Tuesday, August 2, 2016

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

Cancer/Tumor Markers

A-002

Unusual Case of Follicular lymphoma presenting IgM Monoclonal Gammopathy overlapped with Polyclonal peak in Capillary electrophoresis

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BACKGROUND: Follicular lymphoma (FL) represents a third of non-Hodgkin lymphoma (NHL) in western countries. Although the association of monoclonal gammopathy (MG) in B-cell NHL is a well-known phenomenon, the precise incidence rate among subtypes of NHL and the prognostic significance is still unclear. Especially, the association of MG with FL has been rarely reported in asian population. We report a case of follicular lymphoma showing IgM and Kappa chain restriction accompanying with polyclonal gammopathy detected in capillary electrophoresis.

METHODS: We investigated an unusual case of a 63-year-old male patient who was diagnosed to FL presenting IgM MG overlapped with polyclonal peak in capillary electrophoresis. We performed CBC tests using Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY) and biochemical tests with Toshiba chemical analyzer (Toshiba, Nasushiobara, Japan). The monoclonal components were detected in capillary electrophoresis (CE) via capillary 2 (Sebia, Lysse, France) and reconfirmed through a conventional gel electrophoresis (EP) with high-resolution gel EP in a Hydrasys analyzer (Sebia) using Hydragel 15 HR gels (Sebia). To confirm the diagnosis of lymphoma, endoscopic biopsy and bone marrow biopsy were performed in this patient.

RESULTS: Computed tomography of neck revealed highly suggestive of lymphoma with multiple enlarged conglomerated lymph nodes along both internal jugular veins, submandibular, parotid, supraclavicular, superior mediastinal. Endoscopic incisional biopsy of both lingual tonsilar mass was done and a final diagnosis of FL was made. Peripheral blood finding was unremarkable except normocytic normochromic anemia. Bone marrow aspiration showed normocellular marrow pattern except slightly increased plasma cell portions to 5.4%. In the bone marrow clot sections, there were several nodular lesions composed of various-sized lymphocytes, suggestive of lymphoma infiltration. Immunohistochemical staining on this portion of cell aggregations on Rt and Lt clot sections were done and showed CD20, BCL2, BCL6 positive reaction. Serum electrophoresis showed a distinct M-peak in front of a broad polyclonal peak. Due to the partial overlapping between monoclonal and polyclonal peaks, gamma region of serum electrophoresis test presented discrete dual peaks. Following immunotyping electrophoresis tests with immunosubtraction method clearly revealed MG of IgM and Kappa type in spite of overlapped polyclonal gammopathy. The gel electrophoresis showed corresponding result with CE. Finally the patient was diagnosed as stage 4 FL accompanying IgM MG and was started on chemotherapy.

CONCLUSIONS: Our report shows an unusual case of IgM and Kappa type MG overlapped with polyclonal gammopathy in a patient with FL. Although in aggressive B-cell NHL, the presence of MG might be an adverse prognostic factor, the definite clinical significance of MG in B-cell NHL has not been clarified. As reported by others, FL accounts for minor portion of lymphoid neoplasms associated with serum IgM paraprotein. IgM paraproteinemia may also, however, be seen in other B-cell lymphoproliferative disorders including Waldenstrom macroglobulinemia and chronic lymphocytic leukemia. Thus, careful differential diagnosis among these diseases is critical to apply proper treatment. Further studies are also necessary to estimate the value of paraprotein profile as an early indicator for a hidden lymphoma, a tool for evaluating a prognostic outcome and disease severity in lymphoma patients.

A-004

Genomic DNA Breakpoints in *MLL* Gene in Infants with Acute Leukemia

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Background. There are several prognostic factors in infant acute leukemia (AL), including high white blood cell count, presence of 11q23/*MLL* rearrangement, CNS disease, response to initial steroid monotherapy. Although less is known about prognostic factors within group of infant AL patients carrying 11q23/*MLL* rearrangements. So the aim of this study was to evaluate the relation between genomic DNA breakpoints in *MLL* and clinical parameters of infant AL.

Methods. 87 infants (32 boys (37%) and 55 girls (63%) with median age of 4.9 months) with *MLL*-rearranged acute lymphoblastic leukemia (ALL) (n=63), acute myeloid leukemia (AML) (n=22) and mixed phenotype acute leukemia (MPAL) (n=2) were included in the current study. Genomic DNA breakpoint detection in *MLL* gene was performed by long-distance inverse PCR.

Results. Majority of ALL cases was characterized by presence of *MLL-AF4* fusion gene (FG) (n=35;55%), less frequently *MLL-MLLT1* (n=12;22%), *MLL-MLLT3* (n=8;13%) and others were found. The most common breakpoint location within *MLL* gene in ALL patients was intron 11, detected in 31 cases (49%), less frequently breakpoints in intron 10 (n=13;21%) and intron 9 (n=9;14%) were found. The highest variability of *MLL* breakpoints was found in *MLL-AF4*-positive patients: only 15 of 35(43%) had breakpoints in intron 11. The most stable pattern of *MLL* genomic DNA breakpoints was observed in *MLL-MLLT1*-positive patients: 9 of 14 (64%) had breakpoints in intron 11. In AML patients the most prevalent FG was *MLL-MLLT3* (n=8;36%). The most frequent breakpoint location was intron 9 (n=10;45%), less often they were found in intron 10 (n=5;23%) and 11 (n=4;18%). The most stable pattern was revealed for *MLL-MLLT10* FG: *MLL* breakpoints in 4 of 5 (80%) cases were found in intron 9. Distribution of DNA breakpoints in *MLL* gene was similar in boys and girls and did not depend on type of translocation partner gene. ALL patients who had breakpoints in intron 11 were significantly younger (median 3.0 mo, range 0.03-11.6) than all others (median 5.6 mo, range 0.7-11.9) (p=0.025) and than patients with *MLL* breakpoints in intron 9 (median 6.6 mo, range 3.1-11.9)(p=0.017). For AML cases we did not find any relation between age and breakpoint locations. We estimated prognostic significance of *MLL* breakpoint locations in 46 cases of infant ALL uniformly treated by multicenter MLL-Baby protocol. 5-year even-free survival was significantly lower in patients with breakpoints in intron 11 (n=29) in comparison to patients with breakpoint localized from intron 7 to exon 11 (n=17) (0.16±0.07 vs 0.38±0.14 p=0.039). While cumulative incidence of relapse was remarkably higher in the first group of patients (0.74±0.09 vs 0.52±0.17 p=0.045). Median follow-up time was 42 months. Although in Cox regression model including breakpoint location in intron 11 together with age, immunophenotype, initial white blood cell count, initial CNS involvement, type of *MLL* rearrangements, absolute blast number at day 8 of dexamethasone profase, minimal residual disease (MRD) at time point 4 (TP4) of MLL-Baby protocol, the only significant covariate was the presence of MRD at TP4 (HR 5.994, 95% CI 2.209-16.263, p<0.001).

Conclusions. Our data provide new information of molecular genetic features of *MLL*-rearranged infant AL.

A-007

Urinary cell-free microRNA expression signatures serve as novel noninvasive biomarkers for diagnosis and recurrence prediction of bladder cancer

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Background: Cell-free microRNA (miRNA) open up a new field for noninvasive diagnosis and prognosis of bladder cancer (BC) based on their stability in urine supernatant. The aim of the present study was to investigate the role of cell-free miRNA in diagnosing and predicting recurrence of BC.

Methods: Miseq sequencing was performed to identify candidate miRNAs for diagnosing cancer in a screening cohort of 12 participants (BC patients and controls). Real-time polymerase chain reaction was employed to evaluate the expressions of candidate miRNAs which were then analyzed by logistic regression in 540 participants. Meanwhile, urine cytology was conducted for comparison with the miRNA panel and correlation between miRNAs and tumor recurrence was further assessed in the validation cohort. **Results:** We identified a seven-miRNA panel (miR-22-3p, miR-29a-3p, miR-375, miR-7-5p, miR-126-5p, miR-423-5p and miR-200a-3p) that provided high diagnostic accuracy of BC with an AUC of 0.923 and 0.916 for training and validation set, respectively. The corresponding AUCs of this panel for Ta, T1 and T2-T4 were 0.864, 0.930 and 0.978, significantly higher than those of urine cytology, which were 0.531, 0.628 and 0.724, respectively (all at $p < 0.05$). Moreover, nonmuscle-invasive BC (NMIBC) patients with high miR-22-3p level and low miR-200a-3p level had worse recurrence-free survival (RFS) ($p = 0.002$ and $p = 0.040$, respectively). MiR-22-3p and miR-200a-3p were independently associated with recurrence of NMIBC ($p = 0.024$ and $p = 0.008$, respectively).

Conclusion: MiRNA expression signatures from urine supernatant may have considerable clinical value in diagnosis and recurrence prediction of BC.

A-008

Combining prostate cancer antigen 3 (PCA3) and prostate-specific antigen (PSA) improves diagnostic accuracy in men at risk of prostate cancer

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Background: Prostate cancer (PCa) is the most common cancer and the second leading cause of cancer-related death in men of the United States. Currently, serum prostate-specific antigen (PSA) is widely used as an aid for screening, diagnosis, and patient management of PCa. However, the low specificity of PSA results in unnecessary invasive biopsy and high negative biopsy rate. Prostate cancer antigen 3 (PCA3) is a non-coding prostate-specific mRNA that is highly overexpressed in PCa tissue and excreted in urine in PCa patients. The objective of this study is to assess the clinical utility of urinary PCA3, and to compare the performance characteristics of urinary PCA3 and serum PSA test in men at risk of prostate cancer.

