed immunotherapy that either have documented disease progression within 6 weeks of induction or have radiographically stable disease or better in the first 12 weeks of therapy. Immunoreactive spots were detected with a HRP-conjugated, anti-human IgG secondary antibody and developed via ECL reagents. Differentially immunoreactive spots were evaluated via densitometry in PDQuest (BioRad) with a 4-fold difference in expression threshold used to prioritize spots for identification via LCMS at the Mass Spectrometry Facility at the University of Illinois, at Chicago. As a secondary objective, we also performed this same maneuver contrasting autoantibodies in isolated from pretreatment and 12-week post induction time points, from each group. **Results:** We identified series of differentially expressed autoantigens that are candidate biomarkers for prognosticating clinical outcomes for advanced NSCLC patients receiving PD-1/-L1 directed immunotherapy. Our primary endpoint in this study was to identify circulating autoantibody biomarkers in pretreatment sera that have value for prognosticating a "good" versus "poor" clinical outcome. For this, we identified five autoantibodies in pretreatment sera using immunoproteomic methods that were associated uniquely with stable disease in the first 12 weeks of PD-1/-L1 directed immunotherapy. The corresponding autoantigens were identified via LCMS as heat shock protein A4, transitional endoplasmic reticulum ATPase, mitochondrial NADH-ubiquinone oxidoreductase 75 kDa subunit, heat shock protein D, and glyoxalase domain containing protein 4. In parallel, we also identified antibodies against annexin A1 as being uniquely associated with progressive disease. For our secondary objective, we were able to identify a series of circulating autoantigens capable of monitoring treatment response over the first 12 weeks of therapy. Specifically, we found retinaldehyde dehydrogenase 1, stress-induced-phosphoprotein 1, and annexin A2 as being highly correlated with disease progression in patients receiving PD-1/-L1 directed immunotherapy. Only autoantibodies against UDP-glucose 6-dehydrogenase were identified as a candidate biomarker for monitoring patients receiving clinical benefit from immunotherapy over the first 12 weeks of treatment. **Conclusion:** A series of candidate (autoantibody) biomarkers with value for prognosticating patient response to PD-1/L1 directed checkpoint inhibition. These targets are currently being developed into multiplexed immunobead assays for evaluation across appropriate cohort of advanced NSCLC patients receiving immunotherapy that are archived in our institutional biorepository.

A-046

Clinical Correlation between Serum Biomarkers CA27.29 and CA15-3 and Disease Status in Patients with a History of Advanced Breast Cancer

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Background: MUC1 is a biomarker used to aid in detection of recurrence and monitoring treatment response in patients with advanced breast cancer. MUC1 can be measured by two immunoassays: Cancer Antigen 27.29 (CA27.29) and Cancer Antigen 15-3 (CA15-3). While both immunoassays measure the same protein, they are sometimes ordered concurrently for patient management. Changes in MUC1 concentration between sequential measurements are monitored, with a significant percent change (%change) defined as 20–30%. Objective: The purpose of this study was to correlate concurrent CA27.29 and CA15-3 concentrations with clinical disease status to determine if one assay was superior in disease monitoring. Methods: In this IRB-approved study, 314 results were reviewed from 178 Mayo Clinic patients with a history of breast cancer. In each case, CA27.29 (ADVIA Centaur, Siemens Healthineers, Malvern, PA) and CA15-3 (Roche Cobas, Roche Diagnostics, Indianapolis, IN) were ordered on the same day during 2014–2015. Chart review was completed for 25 patients with multiple paired tumor marker (TM) orders and corresponding imaging results (n=63). Concordance between CA27.29 and CA15-3 results was evaluated. Positive results were defined as TM concentrations greater than the reference interval (RI, CA15-3: <30 U/mL, CA27.29: </=38 U/mL). Imaging studies corresponding to the time of TM sample collection were used to define disease status for the first measurement (baseline) and subsequent measurements. Baseline disease was classified as disease present (stable or progressing) or disease absent (no detectable disease). Subsequent statuses were defined as progressing (increase in disease), responding (decrease in disease), or stable (no change in disease). TM %change of 25% was defined as significant and used to correlate with disease status. TM %change was considered concordant with disease status change if: increase >/=25% for progressing, decrease >/=25% for responding, and <25% for stable. Results: The positive concordance between CA27.29 and CA15-3 was 100% (44/44), while the negative concordance was 90% (17/19). Twenty-one patients were classified as disease present and four as disease absent at baseline. CA15-3 and CA27.29 concentrations at baseline correlated with the disease status for nineteen (90%) of the disease present patients (CA27.29/CA15-3 > RI) and 100% of the disease absent patients (CA27.29/CA15-3 < RI). In 71% (27/38) of cases the subsequent measurement showed a %change in both TM's

that correlated with disease status. In the eight instances where neither TM correlated with disease status, six cases showed a decreased TM in the setting of disease progression and two cases had stable disease with a 25% or greater %change in TM at the subsequent measurement. There were six instances (16%) where only one TM correlated with disease status (three cases for each TM). Four of these cases were classified as stable disease. Conclusions: CA27.29 and CA15-3 results have strong concordance and overall similar clinical correlations. In cases where only one of the TM's correlated with the disease status of the patient, each TM correlated 50% of the time. The results from this patient cohort suggest that there is no clinical benefit to ordering both TM's concurrently for breast cancer patient management.

A-047

Serum HE4 levels in pancreatic and gastric cancer.

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Background: Pancreatic and gastric cancers are the seventh and the ninth most common type of cancer in Spain. CEA and Ca19.9 have been the worldwide tumor markers used in clinical practice. Human epididymal protein 4 (HE4) is a marker of ovarian and endometrial cancer, though studies at the tissue level have found an association between this marker and certain neoplasms of the digestive system such as gastric and pancreatic cancer. A little is known about serum levels of HE4 in this kind of pathologies. **Methods:** A total of 112 healthy individuals (47 men, 65 women) recruited as a control group, 49 patients diagnosed with pancreatic cancer (16 men, 33 women) and 33 patients diagnosed with gastric cancer (16 men, 17 women) were selected. None of the subjects presented kidney failure, gynecologic pathology, or other tumor types. The group of cancer was undergoing chemotherapy and/or surgical treatment at the time of the study. Serum determination of HE4, Ca19.9, and carcinoembryonic antigen (CEA) markers was performed in both groups. We compared median values and constructed receiver operating characteristic (ROC) curves, calculating area under the curve (AUC), sensitivity, specificity, and cut-off points for both groups. **Results:** Significant differences in HE4 ($p<0.001$) adjusted by age were observed in the group of pancreatic cancer patients, revealing an AUC of 0.92, a sensitivity of 87.8%, and a specificity of 82.3% with a cut-off point of 63.23 pmol/L. CEA and Ca19.9 showed significant differences ($p=0.001$ and $p=0.028$ respectively) adjusted by age revealing an AUC of 0.85 and 0.77 respectively.Significant differences in HE4 ($p<0.001$) was also found adjusted by age in the group of gastric cancer patients. AUC of 0.9, a sensitivity of 84.8% and specificity of 81.4% with a cut-off point of 62.73 pmol/L. Ca19.9 and CEA showed no differences between case and control group. **Conclusion:** In patients with pancreatic and gastric cancer, HE4 levels are higher when compared to the widely used markers Ca19.9 and CEA, although more studies are needed to clarify this association.