Methods: A cohort of 142 men (mean age 64 years; range 43-79 years) with elevated PSA, and/or strong family history, and/or abnormal digital rectal examination (DRE) were investigated. Urinary PCA3 mRNA level and score were assessed using the Progensa assay and serum PSA was tested on TOSOH automated enzyme immunoassay analyzer AIA-2000. Diagnosis of PCa was confirmed by biopsy using a 12- or 18-core biopsy scheme. The performance characteristics including diagnostic sensitivity, specificity, positive and negative predictive values (PPV, NPV), and test efficiency were evaluated. Area under the receiver-operating characteristic curve (AUC) was quantified using R software to compare the performance of PCA3, PSA, and combination of the two biomarkers. A multivariable logistic regression analysis was conducted to incorporate the two biomarkers and other risk factors such as age, race, family history, DRE, and prostate volume.

Results: Of the 142 patients, 10 (7.0%) were excluded with no biopsy analysis. Among the 132 patients underwent biopsy, 40 (30.3%) were detected with prostate adenocarcinoma. Urinary PCA3 score at the cutoff value of 25 had a diagnostic sensitivity of 77.5%, specificity of 51.1%, PPV of 40.8%, NPV of 83.9%, and test efficiency of 59.1%. Serum PSA had a sensitivity of 87.5% and 25.0%, specificity of 22.8% and 84.8%, PPV of 33.0% and 41.7%, NPV of 80.8% and 72.2%, and test efficiency of 42.4% and 66.7%, at the cut-off of 4 ng/mL and 10 ng/mL, respectively. The AUCs for PCA3 and PSA were 0.697 and 0.577 respectively (P=0.14). A logistic regression algorithm combining PCA3 with PSA increased the AUC from 0.577 for PSA-alone to 0.708 (P= 0.02). Combination of urinary PCA3 score and serum PSA also improved the performance characteristics with a diagnostic sensitivity of 67.5%, specificity of 63.0%, PPV of 37.5%, NPV of 77.3%, and test efficiency of 64.4%. Incorporating patients' demographic and clinical characteristics did not significantly improve the performance of the combined biomarkers.

Conclusion: Our data suggest that PCA3 improves the diagnostic sensitivity and specificity and the combination of PCA3 with PSA gives a better overall performance characteristics in identification of PCa compared with serum PSA alone in high risk population. Implementing the urinary biomarker PCA3 together with serum PSA measurement into clinical practice would guide effective biopsy and lead to a considerable reduction of the number of unnecessary prostate biopsies.

A-009

Circulating plasma microRNAs as potential biomarkers for HCV related hepatocellular carcinoma in Egyptian patients

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Background: Circulating microRNAs are aberrant in cancer patients so the potential use of microRNAs (miRNAs) as ideal tumor markers has been the focus of recent research**.**

Objective: Our hypothesis was that circulating miRNAs are differentially expressed in pretherapeutic sera of HCV related hepatic cancer patients compared to controls and to HCV induced chronic liver disease to find out their ability in differentiate among them.

Materials and Methods: Two stages procedure, the first one aimed to determine which microRNAs are aberrant in HCV related hepatic cancer patients' pool (ten patients) compared to 10 normal donors' pool using Human Liver miFinder miRNA PCR Array. The second stage was done by using real-time quantitative polymerase chain reaction (qPCR) analysis, levels of six candidate miRNAs (miR-122-p5, miR-192-p5, miR-106b-p5, miR-34a-p5, miR-195-p5 and miR-199a-p5), which were chosen based on the previous miRNAs array step, were quantified in sera of 70 HCV related hepatic cancer patients, 50 HCV induced chronic liver disease and 50 healthy controls.

Results: Generally, increased expression levels of some microRNAs (miR-122-p5, miR-192-p5, miR-106b-p5 and miR-34a-p5) were noticed in HCC patients' pool as compared to healthy controls' pool while others (miR-195-p5 and miR-199a-p5) showed decreased expression levels. A diagnostic accuracy of a panel made of combination of 6-serum miRNAs which included in this study was evaluated , ROC curve showed that AUC was 0.990 (95% CI: 0.943 - 1.000, P <

0.001). In discrimination between study groups, this panel showed an excellent diagnostic performance with higher AUC as compared to each studied miRNA separately. When comparing between HCC patients and healthy controls the AUC of 6-serum miRNAs panel was 1.000 (95% CI: 0.951 - 1.000, P < 0.0001). The AUC was 0.977 when comparing between HCC and CLD patients (0.912 - 0.998) and was 0.924 when comparing between CLD patients and healthy controls. logistic regression was made to determine the best predictor miRNA considering P value < 0.05 a probability of entry. MiR-195 and miR-192 were the best predictors (p = 0.0155 and 0.0275 respectively). A second 2-miRNAs (miR-195/miR-192) panel was made and its diagnostic performance is evaluated. The ROC curve of the 2-miRNAs panel showed that AUC was 0.978 (995% CI: 0.925 - 0.997, P< 0.001). The 2-miRNAs panel was also excellent in discriminating between HCC patients and healthy controls (AUC=0.996, 95% CI: 0.942 - 1.000, P < 0.0001) and also between HCC and CLD patients (AUC=0.961, 95% CI: 0.886 - 0.992, P < 0.0001).

Conclusion: These findings suggest that systemic circulating miRNAs have potential use as novel biomarkers for diagnosis of HCV related hepatic cancer patients and at least five of them can be used as early diagnostic marker to differentiate between HCV related hepatic cancer patients and HCV induced chronic liver disease . However, future larger studies are needed to confirm our findings.

A-010

novel flowcytometry-based approach to detect tumor cells in body fluid using sysmex automated hematology analyzer

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Background: Nucleated cells differential analysis of body fluid (BF) samples is important diagnostic tool for several diseases including cancer metastasis. Detection

of tumor cells in BF requires the manual microscopic scanning of slides by the cytopathologist to visually identify cells with suspicious features, which is time-consuming and labor-intensive. Furthermore, the cytological examination of BF for detection of malignancy is not always reliable, because of a relatively low overall sensitivity rates (ranging 40-90%) with the higher false-negative rates for lymphomas and mesotheliomas. This study aimed to develop the scattergram gating analysis for detection of tumor cells in BF using the BF mode on the Sysmex automated hematology analyzer XN-1000 (XN BF mode; Sysmex, Kobe, Japan).

Methods: We used a total of 220 BF samples (53 cerebrospinal fluids, 73 pleural or ascitic fluids, and 94 peritoneal dialysis effluent) obtained from patients with cytological diagnoses (papanicolaou stain) including negative and positive for malignancy, and chronic inflammation with an elevated lymphocyte and histiocyte fractions. As a reference method, morphological manual differential (200 cells counts) was performed by two experienced technologists using cytospin slides stained with the May-Grunwald Giemsa. The gating criteria were based on the WDF scatter plots; #1: detect the cells with larger and clumped cell signal in comparison with general leukocytes, which mainly derived from clustered tumor cells, #2: to detect the middle sized mononuclear cells with less granules rather than neutrophils and similar fluorescence signal to monocytes, which targeting hematological malignant cells and solid tumor cells. BF samples that meet at least one criterion were interpreted as positive for tumor cells.

Results: The malignant BF samples containing tumor cells showed the different scattergram patterns from the benign ones with chronic inflammation. Our scattergram gating analysis achieved an overall sensitivity of 78.6% and specificity of 97.1% in detecting tumor cells positive samples when screening against all samples outcomes. The positive predictive value was 64.7% and the negative predictive value was 98.5%. For the samples of positive for malignancy and/or chronic inflammation (n=125) by morphological manual differential, the sensitivity and the specificity were 78.6% and 94.6%, respectively, with 64.7% of the positive predictive value and 97.2% of the negative predictive value. For the samples with absence of tumor cells and inflammatory observations (=95), no false positive was detected.

Conclusion: A simple measurement of BF by automated hematology analyzer in which cells are minimally handled has a potential to reduce costs and allow routine cell screening in clinical applications. For BF malignancy diagnostics, a scattergram gating analysis is promising to (i) augment diagnostic routines without requiring additional sample preparation procedure, (ii) limit operator bias, and (iii) provide a standardized measurement.

A-011

Development of a New Biochip Array for the Simultaneous Detection of Pepsinogen I, Pepsinogen II and Gastrin 17

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Background: Atrophic gastritis is a condition that is associated with a significantly higher risk of developing gastric cancer; the fifth most common cancer worldwide. Atrophic gastritis involves a loss in the gastric glands, affecting the secretion of pepsinogen II (PGII) from all areas of the stomach and pepsinogen I (PGI) and gastrin 17 (G17) more specifically from the corpus and antrum. During atrophic corpus gastritis, the levels of PGI in circulation are decreased. The ratio of PGI to PGII (which is produced by chief cells in the gastric mucosa) is also lowered. G17 is a crucial peptide hormone of the gastrointestinal tract and is secreted by the gastrin cells in the antrum. During antral atrophy the levels of G17 are ultimately decreased. These serum biomarkers therefore, are valuable in the screening of atrophic gastritis and can provide a comprehensive diagnosis on the condition of the stomach mucosa.

Enzyme-linked immunosorbent assays (ELISAs) have been developed for the single detection of PGI, PGII and G17 in serum and plasma (Biohit Oyj, Helsinki, Finland). Applying ELISA principles, Biochip Array Technology (BAT) allows the multiplex determination of analytes from a single sample. Therefore this collaborative study aimed to develop a biochip array for the simultaneous detection of PGI, PGII and G17 in serum/plasma in order to provide a patient profile to facilitate the non-invasive screening and diagnosis on the condition of stomach mucosa.

Methods: Simultaneous chemiluminescent sandwich immunoassays were employed, the anti-human capture antibodies were immobilised on the biochip surface defining discrete test sites. The immunoassays were applied to the Evidence Investigator analyser.

The multi-analyte calibrators were developed using native human antigen. A correlation study was carried out on a cohort of 76 serum/plasma samples using this biochip array and individual ELISAs (Biohit Oyj, Helsinki, Finland).

Results: Nine-point calibration curves for each individual analyte were simultaneously generated. The assay ranges were 0-200ng/mL for PGI, 0-50ng/mL for PGII and 0-40pmol/L for G17. Cross-reactivity testing demonstrated that each individual assay was specific for its target analyte (<1% cross-reactivity with the other analytes). When 76 serum/plasma samples were tested using BAT and individual ELISAS, the regression analysis showed the following values for the coefficient of

determination (r^2) and slope: PGI assay r^2=0.826, slope 0.7267; PGII assay r^2=0.9439, slope 0.929 and G17 assay r^2=0.9816, slope 1.068.

Conclusions: The results of this collaborative study indicate applicability of BAT to the simultaneous measurement of PGI, PGII and G17 from a single serum/plasma sample. Good agreement was found between this technology and individual ELISAs. The use of this biochip array facilitates the screening and diagnosis of patients at risk of developing gastric cancer and offers advantages over current diagnostic methods such as gastroscopy, which can be highly invasive and costly. This newly developed array uses low sample volume and will offer a cost effective and efficient method of testing for patients.

A-012

LncRNAs expression can distinguish B and T lineage acute lymphoblastic leukemias

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Background: In the late 1990s, gene expression profiling (GEP) based on microarray studies showed that different leukemia subtypes had distinct gene signatures. Microarray technology is not currently applied to leukemia diagnosis because of its high cost and need of deep technical expertise, but it provides a tool for biomarker discover and improvement of the knowledge of the cellular pathways deregulated in the disease. In a previous GEP study, we identified genes differently expressed in acute lymphoid and myeloid leukemias, some of which are long non-coding RNAs (lncRNAs). Recently, lncRNAs have been associated to cancer, including hematological malignancies, although evidences of their role in acute lymphoblastic leukemias (ALL) are still scarce. Objective: The aim of the present study was to investigate lncRNAs that can distinguish B lineage ALL from T lineage.Patients and Methods: 97 Brazilian patients diagnosed with ALL were included in this analysis. RNA was extracted from bone marrow samples obtained at diagnosis using PAXgene Bone Marrow RNA (QIAGEN/BD, Valencia, CA, USA). RNA integrity was determined on Bioanalyzer™ 2100 (Agilent TechnologiesPalo Alto, CA, USA). Gene expression was carried out using the Low Input Quick Amp Labeling kit - One Color and the Sure Print G3 Human GE 8 x 60K array (Agilent Technologies). Data were extracted with the Feature Extraction Software v7.5 and normalized using the Gene Spring software v12.5 (Agilent Technologies). From a set of 32,640 probes, 60 were selected in a supervised analysis using K-Nearest Neighbor (K-NM) algorithm, in order to build an effective classifier based on the differential gene expression signatures of B-ALL and T-ALL patients. We searched those probe´s sequences in genomic databanks, identified the ones that corresponded to lncRNAs and performed an unsupervised Hierarchical Clustering analysis with them to group ALL individuals with close gene expression patterns. Programs were run within a local installation of the GenePattern suite (Broad Institute). Differential expression was validated with TaqMan quantitative reverse transcription real time PCR assays (ThermoScientifc) probes. Results: We identified nine lncRNAs differentially expressed between B-ALL and T-ALL. Six lncRNAs were upregulated in T-ALL (NCBI Genbank sequences XR_248137, NR_034143, NR036502, NR036476, NR_104614, NR_015410), while three were down-regulated (NR_027406, NR_026779, NR_040662). None of them has been previously related to ALL, however, two were reported as associated to cancer: the NBAT1 (CASC14) that suppresses neuroblastoma growth and the CASC15 that is involved in melanoma progression. qRT-PCR confirmed microarray data in four out of the five markers tested ($p<0.05$). B-ALL and T-ALL patients could be clustered separately based solely on the expression data of these lncRNAs. Conclusion: lncRNAs have emerged as an important class of molecules that regulate cell processes. Our findings corroborate the hypothesis that these transcripts are relevant to the biology of acute lymphoblastic B and T leukemias. Besides their role as lineage biomarkers, lncRNAs should be investigated as potential targets for drug and therapy development.

A-013

EGFR analysis in cfDNA reflects tumor heterogeneity and has prognostic value in non-small cell lung cancer

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Background: Mutation analysis of epidermal growth factor receptor (*EGFR*) gene is essential for treatment selection in non-small cell lung cancer (NSCLC). Treatment with EGFR inhibitors is indicated for patients with EGFR activating mutations. Cell-free DNA (cfDNA) has been proposed as a less invasive and more informative alternative to tissue biopsy. We evaluated the clinical utility of *EGFR* mutation analysis in cfDNA from NSCLC patients by droplet digital PCR (ddPCR). Evaluated mutations were the two most prevalent EGFR activating mutations (L858R and delE746-A750) and T790M mutation, associated with resistance to treatment with EGFR inhibitors.**Methods:** We selected 36 NSCLC patients with *EGFR* activating mutations detected in cytological samples obtained by fine-needle aspiration and negative for T790M mutation, including 29 patients with advanced disease (63 ± 22 years, 69% female and 55% never smokers) and 7 patients with early stage NSCLC (64± 24 years, 43% female and 43% never smokers). Wild-type *EGFR* copies and mutated copies for L858R, delE746-A750 and T790M mutations were analyzed by ddPCR in a QX100 system (Bio-Rad) in cfDNA isolated from plasma at baseline and during treatment at best response, pre-progression and progression.

Results: First, we evaluated ddPCR sensitivity and found that for the three *EGFR* mutations, we could quantify maintaining linearity, mutated copies diluted as much as 0.005% in wild-type DNA. *EGFR* mutations were detected in basal cfDNA from 71% of advanced stage patients with positive cytological samples. Concordance between cytological samples and plasma results for EGFR activating mutations was 62%, being only significant for L858R mutation (87%, p=0.001). We detected in cfDNA mutations in patients with negative cytological samples: L858R in 12% of patients, delE746-A750 in 31% and T790M in 13%. We even detected *EGFR* double mutations in 17% of patients, which only presented one of them in cytological sample. Total *EGFR* copy levels in cfDNA in stage I patients were lower than in stage IV (1003 copies/mL versus 3523 copies/mL; $p<0.01$). Patients with basal concentration of *EGFR* activating mutations higher than 94 copies/mL had lower overall (317 versus 805 days; $p<0.05$) and progression free survival (195 versus 724 days; $p<0.05$) than those with lower levels. Similar findings were observed for total *EGFR* copy levels for a cut-off of 3462 copies/mL. Although we observed a decrease in EGFR activating mutations levels for between baseline and best response, this decrease did not reach significance. During follow-up, T790M was detected in 53% of patients. **Conclusion:***EGFR* cfDNA analysis by ddPCR seems a relevant tool for clinical management of NSCLC patients

A-014

A Laboratory Validation of the Dual Measurement of hCG and AFP in CSF

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Background: Primary central nervous system (CNS) germ cell tumors are a rare, heterogeneous and a diagnostically challenging group of neoplasms. The primary diagnosis of intracranial germ cell tumors can be aided by the analysis of cerebrospinal fluid (CSF) for increased concentrations of human chorionic gonadotropin (hCG) and α-fetoprotein (AFP). Following successful treatment new-onset elevations of hCG and AFP in CSF can precede radiologic or symptomatic tumor detection; however, the matrix effect on alternate sample types such as CSF should be taken into consideration prior to analysis as it can impact test results. Assays need to be validated before use in the clinical laboratory. Prior studies have validated measurements of total hCG and AFP in CSF on the Siemens Centaur; here we report on the validation of hCG and AFP assays in CSF on the Roche COBAS 6000 (Roche Diagnostics, Indianapolis, IN, USA). The objective of our study was to perform an in-house validation of total hCG and AFP concentrations in CSF on the Roche COBAS 6000.

Methods: Institutional Review Board approval was obtained prior to beginning the study. Validation testing was performed on remnant CSF sampled from physician-ordered clinical testing at Dartmouth-Hitchcock Medical Center. Serum samples with high AFP or hCG concentrations were used to spike aliquots of pooled CSF. Precision studies, linear range, limit of quantitation and carryover studies of the hCG and AFP assays on the COBAS 6000 analyzer were performed and data analyzed using available templates in EP Evaluator.

Results: Within day precision studies demonstrated acceptable imprecision of 2.4% and 4.7% for AFP concentrations of 12.0ng/mL and 602.8ng/mL and acceptable imprecision of 1.8% and 3.6% for hCG concentrations of 10.5mIU/mL and 118.8mIU/mL. Day-to-Day precision studies demonstrated acceptable imprecision of 5.6% and 3.7% for AFP concentrations of 11.6ng/mL and 538.6ng/mL and acceptable imprecision of 3.4% and 2.1% for hCG concentrations of 9.9mIU/mL and 110.5mIU/mL. The LOB, LOD and LOQ (10% CV) of hCG were 0.4 mIU/mL, 0.8 mIU/mL and 0.8 mIU/mL, respectively. The Linear Range for the hCG assay was established as 1.0 to 10,000 mIU/mL. The LOB, LOD and LOQ (20% CV) of AFP were 1.1 ng/ml, 1.6 ng/ml and 1.6 ng/ml, respectively. The Linear Range for the AFP assay was established to be 1.6 to 1100 ng/mL. The recovery experiment demonstrated no appreciable matrix effect with AFP and hCG recovery differing less than 10% of the target concentration, with the exception of the level one AFP sample. This sample demonstrated over-recovery of 18%; however this over-recovery was deemed analytically acceptable and likely due to a combination of clearer CSF matrix and assay imprecision.

Conclusion: The Roche COBAS 6000 total hCG and AFP assay can accurately quantify hCG and AFP in CSF facilitating the rapid and accurate diagnosis and monitoring of germ cell tumors.