A-048

A Four Kallikrein Panel Test Accurately Predicts Risk of High Grade (Gleason score ≥7) in men with PSA 1.0 - 10.0 ng/mL

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Introduction

Prostate cancer is the most common cancer in men and is projected to account for 164,690 new cases and 29,430 deaths in the U.S. in 2018. Screening for prostate cancer with Total PSA (PSA) has been a significant contributor in reducing the overall mortality from prostate cancer by over 50% in the U.S., but questions remain as to the risk vs. benefit of PSA screening due to complications caused by over diagnosis and over treatment of non-lethal cancer. The complications associated with PSA screening begin with the prostate biopsy, which must be performed to diagnose prostate cancer. About 75% of all prostate biopsies find either no cancer or find Gleason score 6 cancer. A biomarker test is needed to effectively identify men likely to harbor aggressive prostate cancer. Such a test would improve the efficacy of PSA screening by reducing the use of prostate biopsy and the diagnosis of indolent cancer, while still showing high detection rates for aggressive cancers.

Materials and Methods

A new blood test (4Kscore) consisting of a panel of four kallikrein biomarkers (Total PSA, Free PSA, Intact PSA, and hK2), combined with clinical information in an algorithm, has been tested in two prospective U.S. clinical studies involving over 1,300 patients.[1,2] We report here for the first time a combined analysis of 1124 patients, representing a contemporary, racially diverse (19% African American) subgroup from these studies. All the men have a PSA 1.0-10.0 ng/ml. Based on their PSA and clinical information, the cohort represents a group identified in the "grey zone" by screening where the decision for prostate biopsy would benefit from further information of the patient's risk.

Results

The 4Kscore shows significantly better AUC performance in the detection of high grade disease (Gleason score ≥7) vs. use of PSA alone (0.776 vs. 0.638). At a 4Kscore cut point of 7.5%, the sensitivity and negative predictive (NPV) value were 92% and 95%, respectively. The PSA sensitivity and NPV (cut point 4.0 ng/mL) were 85% and 89%, respectively. The 4Kscore would also have spared 379 (34%) of the prostate biopsies. It is noteworthy that 4Kscore detected all Gleason score ≥8, while PSA at 4.0 ng/mL missed 7 out of 64 (11%).

Conclusions

Our study looked at the performance of the 4Kscore test compared to PSA alone to distinguish men with high grade prostate cancer. The cohort was a racially diverse cohort derived from two prospective U.S. clinical studies, and represents an important at risk population in need of further risk stratification. The 4Kscore showed significantly better AUC, sensitivity and NPV performance for predicting high grade prostate cancer at biopsy when compared with PSA.

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[2] Punnen S, et al. J Urology (2018), doi: 10.1016/j.juro.2017.11.113.

A-049

Monitoring EGFR mutations in cfDNA during different treatment lines in Non-small-cell lung-cancer (NSCLC) patients

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Background: The cobas® EGFR Mutation Test v2 is the only FDA approved test to qualitative detection in plasma from non-small cell lung cancer (NSCLC) patients for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR), which can benefit of the treatment with tyrosine kinase inhibitors (TKIs). However, patients under this therapy can eventually develop resistance, mainly due to the presence of T790M mutation. Since repeated biopsies are often an invasive procedure, analysis of EGFR mutations in blood becomes a useful tool to investigate mutational progress. **Objectives:** To assess the ability of cobas® EGFR Mutation Test v2 to detect EGFR mutations, concordance of matched-tissue and plasma and capability of use as a semiquantitative platform for treatment monitoring. **Methods:** Plasma samples from 21 EGFR positive NSCLC patients (13 males, 58 ± 12 years, 8 females, 63 ±11 years) were collected under informed consent. Blood was collected at baseline treatment baseline and at sequential timepoints, including progression and subsequent second line treatments. cfDNA isolation was performed with the cobas DNA Sample Preparation kit (Roche Molecular Systems, Inc. CA, USA). Concentrations of isolated cfDNA were measured in a Qubit 2.0 Fluorometer (Life Technologies) and characterized in a 2200 TapeStation system (Agilent Technologies, CA, USA). Mutations were tested with the cobas® EGFR Mutation Test v2. Mutations were also quantified by digital droplet PCR (ddPCR) (Bio-Rad). **Results:** Mean cfDNA was 0,28 ±0,19 ng/µL. Purity of cfDNA isolated was confirmed by a High Sensitivity D1000 ScreenTape® assay (175,2 ± 8,78 bp) as well as the absence of genomic DNA. We were able to detect EGFR mutations in ctDNA in 12/21 of tissue and blood paired samples. Regarding to plasma positive results, a complete overall concordance was found with tissue (12/12). We could also detect T790M resistance mutation in two patients at the baseline and, very interestingly, one of them could not be detected in its tumor-matched material. When analyzing progression samples, we also observed a gradual increase in Semi-quantitative index (SQI), although changes were not significant at progression. Also, in 3/21 negative cfDNA baseline patients, results turned positive in subsequent progression samples. We analysed results obtained by cobas technology and ddPCR in order to know if a correlation did exist between SQI and number of copies/mL respectively, but no correlation was found in neither of the mutations analyzed: del19, L858R, and T790M. **Conclusion:** cobas® EGFR Mutation Test v2 sensitivity is low but if positive, can avoid tissue biopsy. It can identify the existence of resistance mutations in patients without detectable mutation in biopsy. Sequential analysis at progression should also be taken in account as it can detect the presence of resistance mutation avoiding a rebiopsy.

A-050

Circulating tumor DNA detection with a novel platform for single molecule sequencing validated for targeted and immunotherapy selection

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Background
Comprehensive genomic profiling of solid tumors using circulating tumor DNA (ctDNA) has enabled the detection of all NCCN guideline-recommended somatic genomic classes of alterations from a single, non-invasive blood draw. However, current ctDNA tests still face two major challenges: the inability to reliably identify somatic variants at low mutant allele fraction (MAF), and inconsistency in how the tests have been validated. This study shows how the Single Molecule Sequencing (SMSEQ) platform addresses these challenges. The SMSEQ platform integrates innovative ctDNA extraction methodology, highly optimized library preparation and an error-based variant-calling algorithm to drastically improve sensitivity and specificity. The platform analyzes 5 classes of somatic variants: single nucleotide variants (SNVs), insertions and deletions (Indels), copy number variants (CNVs), fusions and microsatellite instability (MSI).
Methods
We analyzed a 73 gene panel covering NCCN recommended actionable variants for solid tumors in 60 reference ctDNA samples with known variants to establish the limit of detection, sensitivity, specificity, accuracy and reproducibility of the SMSEQ platform. For clinical validation, we tested 36 patients with metastatic colorectal cancer (mCRC) and 34 healthy controls from the Chang Gung Memorial Hospital, and 227 patients diagnosed with solid tumors from Taiwan. Circulating tumor DNA was extracted from plasma followed by library preparation using a highly optimized NGS workflow. Somatic variants in ctDNA are identified using locus-specific modeling to separate tumor variants from normal errors.
Results
Validation according to recently published ACMG/AMP guidelines, shows that the SMSEQ platform allows calling of variants with >99.999% analytical specificity for SNVs, Indels and fusions; and >99% analytical specificity for CNVs and MSI. The platform successfully detected variants at low MAF: 0.1% for SNVs and Indels, <1% for fusions, 5 copies for CNVs, and 1% for MSI.
Somatic variants were identified in 35 of 36 mCRC patient samples (97.2%). No false positives were observed within the targeted region for all 34 healthy controls tested. In paired samples, the SMSEQ platform showed 89.7% concordance with tissue biopsy. Observed gene mutation profiles from ctDNA were consistent with published tissue biopsy data: the most frequently mutated genes were *TP53, APC,* and *KRAS*; KRAS and BRAF variants were mutually exclusive. In addition to mCRC patients, we tested 227 patients diagnosed with various solid tumors from Taiwan. Actionable variants were detected in 170/227 (74.8%) patients.
Conclusion
The CellMax 73-gene liquid biopsy test, using the SMSEQ platform, detects 5 NCCN-guideline recommended variant classes: MSI for immunotherapy as well as SNVs, Indels, CNVs, and fusions for targeted therapy selection at low variant allele fraction/copy number at high sensitivity and specificity.

A-051

Clinical evaluation of HE4 in lung cancer diagnosis

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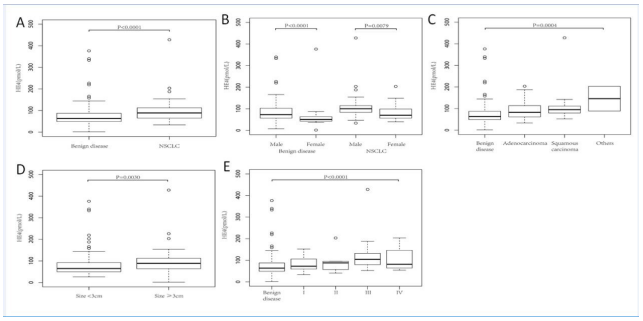
Background and Aims
Lung cancer is the most common cancer and the leading cause of cancer-related death worldwide. With the progress of tumor stage, the prognosis was significantly worse. Thus, early diagnosis of lung cancer is of importance to improve the outcomes of the patients. Human epididymis 4 (HE-4) has been recently shown to be a potential new biomarker for lung cancer. Thus, the present study is to investigate its clinical utility in lung cancer diagnosis.
Methods
A total of 67 non-small cell lung cancer(NSCLC) patients and 112 subjects with benign lung tumors , were recruited from the First Affiliated Hospital of Xiamen University. All the patients' information were recorded and validated. The study received ethical approval from the site. Serum HE4 was examined by the ARCHITECT i2000(Abbott,USA).
Results
Serum levels(Mean±SD) of HE4 in benign disease and NSCLC were 78.88±58.04 pmol/L and 135.89±230.19 pmol/L ($p<0.001$) respectively. Higer serum levels of HE4 were found in NSCLC compared to benign disease($p<0.001$, Fig.1A). Interstingly, HE4 levels in male were siginificantly higher than female in both group, suguesting the gender specific cutoff may be needed when using HE4 in disagnosis(Fig.1B). According to pathological types, HE4 levels in squamous carcinoma were higher than adenocarcinoma and benign disease(Fig.1C). Higher HE4 levels were also detected in larger tumor size (Fig.1D) and late stage(Fig.1E). ROC analysis showed an AUC of 0.675 with sensitivity of 0.612 and specificity of 0.688 at the cutoff of 79.5 pmol/L.
Conclusions
The preliminary data of this study supported the potential clinical application of HE4 in lung cancer diagnosis.



A-052

Multicenter Clinical Evaluation of New Free Light Chain Methods

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Background: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free-light-chain (FLC) determinations for diagnosis and management of clonal plasma-cell disorders. We describe reproducibility, linearity, reference range determination, clinical sensitivity, and specificity data for two new immunoassays that detect FLC-type kappa and lambda, and their ratio.
Methods: Latex-enhanced mouse monoclonal antibody reagents from Siemens Healthcare Diagnostics Inc. for kappa (N Latex FLC kappa) and lambda (N Latex FLC lambda) were assayed on multiple BN™ II and BN ProSpec® Systems. Precision studies were conducted according to CLSI guideline EP5A-2 to estimate repeatability of three sample pools and two controls each for kappa and lambda, using up to three reagent and calibrator lots at four sites. A reference range study on U.S. populations included 201 serum samples from apparently healthy adults (47% female; 53% male; age 21-86 years). A validation reference interval study including 178 donors (59% female; 41% male; age 21-66 years) was conducted to ensure the first study's robustness. Clinical sensitivity of the N Latex assays was investigated against the clinical condition of patients at various disease stages in defined populations consisting of 96 multiple myeloma (MM) and 83 amyloidosis (AL) patients. For comparison, the same populations were investigated using the available FREELITE methods from The Binding Site for the BN II System. The specificity panel applied to the N Latex FLC methods consisted of 163 samples from patients with various immunological conditions. Method agreement for the new FLC and FREELITE BN II assays was evaluated using Passing-Bablok regression analysis.
Results: Between-lot/between-instrument reproducibility for the new kappa assay on the BN II System ranged from 3.5-6.0%/1.2-3.5%. On the BN ProSpec System, the kappa assay's between-lot/between-instrument results were 4.6-7.2%/4.0-7.0%. Between-lot/between-instrument lambda results on the BN II System were 5.9-9.2%/4.1-6.5%. On the BN ProSpec System, between-lot/between-instrument results for lambda were 2.6-7.1%/0.4-3.8%. Reference range studies showed kappa (κ) concentrations (2.5th percentile/median/97.5th percentile) of 8.24/15.1/28.9 mg/L and lambda (λ) concentrations of 9.1/17.3/32.6 mg/L; κ/λ ratio results were 0.53-1.51 (1st-99th percentile). Reference interval validation revealed within-range recoveries of 91.0% for kappa, 93.3% for lambda, and 96.6% for the κ/λ ratio, thereby confirm-

ing the validity of the initial ranges according to CLSI guideline C28-A3. Clinical sensitivity based on the κ/λ ratios for the N Latex FLC methods was 95.8% in the MM and 83.1% in the AL population. The respective results for the comparison methods were 95.8% and 77.1%. Clinical specificity was 96.9% for both N Latex methods. Passing-Bablok regression results between methods were y = 0.794x + 2.1 mg/L (r = 0.943) for kappa (n = 216) and y = 1.17x + 2.16 mg/L (r = 0.975) for lambda (n = 218). **Conclusion:** The new N Latex FLC methods showed acceptable and consistent precision over several reagent lots and instruments, valid reference ranges, and acceptable correlation with an FDA-cleared method. These methods are an attractive alternative for FLC measurement in clinical laboratories. HOOD05162002786441