A-015

Detection and characterization of serum free light chains by MALDI-TOF MS in immunofixation electrophoresis-negative specimens with abnormal free light chain ratios

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Background: Monoclonal free light chains (FLCs) play an important supportive role in diagnosis, prognosis, and monitoring of monoclonal gammopathies. FLCs are secreted in larger quantities by abnormal plasma cells undergoing clonal expansion. Quantitative immuno-nephelometric serum FLC (sFLC) assays are used to measure concentrations of circulating kappa (K) and lambda (L) chains unbound to their heavy chains. An abnormal K/L FLC ratio can indicate a low abundance monoclonal clone which is typically undetected by serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Patients with nonsecretory multiple myeloma, light chain multiple myeloma, primary systemic amyloidosis and light chain deposition disease often are only detected by the FLC ratio. On the other hand, hypergammaglobulinemia patients can also present with abnormal FLC ratios. There is a need to directly detect monoclonal FLCs independent of the K/L ratio. Recently, we have developed a sensitive technique that uses nanobody enrichment-coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for detection of monoclonal proteins in serum.

Objective: The objective of this study was to evaluate the ability of a MALDI-TOF MS method to detect monoclonal FLCs in IFE-negative patient sera with abnormal K/L FLC ratios.

Methods: Residual patient serum specimens (n=48) that were negative by IFE and displayed an abnormal FLC ratio (K/L <0.26 or >1.65) were collected for analysis. Nanobody enrichment was performed with CaptureSelect™ affinity resins to purify IgG, IgM, IgA, K light chains and L light chains. Specimens were reduced to dissociate heavy and light chains. Additionally, FLCs were affinity purified with sepharose beads conjugated with polyclonal antibodies used for the sFLC assay (n=31), which have been shown to have high specificity towards FLCs and low cross-reactivity with light chains bound to heavy chains. Purified specimens were subjected to MALDI-TOF MS in automated acquisition mode (Bruker Microflex). FlexAnalysis software was used to interrogate spectra for isotypes and the molecular masses of monoclonal proteins.

Results: Monoclonal abnormalities were detected in 14 (29%) of nanobody-purified serum samples analyzed by MALDI-TOF MS, thereby supporting positivity by the sFLC assay. These included monoclonal GK isotypes (n=7), free K (n=3), AK (n=2), GK and K FLC (n=1), and a GL (n=1) of varying molecular masses. Seven of these specimens had evidence of monoclonal FLC proteins based on sFLC antibody purification spectra. Interestingly, all three specimens of free K isotype had FLC ratios greater than 100. FLCs were detected in nine additional specimens purified with sFLC assay antibodies. FLC ratios of specimens with detectable FLC were significantly higher than those without (p-value=0.0002, Mann Whitney U test). The remaining specimens (46%) did not harbor any detectable monoclonal protein abnormalities according to MALDI-TOF MS, thereby, corroborating the negative IFE.

Conclusion: MALDI-TOF MS is a rapid monoclonal immunoglobulin isotyping method that generates information rich spectra. This study suggests that IFE-negative serum with abnormal K/L FLC ratios may harbor monoclonal abnormalities undetectable by current routine laboratory methods. Further work is underway to corroborate these findings.

A-016

Two missense mutations in a female patient with a strong familial history of breast cancer: A case report

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Background: Breast Cancer is the most common cancer in women and is the first leading cause of women cancer death in developing countries. Death rates have been declining and it is believed that this decrease is result of earlier detection through increased awareness, screening and improved treatment. Around 5-10% of these tumors present a strong hereditary component due to mutations in highly penetrant genes. *BRCA1* and *BRCA2* are the two most frequently mutated genes and account for up to 45% hereditary breast cancers. Recent advances in sequencing technologies allowed the discovery of several novel genes related to breast cancer increased risk, such as *PALB2*. **Case report:** This report describes a 59-year-old female who was diagnosed with invasive breast cancer at age 38. She presented an important familial history of different types of cancers on both sides of the family. On her mother's side, five cases of breast cancer (three female cousins, one aunt and one great-aunt), one case of ovarian cancer (the same great-aunt with breast cancer), one grandmother with uterine cancer and one male cousin with stomach cancer. On her father's side, only one uncle with bone cancer. Since she was *BRCA* mutation negative, breast cancer screening was done by next generation sequencing in 15 related genes: *ATM, BARD1, BRIP1, CHEK2, CDH1, MRE11A, NBN, PTEN, PALB2, RAD50, RAD51C, RAD51D, STK11, TP53,*and *XRC22*. Two heterozygous mutations were identified: a known benign mutation in exon nine of the *PALB2* gene (rs45551636: c.2993G>A; p.G998E) and a previously undescribed mutation in exon nine of *MRE11A* gene (c.908C>T; p.T303I). Prediction programs SIFT and PolyPhen-2 classified this mutation as possibly damaging. Onlineprediction program Mutation Taster suggested that this variant is a disease causing mutation with a probability value of 0.999 since the protein structure might be affected due to splicing changes. In addition, this is a variant of unknown clinical significance (VUS) and need to be further investigated. We considered as VUS undescribed missense mutations or described variants with minor allele frequency (MAF) <0.02.**Conclusions:** Breast cancer increased risk is linked to genetic factors and shared lifestyle factors. Genetic screening of these novel susceptibility genes in families with a strong history of the disease is of utmost importance for clinical diagnosis, appropriate treatment, prophylactic interventions and genetic counseling, since it significantly impact patient's and family member'swell-being and survival.

A-017

Evaluation of the Immunoassay Reagent Kit for PIVKA-II (ARCHITECT® PIVKA-II) for the Fully-Automated Chemiluminescent ARCHITECT Analyzer

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Background: Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is used as an aid in the diagnosis of hepatocellular cancer (HCC), in monitoring of high risk patients for development of HCC, and in the management of HCC. The goal of this study was to evaluate the analytical performance of the ARCHITECT PIVKA-II assay.

Methods: The ARCHITECT PIVKA-II assay is a quantitative two-step, double monoclonal antibody sandwich assay (3C10 and MCA1-8), for the fully automated chemiluminescent ARCHITECT *i* Systems analyzer. This assay has an assay time of approximately 29 minutes and an analytical range of 0.00 to 30,000.00 mAU/mL. Precision was performed based on guidance from National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-A2. The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) was performed based on guidance from CLSI Document EP17-A2. Linearity was performed based on guidance from NCCLS Document EP6-A. Potential interferents were performed based on guidance from CLSI Document EP7-A2. Correlations to Picolumi and Lumipulse PIVKA-II assays were performed with samples sourced from European and USA sites.

Results: The within-run and total imprecision showed %CVs of 1.9-5.0 and 2.0-8.6 over the range of 38.55 to 26880.54 mAU/mL. The LoB, LoD and LoQ ranged from 0.45 to 0.64, from 1.05 to 1.45 and from 4.93 to 5.06 mAU/mL. The assay is linear up to 30,000.00 mAU/mL. There were no differences between sample types and no interference of common drugs and endogenous substances was observed. The correlation between the Picolumi PIVKA-II and the ARCHITECT PIVKA-II was 1.03 for the regression slope and 1.00 for the Spearman's correlation coefficient. The correlation between the Lumipulse PIVKA-II and the ARCHITECT PIVKA-II was 1.07 for the regression slope and 0.98 for the Spearman's correlation coefficient.

Conclusion: The ARCHITECT PIVKA-II assay demonstrated good analytical performance and compared well with other on-market assays. The ARCHITECT PIVKA-II assay is a convenient fully automated assay with high throughput (200 tests/hour) without the pretreatment of specimens.

A-018

Comparison of Freelite™, N Latex and Luminex serum free light chain assays in subjects with end stage renal disease on haemodialysis

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BACKGROUND: Quantification of serum free light chains (FLC) is important in the diagnosis of plasma cell diseases where abnormal kappa:lambda FLC ratio infers a population of monoclonal plasma cells. Whereas the Freelite™ assay uses a separate renal range for the kappa:lambda ratio compared to the normal population range, N Latex does not require a different range. A third FLC assay based on a multiplex bead array assay (Luminex) and using anti-FLC monoclonal antibodies was compared to Freelite™ and N Latex assay in an end stage renal disease population on haemodialysis.

METHODS: We completed a cross-sectional study comparing the performance of three FLC assays on 104 haemodialysis patients without paraproteinaemia. We quantified FLC pre- and post-dialysis using both the Freelite™, N Latex and Luminex assays.

RESULTS: FLC concentrations were elevated by all assays for pre-dialysis samples. Median kappa FLC was lower by Luminex (77 mg/L) compared to Freelite™ (155 mg/L) and N Latex (130 mg/L). Median lambda FLC was more than 2-fold higher by N Latex (250 mg/L) compared to Freelite™ (110 mg/L) and Luminex (95 mg/L). Of the 104 samples tested pre-dialysis, kappa:lambda FLC ratio was elevated in 29 by Freelite™ (diagnostic range 0.26-1.65), in 2 by Luminex (0.40-1.59) and none by N Latex (0.31-1.56). Only one ratio was above the Freelite™ renal range (0.37-3.1).

Correlation between assays for both pre- and post-dialysis samples was better for kappa FLC (R value 0.869 to 0.939) compared to lambda FLC (R value 0.750 to 0.864). Mean difference for lambda FLC post-dialysis decreased from 140 mg/L (95% C.I. 128-152) to 21 mg/L (95% C.I. 14.8-27.3) for Freelite™ versus N Latex, from 156 mg/L (95% C.I. 142-170) to 59.5 mg/L (95% C.I. 51.6-67.4) for Luminex versus N Latex, and increased from 16.1 mg/L (95% C.I. 8.0-24.2) to 38.5 mg/L (95% C.I. 31.7-45.2) for Luminex versus Freelite™ assay comparisons. Mean differences between assays were minimal for kappa FLC post-dialysis.

Post-dialysis median FLC concentrations decreased for all assays but remained elevated above the reference limit for a normal population. Median kappa FLC decreased 58% for Luminex, 68% for Freelite™, and 66% for N Latex. Median lambda FLC decreased 63% for Luminex, 36% for Freelite™, and 63% for N Latex.