A-053

Reliability of the SPAPLUS® Analyser for the Assessment of Serum Free Light Chains: PathCare Laboratory, South Africa

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Background: Serum free light chain (sFLC) assays (Freelite®) are included in both international and national guidelines for the diagnosis, monitoring and prognosis of multiple myeloma and related disorders. However, historically, sFLC assays run on a wide plethora of analysers exhibited well published antigen excess, non-linearity and over-estimation issues. The manufacturer of the assay has reportedly overcome some of these issues on optimised analysers, including the SPAPLUS. The aim of this study was to evaluate the performance of the Freelite assay on the SPAPLUS, in a large reference laboratory in South Africa, and report on the incidence of antigen excess, sample non-linearity and extremely high sFLC values. **Methods:** sFLCs were measured using κ and λ Freelite immunoassays (The Binding Site Group Ltd., Birmingham UK) on a SPAPLUS analyser (The Binding Site Group Ltd., UK) at PathCare laboratory, Cape Town, South Africa. All results obtained between 01/06/2017 and 18/09/2017 were included. The incidence of antigen excess was calculated based on the proportion of samples flagged with abnormal reaction kinetics by the automatic antigen excess check on the analyser. Extreme sFLC concentrations were defined as κ or λ sFLC concentrations >30,000 mg/L (cut-off based on the subtraction of a typical serum albumin concentration [40 g/L] from a typical serum total protein concentration [70 g/L]). Non-linearity was investigated when samples gave a > value at a 1/10 sample dilution, but a result within the 1/10 measuring range when assayed at a 1/100 dilution. Notable non-linear samples were defined as those that gave results that were >30% different at the two dilutions. **Results:** A total of 1452 serum samples from 1150 patients were included in the analysis. An abnormal κ/λ sFLC ratio (<0.26 or >1.65) was reported in 855/1452 (59%) of samples and indicated monoclonal κ sFLCs or monoclonal λ sFLCs in 87% and 13% of cases, respectively. Of the samples with monoclonal κ sFLCs, the mean κ sFLC concentration was 502 mg/L (range 4.38 mg/L- 34,300 mg/L). Of the samples with monoclonal λ sFLCs, the mean λ sFLC concentration was 1,414 mg/L (range 4.8 mg/L - 26,100 mg/L). In 83% of cases, samples gave a result at the initial online dilution. Antigen excess was flagged in 16/1452 samples (1.1%). Only 1/1452 samples (0.07%) had a sFLC concentration >30,000 mg/L. 72/1452 (5.0%) of samples were non-linear. Of these 72 samples, the median percentage difference between the result at 1/10 and 1/100 dilution was 15% (range 0.41% - 31.4%). Only 2/1452 samples (0.14%) had notable non-linearity. **Conclusion:** We conclude that analytical issues associated with monoclonal sFLC measurement are an infrequent occurrence in routine laboratory practice, when using the Freelite assays on the SPAPLUS analyser. We experienced very little sample non-linearity (0.14%), the incidence of antigen excess was low (1.1%) and very few samples had extremely high sFLC values (0.07%).

A-054

Establishment and validation of a predictive nomogram model for non-small cell lung cancer patients with chronic hepatitis B viral infection

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Background: This study aimed to establish an effective predictive nomogram for non-small cell lung cancer (NSCLC) patients with chronic hepatitis B viral (HBV) infection. **Methods:** The nomogram was based on a retrospective study of 230 NSCLC patients with chronic HBV infection. The predictive accuracy and discriminative ability of the nomogram were determined by a concordance index (C-index), calibration plot and decision curve analyses and were compared with the current TNM staging system. **Results:** Independent factors derived from Kaplan-Meier analysis of the primary cohort to predict overall survival (OS) were all assembled into a Cox's proportional hazards regression model to build the nomogram model. The final model included age, tumor size, TNM stage, treatment, apolipoprotein A-I, apolipoprotein B, glutamyl transpeptidase and lactate dehydrogenase. The calibration curve for the probability of OS showed that the nomogram-based predictions were in good agreement with actual observations. The C-index of the model for predicting OS had a superior discrimination power compared with the TNM staging system [0.780 (95% CI: 0.733-0.827) vs 0.693 (95% CI: 0.640-0.746), $P < 0.01$], and the decision curve analyses showed that the nomogram model had a higher overall net benefit than the TNM stage. Based on the total prognostic scores (TPS) of the nomogram, we further subdivided the study cohort into 3 groups: low risk (TPS ≤ 13.5), intermediate risk (13.5 < TPS ≤ 20.0) and high risk (TPS > 20.0). **Conclusion:** The proposed nomogram model resulted in more accurate prognostic prediction for NSCLC patients with chronic HBV infection.

A-055

Prognostic nomogram for patients with Nasopharyngeal Carcinoma incorporating hematological biomarkers and clinical characteristics

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Background: Predictive models for survival prediction in individual cancer patients following the TNM staging system are limited. The survival rates of the patients who have the same TNM stage disease were diversified. Therefore, we construct a nomogram that incorporates hematological biomarkers and clinical characteristics for predicting the overall survival (OS) of nasopharyngeal carcinoma (NPC) patients. **Methods:** The clinicopathological and follow-up data of 460 NPC patients who were diagnosed histologically in Sun Yat-sen University Cancer Center between July 2007 and December 2011 were retrospectively reviewed. The data was randomly divided into primary and the validation groups. Cox regression analysis was used to identify the prognostic factors for building the nomogram in the primary cohorts. The predictive accuracy and discriminative ability of the nomogram were measured by concordance index (C-index) and decision curve and were compared with the TNM staging system, Epstein-Barr virus DNA copy numbers (EBV DNA) and TMN stage plus EBV DNA. **Results:** The results were validated internally by assessing discrimination and calibration using the validation cohorts (N = 230) at the same institution. Independent factors for overall survival including Age [hazard ratio (HR): 1.765; 95% confidence interval (CI): 1.008~3.090)], TNM stage (HR: 1.899; 95% CI: 1.023~3.525), EBV DNA (HR: 1.322; 95%CI: 1.087~1.607), lactate dehydrogenase level (LDH) (HR: 1.784; 95%CI: 1.032~3.086), high sensitivity C-reactive protein (hs-CRP) (HR: 1.840; 95%CI: 1.039~3.258), high-density lipoprotein cholesterol (HDL-C) (HR: 0.503; 95%CI: 0.282~0.896), hemoglobin (HGB) (HR: 0.539; 95%CI: 0.309~0.939) and lymphocyte to lymphocyte ratio (LMR) (HR:0.531; 95%CI: 0.293~0.962) were selected into the nomogram for survival. The C-index in the primary cohort and validation cohort were 0.800 and 0.831, respectively, which were statistically higher than the C-index values for TNM stage (0.672 and 0. 716), EBV DNA (0.668 and 0.688), and TNM stage+ EBV DNA (0. 732 and 0. 760), $p < 0.001$ for all. And the decision curve analyses showed that the nomogram model had a higher overall net benefit than the TNM staging system, EBV DNA and TNM stage+ EBV DNA. Then we stratify patients into three distinct risk groups for OS based on the total points (TPs) of nomogram: a low risk group (TPs ≤ 19.0), an intermediate risk group (19.0 < TPs ≤ 25.5) and a high risk group (TPs > 25.5), respectively. **Conclusion:** We have generated nomogram predicting prognosis for NPC patients with a higher predictive power than the TNM staging system, EBV DNA and TNM stage+ EBV DNA.