CONCLUSIONS: Significant differences in FLC concentration existed between the three assays in an end stage renal disease population, pre-dialysis. This affected the kappa:lambda ratio, which was lowest by N Latex and highest by Freelite™. Markedly elevated lambda FLC contributed to the low N Latex ratios. Clearance of FLC by dialysis reduced kappa FLC by two-thirds in all assays and lambda FLC by a similar amount in N Latex and Luminex assays compared to just over a third reduction of lambda FLC by Freelite™. This difference in clearance of lambda FLC by Freelite™ possibly reflects a difference in antibody reactivity of various molecular forms of lambda FLC that may be present in renal disease.

A-019

circulating free DNA assessment of prognostic biomarkers in prostate cancer.

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Background: Cell-free DNA (cfDNA) is of crucial interest in oncology. Several studies have shown the potential role of cfDNA levels in the prognostic assessment of different solid malignancies. However, the quantification of pure cfDNA is a prerequisite for a reliable genotype analysis focused on the detection of cancer-specific DNA mutations signatures and/or epigenetic modifications. In the present study, the quality and quantity of the cfDNA were assessed by two different quantification procedures, Qubit 2.0 and Nanodrop fluorometer measurements, in order to identify the best and most cost-effective procedure in relation to the tumour stage. Further aim of this study was to evaluate the total cfDNA level and the cancer-specific DNA mutations as prognostic biomarkers in prostate cancer patients. **Methods:** We collected blood samples of patients affected by prostate cancer and healthy individuals. Blood samples were collected at the diagnosis of advanced cancer confirmed by biopsy, and at 6 and 12 months following the diagnosis. Blood collected was processed within one hour and frozen at - 80°C; cfDNA was extracted from plasma through Qiagen kit and Promega automatic extractor. Qubit 2.0 and Nanodrop were applied for measurements of total amount cfDNA before qPCR quantification performed targeting of the single copy gene *APP*. Methylated *GSTP1* and *RASSF1A* tumour specific cfDNA markers were determined in patients with prostate cancer. **Results:** A total of 25 prostate cancer patients and 30 aged matched healthy controls were evaluated. Automated DNA extractions resulted to be more accurate and cost-effective than the manual procedures. The pre-PCR quantification by Qubit and Nanodrop measurements revealed differences between the two procedures, highlighting the highest sensitivity of Qubit in the detection of small amounts of pure double strand cfDNA. On the other hand, Nanodrop spectrophotometric measurements showed to be more apt to perform quality and purity assessment of extracted DNA. Concerning the cfDNA levels in our cancer patients, preliminary data showed that patients with high cfDNA concentration at baseline had worse disease free time and overall survival, in comparison to those with a lower concentration. **Conclusions:** The automated cfDNA extraction associated to the quantification by Qubit 2.0 seems to be the best approach to quantify the patient's cancer-specific DNA mutations by qPCR assay. The spectrophotometric Nanodrop approach could be used for the evaluation of plasma samples with potentially higher cfDNA quantity in advanced cancer patients. The combination of multiple mutational/methylation and distinctive antigenic cancer biomarkers including prostatic markers is suitable to determine the total amount of cfDNA in prostate cancer patients. Cancer progression correlates with the changes in the level of cfDNA in plasma. Therefore cfDNA detection can be used as a prognostic and predictive tool for the stratification, the clinical management and the follow-up of patients with malignant melanoma and prostate cancer.

A-020

Combination of hK2, CCL11 and PSA in prognosis of prostate cancer patients

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Backgrounds: Human glandular kallikrein 2 (hK2) and prostate specific antigen (PSA or hk3) are members of kallikrein family produced by prostatic epithelium. While the production of hK2 is often increased in early stages and/or less well differentiated prostate cancer (PCa), PSA showed a decreased production. CCL11, a member of chemokines, plays an important role in regulation of tumor growth, progression and metastasis. The screening test need to be high sensitive to detect patients with early-stage disease with a sufficient specificity to prevent false-positive patients from undergoing invasive and unwarranted diagnostic evaluations. Although PSA is the best and most sensitive available screening test for PCa, there is a large overlap between PCa and benign prostatic hyperplasia (BPH) especially with PSA range 4-10 ng/ml which provoke the necessity to reveal, validate and advocate potential adjunct markers to improve PCa specificity with respect to sensitive detection. **Objective:** This study aimed to explore the diagnostic and prognostic value of hK2, CCL11 and PSA combination to improve the overall value of sensitivity, specificity and diagnostic accuracy of PCa patients. **Patients and Methods:** This study included 64 newly diagnosed PCa patients, 72 BPH and 65 apparently healthy men with matched age. Digital rectal examination (DRE) and transrectal ultrasound (TRUS) guided biopsy with volume measurements of the prostate were performed for all PCa patients. Clinical and pathologic distribution of stages were found 70.3% T2 (n=45), 21.9% T3 (n=14) and 7.8% T4 (n=5). Stages T3 and T4 were combined as advanced PCa group and compared with T2 (early PCa) to validate each biomarker in identifying early stage disease. Serum samples were collected from all patients after at least one-week gap following DRE and prior to any prostate biopsy. Serum levels of hK2, CCL11, tPSA and f/tPSA were measured. Validity (sensitivity and specificity) of each biomarker and their combination as well as possible association between parameters were assessed by analysis of ROC curve. **Results:** PSA had a sensitivity of 82% at 77% specificity with a diagnostic accuracy of 81% while f/tPSA ratio attained a sensitivity, specificity and accuracy of 81%, 82% and 85% respectively. Serum hK2 and CCL11 levels differentiated significantly among PCa, BPH and control groups ($p<0.05$). Although hk2 and CCL11 had no statistical differences among PCa stages ($p>0.05$), their ratios with tPSA significantly differentiated between early and advanced PCa stages ($p<0.05$) with a sensitivity, specificity and accuracy of (69%, 71%, 80%) and (88%, 82%, 85%) respectively. Combination of hK2, CCL11 and f/tPSA ratio seems to improve the overall value of sensitivity, specificity and diagnostic accuracy to 93%, 84% and 88% respectively. **Conclusion:** hK2 and CCL11 may provide a useful diagnostic information helping distinguish between BPH and PCa. Combined use of these biomarkers with the standard ones, PSA, can improve the overall value of sensitivity, specificity as well as accuracy of PCa especially in PSA overlap zone eventually sparing unnecessary prostate biopsies. However, larger prospective studies are warranted to validate the diagnostic value of combining these markers.

A-021

Quantification of Death Receptors as Tumor Markers for the Prediction of TRAIL Sensitivity by Flow Cytometry

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Background: The clinical evaluation and classification of biopsied tumors for genotypic and phenotypic characteristics allows for the application of appropriate anti-cancer treatments for specific tumor types. Personalized cancer treatments are critical to tailor therapies to individuals resulting in more efficacious therapies while minimizing negative side effects. Individualized cancer plans can be applied to the utilization of death ligand Tumor necrosis factor-Related Apoptosis Inducing Ligand (TRAIL) as an anti-cancer drug. Administration of TRAIL has been shown to be highly effective in selectively killing cancer cells. The selectivity of TRAIL is through its interaction with extracellular death receptors (DR), DR4 and DR5, the binding to which ultimately leads to apoptosis or programmed cell death in cancer cells. In Phase I studies, TRAIL was found to be safe and well-tolerated, however, Phase II studies only show a small cohort of patients responded to TRAIL therapy while others displayed resistance. Consequently, we propose the pre-treatment analysis of biopsied tumors for expression of death receptors to determine patient's suitability for TRAIL treatment.

Methods: An analytical flow cytometry method is described to determine tumor expression of death receptors and predict an individual's sensitivity to TRAIL. We employed various established malignant melanoma cell lines that have been reported to show varied sensitivity to TRAIL. Cells were stained with saturating amounts of anti-human CD261 (DR4) and anti-human CD262 (DR5) conjugated to phycoerythrin (PE) along with an IgG1κ isotype antibody and analyzed on the BDFACS Canto II using FACS Diva software. Additionally, we measured the degree of apoptosis experienced by the malignant melanomas in response to TRAIL treatment. Post treatment cells were collected and stained with FITC-AnnexinV and Propidium Iodide (PI) and analyzed by FACS.

Results: Three malignant melanoma cell lines, A375, MeWo and WM164 were analyzed for membrane expression of DR4 and DR5. Cell line A375 had the highest expression of both DR4 and DR5 with a mean fluorescence intensity (MFI) of 42.0±2.7 (n=9) and 1958.0±25.9 (n=9), respectively. Compared to A375, MeWo and WM164 had significantly less expression of DR4 with a MFI of 15.7±3.6 (n=9) and 18.0±1.4 (n=9), respectively, and DR5 with a MFI of 928.4±47.0 (n=9) and 552.7±10.8 (n=9), respectively. Cell line A375 was the most sensitive to TRAIL-induced apoptosis. Treatment with 50 ng/ml TRAIL resulted in the apoptosis in 30.7%±0.6 (n=9) of the cells, whereas, MeWo and WM164 were highly resistant to TRAIL and there was no significant induction of apoptosis.

Conclusion: A flow cytometry technique is proposed to measure DR expression of biopsied tumors to predict their sensitivity to TRAIL-induced apoptosis. Preliminary results show a direct correlation between DR expression and sensitivity to TRAIL-induced apoptosis as TRAIL-resistant cells lines, MeWo and WM164 had 59.9%±4.5 (n=18) less DR4 and 62.2%±2.6 (n=18) less DR5 membrane expression compared to the TRAIL-sensitive cell line, A375. These data provide the rationale for personalized cancer treatments and the analysis of biopsied tumor pre-treatment to determine the best anti-cancer therapy that will be most effective and possess minimal side effects.

A-025

LINE_1 Open Reading Frame 1 (ORF1) Protein Concentrations in Men with Prostate Cancer

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Background: Retrotransposones are repetitive DNA sequences capable of copying and moving themselves and other sequences to new location throughout the genome. Long interspersed nuclear element 1 (LINE1) is the most abundant and active group of these retrotransposons. Methylation of the LINE1 promoter regulates its activation and the generation of ORF1 and ORF2 proteins. LINE1 hyper-activation has been demonstrated in many types of cancer including, colon, prostate, lung and breast. We investigated whether L1 ORF1 concentrations in serum from men with borderline PSA concentrations were different based on the clinical decision of performing a biopsy or not. We further compared L1 ORF1 values in men who had undergone biopsy and had confirmed cancer versus those who did not have malignancy.

Methods: Remnant serum samples from 63 men >50-year-old with clinically measured PSA values of 4 -14 ng/mL (borderline) were included in this study. The clinical decision of having biopsy (n=20) or not (n=43) had been made during their routine clinical workup. Of the 20 biopsied subjects, 9 had confirmed prostate cancer, one had suspected atypia, 7 had no abnormality, and 3 had prostatitis. In order to measure ORF1 protein concentration in serum, we used an in-house competitive ELISA using a custom-made antibody against the select amino acid sequence of ORF1 protein. The same peptide sequence was coupled with biotin and used as anchor in streptavidin coated 96-well plates. Then, a secondary antibody (GAR-HRP) and colorimetric substrate were used to generate a blue color. Absorbance values at 450 nm were measured and patient sample concentrations were calculated based on a logistic 4-parameter standard curve generated from calibrators of known ORF1 concentrations.

Results: The mean ORF-1 protein concentration in biopsied and non-biopsy group were 26.60 (SD=14.9) and 16.40 (SD=9.2) ng/ml respectively. The difference between these groups was significant (P <0.003). The mean ORF-1 protein concentration in biopsy-confirmed prostate cancer subjects (n = 8) was 33.7 (SD=15) ng/ml, and 23.7 (SD=10) in biopsy-confirmed normal subjects (n=7). ORF1 protein concentration in biopsy-confirmed cancer subjects was significantly higher than non-biopsy group (P <0.001). **Conclusion:** ORF1 is a novel biomarker of cancer that can potentially serve as an aid in making the decision of whether a man with borderline PSA values should undergo biopsy or not. More data is required to confirm this finding as well as to the effect of smoking status of the subject at the time of sample collection.

A-026

Mutation spectrum and frequencies of *BRCA1* and *BRCA2* genes among 1,011 Brazilian patients

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Background: Breast cancer is the most common cause of mortality among women worldwide and this rate has increased rapidly in all countries. Around 10% of women who are diagnosed with breast cancer report a family history of the condition and a significant proportion of individuals with hereditary breast cancer have mutations in *BRCA1* and *BRCA2* genes. Several studies of *BRCA* mutations spectrum and frequencies have been done in populations from distinctive geographic regions. However, most are restricted to North America and Europe and studies in Brazil are scarce and restricted to a few reports with limited sample sizes. **Objective:** In this context, the aim of this study was to survey the spectrum and prevalence of *BRCA* mutations in Brazilian patients with breast/ovarian cancers or with family history of the condition, based on mutation analysis of DNA. **Methods:** Coding and flanking regions of the *BRCA1/2* genes from 1,011 patients were sequenced by next generation sequencing at Progenética Laboratory/ Hermes Pardini Institute. Patients come from different regions of the country and have anaverageage of 45.74 (range 17-84 years).**Results and Discussion:** We identified a total of 259 mutations: 115 in *BRCA1* and 144 in *BRCA2* gene. In *BRCA1*, 80 mutations were classified as pathogenic mutation, five as probably pathogenic and 29 as variants of unknown clinical significance (VUS).For *BRCA2*, 52 were classified as pathogenic, 11 as probably pathogenic and 76 as VUS. Overall, 15% of patients (148/1011) harbored a pathogenic/ probably pathogenic mutation in these genes, 57% of them in BRCA1 and 43% in BRCA2. This data is consistent with previously observed frequency mutation in BRCA genes for the Brazilian population, since *BRCA1* presents a higher mutation rate than *BRCA2*. Among 115 mutations in *BRCA1*, we observed 63 distinct mutations and 16 were identified more than once, including 27 women that carried the mutation 5382insC (the most common mutation identified worldwide and found at Ashkenazi Jews),nine with the 3450del4 mutation and five with 917delTT mutation, strengthening the relevance of these mutations at Brazilian population. For *BRCA2*, 92 mutations were identified once and 52 were identified more than once, which the more frequent one, rs80358547 (c.2T>G), was identified in nine patients.We were able to identify 61 previously undescribed mutations, 17 at *BRCA1* (threepathogenic and threeprobably pathogenic) and 44 at *BRCA2* (11 pathogenic and nine probably pathogenic). **Conclusion:** These results reveal the importance of this kind of survey to discovery new pathologic variants at countrieswith high rate of miscegenation and with restricted mutational information of *BRCA1/2*. Furthermore, knowledge of the proportion of normal individuals and cancer patients carrying mutations and the frequency of these mutations are needed for determining relevant mutations for the prevalence of hereditary breast cancer in the population studied, as well as defining public health and screening strategies for these patients. With these data,we will be able to offer a better genetic screening and counseling of patients with breast cancer and/or a family history for the disease.

A-027

Brazilian women with double heterozygosity for *BRCA1* and *BRCA2* mutations: two rare case reports

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Background: Breast Cancer is the most common cancer in women worldwide. Around 5-10% of these tumors are a result of mutations in *BRCA1* (MIN #113705) and *BRCA2* (MIN #600185) genes. Since *BRCA1/2* are associated with a large number of DNA repair pathways, mutations in these genes increase the probability to develop genetic alterations that can lead to malignant transformation and cancer. Among general population, the mutation frequency of these genes is very low (0.2%). However, it is higher in high-risk families, reaching until 20%. In recent years, several families have been described with more than one *BRCA* mutation. Nevertheless, the identification of individuals with two independent mutations in both genes is still rare. Only few individuals or families have been reported to have more than one non-Ashkenazi *BRCA* mutation. **Objective:** This report describes two independent cases of two non-Ashkenazi mutations in each BRCA1 and BRCA2 genes by next-generation sequencing (NGS) analysis. **Methods:** Genomic DNA was extracted from blood samples of the patients, using DNA micro kit (Qiagen). Mutation screening in the entire coding regions of BRCA1/2 genes was carried out on the Ion Torrent PGM™ sequencer. All procedures for library preparation, emulsion PCR and next-generation sequencing were performed with Ion Torrent equipment and Ion Torrent kits following the manufacturer's instructions. The sequence data were processed using standard Ion Torrent Suite™ Software running on the Torrent Server. Reads were aligned to the human genome reference (hg19/GRCh37) and variant calling were performed by Ion Reporter™ Software. Mutations were also validated by conventional Sanger sequencing. **Results and Discussion:** In a 49-year-old woman, we detected two independent deletions resulting in frame shifts: a *BRCA1* mutation in exon 11 (c.798_799delTT/p.V266fs/ rs80357724) and a *BRCA2* mutation in exon 11 (c.2808_2811delACAA/ p.K936fs/ rs80359352).In a 48-year-old woman, we identified a *BRCA1* mutation in intron 13 (c.4357+1G>A/IVS13+1G>A/ rs80358027) and a *BRCA2* mutation in exon 11 (c.6402_6406delTAACT/ p.N2134fs/rs80359584). All these mutations have been reported previously and classified as pathogenic. Since these mutations have a very low mutation frequency, the identification of two of these mutations in the same individual has never been described before. **Conclusion:** These findings reinforce the recommendation for mutation screening in both BRCA genes into clinical practice, avoiding misleading caused when only a familial mutation or a single gene is tested. In these cases, relatives of these patients could be falsely reassured if only one mutation or gene is excluded while an unrecognized one could still be present.

A-028

Prevalence of *EGFR* mutation in non-small cell lung cancer patients from Brazil: A personalized medicine for EGFR-TKI treatment

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Background: Lung cancer is the leading cause of both new cancer diagnoses and cancer-related deaths all over the world and approximately 85% of this kind of cancers are diagnosed as non-small cell lung cancer (NSCLC). Clinically, most of NSCLC patients are diagnosed at the advanced stages of disease, leading to a short survival. However, target therapy recently has achieved promising successes in NSCLC patients harboring Epidermal Growth Factor Receptor (EGFR) active mutations. Tyrosine kinase inhibitors (TKIs) can inhibit the EGFR TK domain reversibly through competitive binding with ATP. In this way, TKI shave been used to treat cancers harboring *EGFR* mutations or aberrant activation of EGFR, significantly prolonging patients's survival. For EGFR-TKI therapy, EGFR mutations need to be first detected in patients to allow oncologists to decide which first-line treatment should be offered to improve the efficacy of the treatment. **Objective:** In this context, the objective of this study was to survey the spectrum and prevalence of *EGFR* mutations identified in genomic DNA samples obtained from tumor tissues, using Cobas® EGFR Mutation Test real time PCR. This study was conducted in the Progenética Laboratory/ Hermes Pardini Institute. **Results and Discussion:** This study involved a total of 2,009 patients, with mean age of 64.8 years (range 52 until 77), with53% women and 47% men. Ten percent of the patients showed invalid results and these samples were associated with poor DNA quality and/or quantity (p>0,01).From the remaining patients, we identified mutations at 432(24%).A total of 416 patients presented only one mutation in *EFGR* gene and 16 were double mutant. Mutations in exon 19 (19Del) and exon 21 (L858R) were the most frequent, accounting for 53.7% and 32.9%, respectively. According to the literature, these mutations account for up to 90% of all *EGFR* mutations. Other concordant result was the prevalence of *EGFR* mutations in women, representing 64% of the mutated patients (p>0.0001). The mutations associated with TKIs resistance (S768I e T790M) were identified in 4% of the patients and in most cases (75%) they were associated with a sensitivity mutation (19Del or L858R). **Conclusion:** In conclusion, this work revealed similar results for mutation prevalence and spectrum with other previously analyzed European and American populations. Additionally, our results regarding the influence of DNA quantity and quality in obtaining conclusive test results reinforce the need of proper pre-analytical sample handling for paraffin-embedded tissue, especially when a limited tissue size is available. A recent alternative standing emerging is the utilization of circulating free DNA (cf-DNA) in the blood originating from tumor lesions, as surrogate sample for detecting *EGFR* mutations. This alternative is a less invasive source for obtaining genomic samples than surgical biopsy.