A-056

Molecular analysis of *MEN 1 gene* in suspected carriers of Multiple Endocrine Neoplasia type 1 born in Argentina

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Background: MEN 1 is an autosomal dominantly inherited syndrome characterized by parathyroid, gastroenteropancreatic and anterior pituitary tumors. Familial MEN1 (F) is defined in an individual who has at least one first-degree relative with one or more main endocrine tumors and Sporadic MEN1(S) when one individual is affected within a family with no history of the disease. The gene related to this syndrome is *MEN1*,it is a tumor suppressor gene located in chromosome 11q13 and codes for menin, a nuclear protein of 610 amino acids. Genetic diagnosis requires the sequencing of the whole DNA coding sequence and is warranted in patients with two or more of the characteristic tumors, their first-degree relatives, or young patients carriers one of

the tumors. AIM: To evaluate the *MEN1* germline mutations in patients with clinical features of MEN 1 born in Argentina. Subjects: We studied 127 potential carriers: 56 were index-cases (31 female and 25 male) and 71 first-degree relatives. The DNA of 66 healthy subjects was analyzed as a control group. All subjects gave informed consent to genetic studies. Methods: Genomic DNA was obtained from peripheral blood leukocytes. Coding region from the promoter to exon 10 and intronic flanking regions were amplified by PCR. The DNA fragments were sequenced after being manually labeled with ddNTP33 and since 2009 by automatic Sanger Sequencing. Long Range PCRs were performed in patients in whom no mutation was found and actually MLPA assay was evaluated. Pathogenic variants were confirmed in another DNA sample. Novel pathogenic variants were eventually confirmed by RFLP-PCR. Missense novel variants were verified by sequencing 104 alelles of a normal control population to evaluate the clinical significance. Results: We found germline mutations in 68% of index-cases: 55.3% were Familial MEN 1 and 44.7% were Sporadic MEN 1. Within the group of the Familial MEN 1 germline mutations were in 84% and 55.0% in the Sporadic MEN1 group. Germinal mutations were in the 38% of the first-degree relatives. The pattern of mutation type was: frameship(FS)(47%), missense(MS)(23.7%), nonsense(NS)(23.7%) and 5.3% of splice site(SS). The germline Variants/Exon found were:c.1060_1063dupTGCC/8;c.1340T>C/9,c.1102 delG/8,c.22A>T/2; c.471delG/3; c.487delG/3; c.791T>C/5, c.1350+1G>A/9, c.249-252delGTCT/2, c.1045C>T/7, c.551T>A/3, c.244delG/2, c.625_628delCAGA/3, c.1127T>C/8, c.1405G>T/10, c.378delG/2, c.672 delA/4, c.377G>A/2, c.828 C>G/6, c.1546_1547insC/10, c.784-9G>A/int4, c.1378C>T/10, c.1243 C>T/9, c.655 -1G>A/3, c.1102 del G/8, c.466 G>T/3, c.286 C>T/2, c.652 C>T/3, c.1243 C>T/9, c.483_495 del 13/3, c.1664G>T/10; c.792delC/5. The SNPs in our population were: c.1621G>A , c.512G>A, c.1254C>T, c435C>T, c.1080C>T and c.-533T>A. Conclusions: Germline mutations were detected in 67.9 % of MEN1 index cases and 38% in first-degree relatives. MEN1 mutations were distributed throughout the entire gene and included NS, FS, MS and SS mutations like other series that has been published. No correlation between phenotype and genotype was observed. The high frequency of novel mutations in the first series of patients with MEN1 born in Argentina and their presence in exons other than those reported in the literature it could be related to the ethnic and environmental factors from our population.

A-057

Agreement of Hevylite-based response assessment vs standard Electrophoresis/Immunofixation-based assessment: Follow-up of 26 Multiple myeloma patients

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Background: Protein electrophoresis (PE), immunofixation electrophoresis (IFE) and serum free light chains are the current gold standards for monitoring monoclonal proteins (MP). Precise evaluation of the changes on the concentration of MP is key to the management of Multiple Myeloma (MM) patients allowing clinicians to assess the success of the previously elected therapy. However, under certain conditions these techniques may present limitations rendering importance to the study of new biomarkers. **Objective:** to validate the use of the Hevylite® (The Binding Site) assay as a monitoring technique of MM patients by comparing it with the gold standard techniques. **Methods:** Quantification of monoclonal proteins in 26 MM patients during several courses of treatment and comparison of response criteria by Hevylite vs PE and IFE. The Hevylite assay quantifies immunoglobulins by their heavy/light chain pairs. Response assessment by Hevylite is based on the percentage of reduction of MP indicated by the International Myeloma Working Group (IMWG) criteria for PE/IFE. **Results:** 201 results obtained with Hevylite corresponding to a median of 10 (range: 6-19) determinations/patient. Response assessment based only on serum monoclonal evaluation (Table 1) showed an 83% overall agreement of Hevylite relative to SPE/IFE (only 1/172 samples showed more than 1 level of response difference). Hevylite identified all patients with progressive disease. Contingency analysis shows a progressive and statistically significant increase ($p<0.001$) in the number of patients with abnormal Hevylite Ig'k/Ig'ʎ ratio, as the quality of response decreases. Biochemical relapse is identified in 5 occasions by Hevylite and not by PE/IFE. Conversely, in 4 occasions IFE shows relapse from complete response while Hevylite does not. **Conclusion:** Long-term biochemical follow-up of the present cohorts shows good indications that Hevylite could work as a valid alternative assay for monitoring MM patients in case of limitations of the traditional techniques.

Table 1.Concordance of response assessment based on Hevylite vs PE/IFE

		PE/IFE assessment				
		MR	PR	VGPR	CR	
Hevylite assessment	MR (<50% MP decrease)	17	1	0	0	
	PR (>50% and <90%)	0	55	1	1	
	VGPR (>90% MP decrease)	0	7	17	5	
	CR (normal ratio)	0	0	14	54	
Agreement		**100%**	**87%**	**53%**	**90%**	**83%**

MR: minimal response, PR: partial response, VGPR: very good partial response, CR: complete response.

A-058

Development of a 2-Color biosensor-based ensemble FRET assay for discovery of potential substrates of the Cancer-implicated Human Multidrug Resistance Protein-1

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Human ATP-binding cassette (ABC) transporters are a superfamily of trans-membrane proteins responsible for efflux of drugs and other xenobiotics from the cell in an ATP dependent process. Overexpressed by cancer cells, Multidrug resistance-associated protein 1 (MRP1/ABCC1), a sub-member of ABC transporters, actively pumps out anticancer drugs, reducing the efficacy of the drugs and leading to failure of chemotherapy. The objective of the project is to identify potential substrates of MRP1 protein using a 2-color biosensor-based ensemble FRET technique. Since MRP1 affects the efficacy of drugs, profiling these drugs early for their MRP1-substrate status will inform the withdrawal of hit-drugs before they reach the more laborious and expensive clinical trials stages, thereby saving billions of dollars. This underscores the relevance of this project. Currently, there is no high throughput assay available to identify potential drug substrates of MRP1 protein. Previously, our group engineered a single 2-color MRP1 which reported intramolecular FRET as a function of structural changes and identified eight compounds as hits following a screening of NIH library of clinically tested compounds. The 2-color MRP1 construct was genetically modified by fusing a green fluorescent protein (GFP) and a red fluorescent protein (RFP) to the transporter. Here, we engineered a set of four functional 2-color MRP1 biosensors by switching the GFP positions to determine which GFP position improves FRET and investigate the constructs' interaction with the 8-compound hits using a steady state FRET-based assay. HEK 293 cells were transiently transfected with the 2-color constructs followed by confocal microscopy to observe the expression and localization of the proteins. Functional characterization of the 2-color biosensors was done through doxorubicin accumulation assay. 20 µg of each 2-color protein in tris sucrose buffer was prepared and incubated independently with 10 µM of the 8-test compounds and/or 4mM/5 mM ATP/$MgCl_2$ at 37 ^{0}C for 10 minutes prior to FRET Measurements using Fluorimeter model FL3-11. FRET efficiencies of compound conditions were normalized with that of apo condition. Fifty other drugs from an anti-cancer library were also screened by the same protocol using the most FRET sensitive construct, GR-888. 2-color MRP1 constructs GR-881, GR-888 and GR-905 demonstrated a change in FRET in the presence of meropenem, mesalamine and EGCG and/or ATP when compared to their ligand-free conditions. Changes in FRET by compounds alone were in some cases repressed by the addition of ATP suggesting biosensors could detect MRP1 modulators without ATP induction. Of the fifty anticancer drugs, ten hits showed percentage FRET change typical of MRP1 substrates. Overall, we have shown that except for GR-638, the 2-color MRP1 biosensors interact with potential MRP1 modulators and do hold promise for the discovery of novel MRP1 substrates using steady state FRET analysis. The long-term plan is to upscale this strategy to a high-throughput standard by using a regular plate reader to identify novel substrates of MRP1. These data, when published, are expected to have considerable impact on oncology.

A-059

Serum lactate dehydrogenase and C-reactive protein levels for the diagnosis of prostate cancer

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Background: Prostate cancer (PC) is a major health concern worldwide, being the second most common neoplasm and sixth cause of cancer-related death in the en-

tire world. Serum total prostate specific antigen (PSA) has become the most clinically useful tumour marker for the diagnosis and subsequent monitoring of PC. The free-to-total serum prostate specific antigen ratio (%fPSA) has been proposed to differentiate benign from malignant prostate disease in patients with nonspecific elevations of serum PSA levels (range between 4 and 10 ng/mL). Serum lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels can increase in many inflammatory processes and cancer. The aim of this study was to evaluate the utility of serum LDH and CRP levels for diagnosis of PC in men with nonspecific elevations of serum PSA levels.**Methods:** We studied men with no known history of PC and serum PSA levels between 4 and 10 ng/mL, who underwent 12-core transrectal ultrasound guided prostate biopsy. The following serum biomarkers were measured: PSA and free-PSA by electrochemiluminescence immunoassay on Hitachi Modular E-170 analyzer (Roche Diagnostics, Basel, Switzerland); LDH by enzymatic photometric method according to the International Federation of Clinical Chemistry and CRP by immunoturbidimetric test with monoclonal anti-CRP antibodies on Hitachi Modular cobas c 702 analyzer (Roche Diagnostics, Basel, Switzerland). The %fPSA was calculated using the following formula: (free-PSA/PSA)x100(%). Patients were classified into two groups according to the diagnosis of prostate biopsy: PC and NOT PC patients. Logistic regression was used for develop a probabilistic model to predict patients with PC and determine the importance of each biomarker by calculating the odds ratio. The diagnostic accuracy was determined using receiver operating characteristic curves (ROC), calculating the area under the ROC curve (AUC). **Results:** We studied 232 patients with ages between 43 and 98 years old (median=72), 200 NOT PC and 32 PC patients. Serum PSA and CRP levels were similar in the PC and NOT PC patients, in contrast serum LDH levels were higher in the PC patients and %fPSA values were higher in the NOT PC patients. Serum PSA and CRP levels were not statistically significantly to differentiate between PC and NOT PC patients ($p>0.05$). Serum LDH levels and %fPSA values were included in the probabilistic model to predict patients with PC. The odds ratios were 0.8530 and 1.0071 for %fPSA and LDH, respectively. The probabilistic model to predict patients with PC was: $(1+e^{-Z})^{-1}$; ($Z=0.0070xLDH-0.1589x\%fPSA-1.4898$). The AUCs were 0.657($p=0.0048$), 0.802($p<0.0001$), and 0.844($p<0.0001$) for serum LDH levels, %fPSA values and probabilistic model, respectively.**Conclusions:** Serum CRP levels were not useful to differentiate benign from malignant prostate disease, in contrast serum LDH levels could be used for diagnosis of PC in patients with serum PSA levels between 4 and 10 ng/mL. A probabilistic model to predict patients with PC using serum LDH levels and %fPSA values may improve the diagnostic accuracy compared to using %fPSA alone.