A-029

Light Chain Escape: A infrequent relapse in Multiple Myeloma

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Background: Free light chain escape (FLC escape) is defined as an increase in monoclonal serum free light chain (sFLC) without a corresponding increase in monoclonal intact immunoglobulins in a patient diagnosed with intact inmunoglobulin Multiple Myeloma (IIMM). The frequency at which IIMM patients relapse with FLC escape may be as high as 20%. We reported the clinical case of a patient with an IIMM where the serial measurement of sFLC after autologous stem cell transplantation (ASCT) allow us to detect a relapse as a FLC escape.

Case presentation: A 64 years old man was diagnosed with IgA Kappa MM in ISS stage 3. At diagnosis, he presented a monoclonal protein of 1,90 g/dL detected by serum protein electrophoresis (SPE) which was typed by serum immunofixation (IFE) as IgA Kappa. The sFLC levels were 510.5 mg/L for kappa and 13.8 mg/L for lambda with a ratio of 36.9. The Bence Jones proteinuria (BJP) was positive for kappa. A bone marrow biopsy showed a 20% of plasma cells and multiple lesions in axial skeleton and skull were found by PET/CT. After induction therapy with 6 cycles of VD (bortezomib and dexamethasone) he received an ASCT and achieved a status of complete response with negative IFE, 0.1% of plasma cells in bone marrow, an abnormal sFLC ratio of 9.5 (kappa=11.5 mg/L and lambda 1.2 mg/L) and the presence of a selective IgA deficiency (<10 mg/dL). Two months after ASCT, sFLC kappa began to increase with levels of 33 mg/L (ratio of 14.7) and normal SPE and BJP. Five months after ASCT, sFLC kappa levels were 150 mg/L (ratio of 57) with negative SPE and BJP. The sFLC kappa rapidly increased to a maximum of 21.282 mg/L (ratio 41.7) with negative SPE at seventh month after ASCT. Only sFLC kappa was identified by IFE with absence of IgA and the selective IgA deficiency was persistent since ASCT. A biological relapse was documented consistent with FLC escape. At this time, the bone marrow plasma cells were 42% and a PET/CT was performed showing a hypermetabolic focus confirming a clinical relapse. The patient began treatment with lenalidomide, dexamethasone and clarithromycin. After first and second cycles, sFLC kappa levels decreased to 1121 mg/L (ratio of 124.6 mg/L) and 379.9 mg/L (ratio of 61.8), respectively but the patient presented a severe thrombocytopenia during this period with adverse outcome.

Conclusion: In patients with IIMM that achieved a status of remission is very important the periodic assessment of sFLC to detect early if a FLC happens. Without the quantification of sFLC levels after remission or ASCT, the FLC escape couldn´t be detected and therefore the relapse of the disease. Furthermore, this case is an example of the clonal heterogeneity in MM with different clones at diagnosis (IgA Kappa) and relapse (Kappa) due to the different sensitivity of clones to the treatments, remaining the more resistant clones.

A-030

Improving quantification of M-protein Using Capillary Electrophoresis Immunosubtraction

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Background:

M-protein quantification is routinely performed by demarcating serum protein electrophoresis (SPE) regions. However, quantification of beta-migrating M-proteins is difficult due to overlapping non-immunoglobulin proteins. Therefore, some groups using techniques that do not separate beta-1 and beta-2 regions only quantify symmetric beta-migrating M-proteins > 2 g/dL. For this reason, recent guidelines have recommended following beta-migrating IgA M-proteins with total IgA levels. Immunosubtraction on capillary zone electrophoresis is a method currently used qualitatively to subtract out (and therefore highlight) immunoglobulins in serum, thus reducing the masking effect of normal serum proteins. This study expands on traditional immunosubtraction by developing a quantitative immmunosubtraction (qIS) suitable for measuring beta-migrating M-proteins as low as 0.1 g/dL.

Methods:

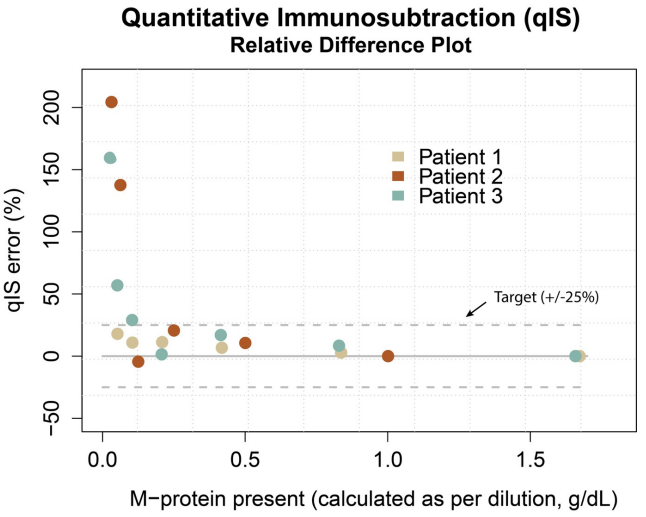
qIS is achieved by quantifying the subclass-specific immunoglobulin contribution to the SPE region containing the M-protein. We performed a comparison study with serial dilutions from three patients with beta-region M-proteins > 1 g/dL (n=20) as measured by SPE. We performed SPE and immunosubtraction on each dilution with the Sebia Capillarys 2™. Capillarys 2 immunosubtraction produces only qualitative traces. To quantify, we used traditional SPE analysis to calculate protein concentration in a region including both M-protein and normal protein. We then imported immunosubtraction images into Image J™ and performed region of interest analysis to calculate the involved immunoglobulin subclass contribution to the SPE region. In this way, we quantified pure immunoglobulin concentrations within a band of restriction without contamination by non-immunoglobulin proteins.

Results:

Passing-Bablok regression between qIS and the expected M-protein recovery produced a slope of 0.98 (95% CI 0.96 1.03), r = 0.999. Using a quality target of 25% error, our analytical measurable range spanned the maximum concentration tested (1.6 g/dL) to 0.10 g/dL (Figure).

Conclusion:

qIS achieves quantification of beta-migrating M-proteins at concentrations an order of magnitude lower than traditional SPE methodology, thus allowing earlier detection of M-protein recurrence or reduction.



A-031

Serum heavy/light chain analysis and specific isotype pair suppression in the monitoring of multiple myeloma

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Background: The immunoglobulin heavy/light chain (HLC) analysis has been recently proposed for monitoring the monoclonal protein (MP) in multiple myeloma (MM). The HLC ratio (rHLC) has been suggested as a new marker of monoclonality and early indicator of biological progression of the disease. **The aim** of this study is to compare HLC and its ratio in the follow-up of MM patients with standard laboratory techniques **Methods:** 24 diagnostic and 239 post-treatment serum samples from 26 MM patients (15 IgG, 10 IgA, 1 IgAK-GK) were included, with a median follow-up time of 37.5 months (range 21-67) and 9.5 samples per patient on average (range 6-19). Samples were analyzed for HLC and standard tests. Serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) were performed on the Capillarys and Hydrasys (Sebia) respectively, total immunoglobulins were measured on the Immage 800 (Beckman Coulter), HLC and serum free light chain (Hevylite®, Freelite®, Binding Site) on the BN proSpec (Siemens). **Results:** At **diagnosis**, all the samples presented an abnormal rHLC, in agreement with the IFE, elevated involved HLC (iHLC) and suppressed uninvolved HLC (uHLC) levels. During **follow-up**, 97.4% of the samples with MP detected by SPE had an abnormal rHLC, 83,5 % presented increased iHLC and 80.7 % decreased uHLC. Moreover, iHLC levels correlated well with the MP measured by SPE (y=0.46+1.06x, r=0.943). In 96 samples with normalized SPE, there was a moderate agreement between the IFE and the rHLC and uHLC suppression (kappa coefficient, 0.510 and 0.439, respectively). 25/42 IFE positive and 5/54 IFE negative samples had an abnormal rHLC. 5 IgG MM samples presented oligoclonal bands (OCB), impacting the IFE agreement with the HLC parameters (kappa coefficient excluding OCB samples, 0.591-0.593). After **treatment**, 11 patients achieved Complete Response (CR), of which 3 relapsed. rHLC was normal and in agreement with SPE and IFE in 6 patients. In 4 patients (1 IgG and 3 IgA) the rHLC normalized 2-5 months before the IFE became negative, and in 1 IgA patient the rHLC indicated relapse 3 months before IFE. In 10 patients who achieved Partial Response (PR) and 5 Very Good Partial Response (MP detected only by IFE), the rHLC was in agreement with the conventional tests, except for 9 samples from 3 IgG patients with normal rHLC that presented OCB and low levels of MP (< 3 g/L).

In PR patients, an increase in the iHLC was usually observed at progression.

In the 5 VGPR patients, the rHLC abnormality was mainly attributable to the suppression of the uHLC, while the iHLC is within the reference interval in most of the samples. The 5 patients subsequently progressed. **Conclusion:** These results confirm rHLC as a monoclonality marker, which may be an early indicator of degree of response to treatment and relapse. Despite the effect of oligoclonal bands on the IFE vs HLC agreement, HLC correlated with the clinical outcome. HLC is useful and complementary to other techniques for monitoring response, and adds information about the suppression of the uninvolved immunoglobulins

A-036

Clinical value of ANNA-1 antibodies in patients with paraneoplastic syndromes

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Background: The ANNA-1 or anti-Hu antibodies are directed against an antigen localized in the nucleus of all neurons. They are directed against a family of RNA binding proteins with a molecular size of 35-40 kDa. They are expressed in the nuclei of neurons of the central and peripheral nervous system. Paraneoplastic syndromes associated with this antibody are sensory neuropathy, encephalomyelitis, cerebellar degeneration with autonomic dysfunction and limbic encephalitis. The tumours associated with the presence of this antibody are small cell lung cancer, prostate cancer, breast cancer, neuroblastoma and sarcoma. The aim of this study is to show the clinic value of this antibody in the study of paraneoplastic syndromes.