A-060

Fecal Immunochemical Test (FIT) Specimen Stability near the Clinical Cut-off

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Objective: The goal of this study was to investigate the stability of FIT specimens and to retrospectively assess potential patient impact of poor stability. **Background**: The Fecal Immunochemical Test (FIT) detects colonic bleeds, and is the most prevalent test for colorectal cancer screening across Canada. In Alberta, ≥75 ng hHb/mL is the clinical cut-off value used to reflex middle-aged patients for colonoscopy to search for neoplastic growth. FIT specimens are generally collected by the patient from home using a concealed, easy to use collection device. Patients are instructed to bring the collection to a laboratory site within 7 d following manufacturer claims of 14 d stability at ambient temperature. **Methods**: Specimens near the clinical cut-off (75 ng hHb/mL) values were considered to be most likely affected by instability or degradation, and thus were selected for this study. OC-Sensor Diana instrument (Somagen) ("give the address) was used in this study. Both QC material and patient specimens (n=9) were used in the following experiments. In experiment 1, FIT stability was assessed by selecting patient specimens (n=9) arriving to the laboratory on the date of home collection (day 0) that were initially 75-100 ng hHb/mL Specimens were stored at ambient temperature between measurements on 3, 5, 7 and 10 day post-collection. In experiment 2, specimens stored at ambient temperature for 7 and 14days post-collection (n=23) were tested. In experiment 3, retrospective analysis investigated turn-around time and the frequency of FIT results 50-100 ng hHb/mL obtained from laboratory information system (Cerner Millennium) under institutional data policies. **Results**: Within-day (using QC material) and within-run (serial measurement of patient specimens, n=9) precision were 13.5%, and 16%, respectively. In experiment 1, the pooled specimen mean on day 0 was 83.9 ± 7.3 ng hHb/mL, which consistently decreased with ambient storage. Indeed, the median percent decrease relative to day 0 was 16.5%, 24.6%, 46%, and 73.5% on d3, 5, 7, and 10, respectively. In experiment 2, 83% of specimens were initially positive (84 ± 13.1 ng hHb/mL). However, 7 d after collection and storage at ambient temperature, 26% of specimens remained positive; and after 14 d, only 16% of samples were above the clinical cut-off. Experiment 3 investigated 381,702 results reported from 2011-2017; and 87% were measured within 2d of collection. Cumulatively, 9.1% of patient results were ≥75 ng hHb/mL, and 1.7% were 75-100 ng hHb/mL. Importantly, 11,973 specimens were 'weak' negative (50-75 ng hHb/mL), of which, 2.5% were measured ≥5d post-collection. The potential patient impact was assessed in 2016 data from 3971 patients with results between 75-100 ng hHb/mL. 77% of those patients underwent colonoscopy, and colorectal cancer was diagnosed in 36 patients in this group (~1%), however, the presence of benign neoplasms requiring more intense follow-up could not be determined. **Conclusion:** Specimen stability is of great importance for the FIT testing system used in this study. Negative results in specimens stored at ambient temperature for more than 4 days should be repeated or interpreted with caution.

A-061

Reference Interval Determination and Method Comparison of the µTASWako and Beckman Access Total AFP Assays

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INTRODUCTION: Alpha-fetoprotein (AFP) can serve as a marker for a variety of tumors, including hepatocellular carcinoma, hepatoblastoma, and germ cell tumors. As a marker for hepatocellular carcinoma, AFP can be utilized either independently or as part of a L3-AFP index calculation [(AFP-L3 variant ÷ AFP Total) x 100%]. The µTASWako AFP-L3 assay (Wako Life Sciences, Inc.) instructions for use (IFU) lists reference intervals for the AFP-L3 assay, but no reference intervals are provided for the associated total AFP assay. As total AFP is often reported as part of an AFP-L3 index, this may lead to potential misinterpretation if no reference interval is given. **OBJECTIVE:** A method comparison was performed between the total AFP of the µTASWako AFP-L3 assay (Wako total AFP), and the Access total AFP assay (Beckman Coulter, Inc.; Beckman AFP). A reference interval determination was performed for both assays. **METHODS:** The reference interval study consisted of 140 apparently healthy individuals (120 adults and 20 pediatrics, evenly split by gender). These samples were collected from non-fasting patients, and the exclusion criteria included kidney disease, hepatic disease, autoimmune disorders, malignant neoplasms, pregnancy, and inflammatory bowel disease. A method comparison study was performed using these samples. Reference intervals at the 95th, 97.5th, and 99th percentiles were determined by non-parametric estimates. A separate method comparison was also performed using residual serum specimens (n =95) that extended a concentration range above the reference interval samples comparison. The analytical measuring range (AMR) of the Wako total AFP is 0.8 – 1000 ng/mL and is 0.5 – 3000 ng/mL for the Beckman AFP assay. **RESULTS:** The Passing Bablok regression fit analysis using the 140 reference interval specimens yielded the following total AFP equation: Wako total AFP (ng/mL) = 0.69 (Beckman AFP, ng/mL) - .0035 with a r^2 of 0.79. The data spanned ≤0.8 – 8.3 ng/ml for the Wako total AFP and 1.03 – 17.4 ng/mL for the Beckman AFP. Seventeen results were below the AMR of the Wako total AFP assay and were not included. The method comparison using the residual serum specimens (n=95) gave the following equation: Wako total AFP (ng/mL) = 0.99 Beckman AFP (ng/mL) - .3756 with a $r^2$of 0.99. This method comparison spanned 0.8 – 782 ng/ml (Wako AFP) and 1.24 – 877 ng/ml (Beckman AFP). The reference interval for Wako total AFP determined in the study was ≤3.3 at the 95th percentile, ≤3.4 at the 97.5th percentile, and ≤4.6 at the 99th percentile. The reference interval for the Beckman AFP determined in this study was ≤5.3 at the 95th percentile, ≤7.3 at the 97.5th percentile, and ≤8.3 at the 99th percentile. No significant differences were seen when partitioned by age or gender. The reference interval given in the Beckman IFU is <9.0 ng/mL (98.9th percentile, n=1126). **CONCLUSIONS:** While the methods agree well across the AMR, measured total AFP concentrations in the Wako assay differ significantly from the Beckman AFP assay in concentrations associated with healthy individuals. Reporting a separate reference interval for the Wako total AFP in the determination L3-AFP should be considered.

A-062

Serum YKL-40 as Biomarker for AFP-negative Hepatocellular Carcinoma

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Background: Alpha-fetoprotein (AFP) is the most widely used serum biomarker for hepatocellular carcinoma (HCC), despite its limitations. As complementary biomarkers, YKL-40, also called human Chitinase 3-like1, is a new biomarker for malignant tumor. Whether it may serve as a biomarker for AFP-

negative HCC remains unclear. This study aimed to investigate the usefulness of serum YKL-40 level as a biomarker for hepatocellular carcinoma (HCC). **Methods:** Enzyme linked immunosorbent assay was used to detect the serum YKL-40 level in 64 AFP-positive HCC patients ,93 AFP-negative HCC patients, 55 benign liver diseases (BLD) patients and 72 healthy controls (HC), respectively. The areas under the receiver operating characteristic(AUROC) curves of YKL-40, AFP and their combination were calculated and compared respectively. **Results:** It is showed that the optimal cut-off value to confirm a positive diagnosis of HCC was 69.48 ng/mL for YKL-40. The serum YKL-40 levels of all HCC patients (median 132.38 ng/mL, range 92.07-194.00) had significantly higher (P<0.001) than those patients with benign liver diseases (93.63 ng/mL, 57.94-136.22) or of a healthy control group (34.04 ng/mL, 18.10-59.40). The AUC of YKL-40 in AFP-negative HCC patients, AFP-positive HCC patients, and all HCC patients were 0.855 (95% CI, 0.804-0.905, P<0.01), 0.820 (95% CI, 0.756-0.884, P<0.01), and 0.841 (95% CI, 0.793-0.888, P<0.01), respectively. The sensitivity and specificity of YKL-40 for AFP-negative HCC patients, AFP-positive HCC patients, all HCC patients were 96.8% and 69.4%, 79.7% and 72.6%, 91.1% and 69.4%, respectively. When combining YKL-40 with AFP, the sensitivity and specificity were 96.8% and 72.6%, the AUC was 0.907 (95% CI, 0.873-0.942, P<0.01), which was statistically higher than that of AFP alone (AUC=0.862, 95% CI, 0.819-0.904, P<0.01). The results indicated that the diagnostic power improved significantly compared with either AFP or YKL-40 alone for HCC, and that YKL-40 had a better sensitivity for AFP-negative HCC diagnosis. **Conclusion:** Serum YKL-40 might be a better biomarker than AFP, and its combination with AFP may enhance the sensitivity of HCC. YKL-40 overexpression in serum had significant diagnostic power for AFP-negative HCC.

A-063

Diagnostic and high-grade cancer prediction performance of LDN-PSA glycosylation isoform

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Background: Although prostate specific antigen (PSA) is a widely used for prostate cancer (PCa) screening, the overdiagnosis and overtreatment of PCa due to low specificity of PSA is a major issue worldwide. To improve specificity, we focused PCa associated aberrant glycosylated PSA, LacdiNAc (LDN)-PSA, which has LacdiNAc structure on its *N*-glycan terminal. We have demonstrated a pilot study of LDN-PSA by automated immunoassay system which utilizes surface plasmon field-enhanced fluorescence spectroscopy (SPFS) as its detection principle. The aim of this study is to evaluate the diagnostic and high-grade PCa prediction performance of serum LDN-PSA directly compared to conventional PSA-based testing and indirectly compared PHI and PCA3 test. **Methods:** The serum LDN-PSA was measured by SPFS-based automated two step sandwich immunoassay system [Kaya, T. *et al.* Anal. Chem. 2015;87:1797-1803.]. Serum total and free PSA was tested on automated immunoassay analyzer Architect i1000 (Abbott Japan). All serum samples were collected before prostate biopsy (Pbx) and store at -80°C until use. To evaluate diagnostic and high-grade PCa prediction performance, a total of 528 patients with Pbx-proven benign prostatic hyperplasia (BPH, n=238) and Pbx-proven PCa (n=290) were enrolled. The grade group (GG) of Pbx specimens were evaluated according to the International Society of Urological Pathology guidelines. Predictive performance of each test was evaluated by ROC analysis. **Results:**Serum LDN-PSA levels in the range of total PSA <10 ng/mL and any PSA range were significantly higher in patients with PCa (median: 0.1175 U/mL and 0.2060 U/mL, respectively) than BPH (median: 0.0650 U/mL and 0.0670 U/mL, respectively), p<0.0001. At the cutoff LDN-PSA level (0.062 U/mL) for the prediction of PCa, the avoided biopsies rate was 45.4% at its 90% sensitivity, which was much higher than that of F/T ratio (32.5 %) and total PSA (18.8%). The AUC of LDN-PSA predicting PCa (0.8324; all range and 0.7462; <10ng/mL) was significantly higher than that of total PSA (0.7132 and 0.5715) and F/T ratio (0.7462 and 0.6899). Although, the patients background was different, we performed indirect comparison of diagnostic performance between LDN-PSA and FDA-approved markers. The diagnostic performance of LDN-PSA (AUC 0.74-0.83, NPV 82%, risk of missing PCa 10% and avoided biopsies rate 36-45%) is comparable to those reported value of PHI (AUC 0.70-0.77, NPV 67-92%, risk of missing PCa 8-33% and avoided biopsies rate 36%) and PCA3 (AUC 0.66-0.69, NPV 88-90%, risk of missing PCa 10-12% and avoided biopsies rate 44%). Serum LDN-PSA levels of PCa patients were much higher at Pbx GG 3 (median: 0.2100 U/mL) than Pbx GG 2 (median: 0.0915 U/mL), p=0.0001, while total PSA could not discriminate between Pbx GG 2 and 3. F/T ratio also much lower at Pbx GG 3 (median: 0.1765) than Pbx GG 2 (median: 0.3405), p=0.0004. The AUC of LDN-PSA predicting > Pbx GG3 (0.7947) showed quite better performance than that of total PSA (0.6005) and F/T ratio (0.7440). **Conclusion:**These data suggest that LDN-PSA improves diagnostic accuracy of PCa detection, which lead to large reduction of unnecessary biopsies. Predicting high-grade PCa patient by LDN-PSA could be used as a clinical index of patients under active surveillance.

A-064

Exosomal long non-coding RNA HOTTIP as a novel serum-based biomarker for diagnosis and prognosis of gastric cancer

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Objective: Long non-coding RNA HOTTIP plays important roles in the generation and progression of human cancers. Exosomes participate in cellular communication by transmitting molecular between cells and are regarded as suitable candidates for non-invasive diagnosis. However, the existence of HOTTIP in the circulating exosomes and the potential roles of exosomal HOTTIP in gastric cancer (GC) was poorly understood. This study aims to evaluate the existence of HOTTIP in the circulating exosomes and the potential roles of exosomal HOTTIP in GC. **Methods:** Exosomal HOTTIP was firstly detected in cell culture of GC SGC7901 cell line. Then, the stability of serum exosomal HOTTIP was evaluated by prolonged exposure to room temperature and treated with multiple freeze-thaw cycles. Finally, exosomal HOTTIP levels were detected by reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) in sera of 246 subjects (126 GC patients and 120 healthy people). Receiver operating characteristic (ROC) curves and Cox analyses were used to evaluate its diagnosis and prognosis value, respectively. And the clinical value of exosomal HOTTIP were compared with traditional biomarkers, including CEA, CA 19-9 and CA 72-4. **Results:** Exosomal HOTTIP could be detected in culture medium of GC cell line, and the levels were increased with the incubation time extended. Exosomal HOTTIP were not affected after treated with prolonged exposure to room temperature or 3 freeze-thaw cycles. Levels of exosomal HOTTIP were also upregulated in GC patients than in normal controls (P < 0.001). And the levels were significantly correlated with invasion depth (P = 0.0298) and TNM stage (P < 0.001). The AUC for exosomal HOTTIP was 0.827, which demonstrated a higher diagnostic capability than CEA, CA 19-9 and CA72-4 (AUC = 0.653, 0.685 and 0.639, respectively) (P < 0.001). The Kaplan-Meier analysis showed a correlation between increased exosomal HOTTIP levels and poor overall survival (OS) (logrank P < 0.001), while no significant relationship was observed between OS and traditional tumors (CEA, CA 19-9 and CA72-4). And univariate and multivariate COX analysis revealed exosomal HOTTIP overexpression was an independent prognostic factor in GC patients (P = 0.027). **Conclusion:** Exosomal HOTTIP is directly released from GC cells, and may be a better biomarker for GC in diagnosis and prognosis than CEA, CA 19-9 and CA72-4.