Methods: We report four patients with paraneoplastic syndromes and the presence of anti-Hu antibodies were detected. Onconeural antibodies were identified in serum sample by indirect immunofluorescence (Euroimmun AG) based on primate tissues (cerebellum, nerves and intestine). The positive results were confirmed on recombinant immunoblot assay (Ravo-Diagnostika) that detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies.

Results: The results obtained are shown in the table.

Gender	Age (years)	Paraneoplastic syndromes	Antibody	Diagnosis of the patient after study	Survival
Male	79	Limbic encephalitis	Anti-Hu 1/100	Squamous cell lung cancer	Deceased (2 months)
Female	46	Sensory neuropathy	Anti-Hu 1/100	Multiple Sclerosis	Alive (25 months)
Male	67	Paraneoplastic encephalitis	Anti-Hu 1/1000	Lung adenocarcinoma	Deceased (19 months)
Female	50	Paraneoplastic encephalitis	Anti-Hu 1/1000	Small cell lung cancer	Deceased (7 months)

Conclusion: The presence of anti-Hu antibodies was associated to lung cancer in three patients while in the remaining patient was not found a tumour. In these three patient, the presence of anti-Hu antibodies was associated with a poor prognosis with short survival time. In the patient with multiple sclerosis and positive anti-Hu was not found a tumour despite the presence of continuous positive anti-Hu. In this patient, the possible interference due to anti-nuclear and anti-mitochondrial antibodies was studied with negative results. In summary, the presence of this antibody should help the clinician towards finding a hidden tumour, foremost among them, small cell lung cancer presents in 80% of cases of positivity for this antibody.

A-038

Lumipulse *G* HE4 Assay for Monitoring of Patients with Epithelial Ovarian Cancer

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Background: Human epididymis protein 4 (HE4) demonstrated to be a useful biomarker for ovarian cancer (Hellström I, 2003; Drapkin R, 2005; Moore RG, 2007). This study was to analytically verify the Lumipulse ***G*** HE4 assay and clinically validate the assay for monitoring recurrence and progression of epithelial ovarian cancer (EOC).

Methods: Lumipulse ***G*** HE4 assay is a Chemiluminescent Enzyme Immunoassay for the quantitative determination of HE4 in human serum and plasma on the Lumipulse ***G***1200 System via a two-step sandwich immunoassay method using two monoclonal antibodies against HE4. The amount of HE4 in the specimen is obtained from the luminescence signals derived from the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1,2-dioxetane disodium salt).

Results: The Lumipulse ***G*** HE4 assay demonstrated linearity from 20 to 1,500 pM, and an analytical sensitivity with the LoQ (Limit of Quantitation) ≤ 20.0 pM. The precision study of 2 controls and 8 sera (n = 120 for each sample) revealed a total %CV ≤ 6.1% at 3 testing sites using 3 lots of reagents. There was no High Dose Hook effect with up to 300,000 pM of HE4 antigen in samples. Interference studies showed an average percent difference ≤ 10% between test and control samples for potential interferents, including 9 endogenous substances (human anti-mouse antibody, rheumatoid factor, conjugated bilirubin, unconjugated bilirubin, human immunoglobulin G, biotin, triglycerides, hemoglobin, and human serum albumin) and 23 drugs, which were spiked individually into sera (test samples). In the monitoring study, changes in HE4 levels in serial serum samples collected in SST tubes from 72 subjects with EOC were compared to changes in disease status, that is, progression or no progression. A total of 330 observations were undertaken with an average number of 5.6 observations per subject. A positive change in the HE4 value was defined as an increase of HE4 in the observation value that was at least 18% greater than the previous observation value. Of the 61 samples with a positive change, 49% of them correlated with the progression of EOC while 80% of the 269 subject serial samples with no significant change in the HE4 value correlated with no progression. The total concordance with diagnosis was 74%, PPV (positive predictive value) 35%, and NPV (negative predictive value) 87%. A comparison of Lumipulse ***G*** HE4 with the predicate device, HE4 EIA, was analyzed using weighted Deming regression. The slope and correlation coefficient (r) obtained were 1.03 and 0.9891, respectively, for the tested specimens (n = 143) which ranged from 33.4 - 969.5 pM, and slope and r of 1.03 and 0.9917, respectively, for the tested specimens (n = 168) ranged from 33.4 - 4602.0 pM.

Conclusion: The Lumipulse ***G*** HE4 assay has demonstrated to be accurate, precise, and sensitive for the quantitative determination of HE4 antigen in human serum and plasma, and is useful in monitoring the course of disease in women with epithelial ovarian cancer.

A-039

A Risk of Ovarian Malignancy Algorithm (ROMA) Derived from Lumipulse *G* HE4 and Lumipulse CA125II Assays

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Background: The FDA-cleared ROMA, a combination of HE4 EIA and ARCHITECT CA 125 II, is intended to assess the likelihood of finding malignancy on surgery of a woman with an ovarian adnexal mass (Chudecka-Glaz, 2015). The current study was to evaluate the clinical utility of Lumipulse ***G*** ROMA, a new ROMA assay under development.

Methods: Lumipulse ***G*** ROMA is a qualitative serum and plasma test that combines the measurements of Lumipulse ***G*** HE4 and Lumipulse ***G*** CA125II assays as a ROMA score = exp(PI) / [1 + exp(PI)]*10, where PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125] for a premenopausal woman, and PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125] for a postmenopausal woman. The cut-points for defining a high likelihood of finding malignancy from a low likelihood was set as ≥ 1.31 and ≥ 2.77 for a pre and postmenopausal woman, respectively. Lumipulse ***G*** ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. Lumipulse ***G*** ROMA is for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. Lumipulse ***G*** ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay. PRECAUTION: Lumipulse ***G*** ROMA should not be used without an independent clinical /radiological evaluation and is **not** intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of Lumipulse ***G*** ROMA carries the risk of unnecessary testing, surgery, and/or delayed diagnosis.

Results: A precision study of 5 panels spanning the range of Lumipulse ***G*** ROMA scores revealed a total %CV ≤ 8.1 (n = 120, 3 sites). The method comparison between the Lumipulse ***G*** ROMA and the FDA-cleared ROMA showed a Deming regression slopes of 1.0, and a correlation coefficient (r) of 1.0 (n = 130). To assess the likelihood of finding malignancy on surgery of a woman with an ovarian adnexal mass, samples from patients presenting to a generalist with an ovarian adnexal mass were tested. For diagnosis of EOC only, a sensitivity of 93.6% (n = 47), specificity of 76.0% (n = 366), PPV of 33.3% (n = 132), NPV of 98.9% (n = 281), PLR of 3.894 and NLR of 0.084 was achieved at an EOC prevalence of 11.4% (n = 413). For diagnosis of EOC + LMP, a sensitivity of 87.7% (n = 65), specificity of 76.0% (n = 366), PPV of 39.3% (n = 145), NPV of 97.2% (n = 286), PLR of 3.647 and NLR of 0.162 was achieved at an EOC + LMP prevalence of 15.1% (n = 431).

Conclusion: The Lumipulse ***G*** ROMA under development appeared to be precise and sensitive for assessing whether a woman with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery.

A-040

Development of RT-qPCR Gene Expression Assays for Multiple New Cancer Therapies

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Background: Gene expression profiling to identify candidates for targeted cancer therapies is a growing need. Once molecular biomarkers are identified using massive parallel processing technologies such as microarrays or NGS, development of a robust quantitative assay for biomarker expression, such as RT-PCR, is the logical next step. In addition to their established and reliable use in in-vitro diagnostics, quantitative PCR (qPCR) assays have the advantage of being cost-effective. Here we present biomarker A and biomarker B RT-qPCR gene expression assays for targeted antibody-drug conjugate therapies that accept FFPE tissue as input and demonstrate, with initial research, high performance characteristics for measuring gene expression (i.e., RQ).

Methods: RT-qPCR was performed using the LifeTech QuantStudio 7 employing low-density (384-well) array cards, allowing for evaluation of multiple target and reference genes (2 target and 12 reference genes in this study). Stable normalization genes for three different tissue types (breast, ovary, lung) were selected from 12 candidate reference genes across 4 normal and 17 clinical tumor FFPE samples. Reference gene normalization was achieved using the geNorm algorithm. RNA extraction from 10 µm sections of FFPE tissue was performed using the automated Tissue Preparation System (TPS) from Siemens Healthcare. PCR amplicon specificity for the different assays was assessed using electrophoresis and NGS of end-point PCR product. Cell line controls were selected for low and high expression of target genes. Analytical reproducibility was assessed using both cell line control and clinical samples. Analytical sensitivity was assessed using mixtures of normal and high-level expressors of each biomarker. Differential expression of biomarkers A and B was assessed across 4 normal and 17 clinical tumor FFPE samples for each tissue type.

Results: The Siemens TPS System showed highest yield (>700 ng/sample), reproducibility (<0.25 SD between runs and operators), and scalability (up to 48 samples/run) over other manual RNA extraction methods. PCR efficiency for all primer/probe sets was found to be 100 ±6%. Electrophoresis of amplicons showed >95% on-target products based on size, while results from NGS showed mapping to the gene of interest >90% for the majority of assays, including selected target and references assays. Of 132 data points per indicated target or reference gene, normalized Cq values were below 0.2 SD for triplicates, and Cq values were <31 for control and clinical FFPE samples. The minimum detectable level of elevated biomarker expression, differentiated from normal, also known as lower limit of quantification (LLoQ), was determined to be 1.22 for biomarker A and 1.34 for biomarker B. Fold difference for mean expression values (i.e. RQ) between normal and tumor samples was highly significant in lung (fold difference = 3.7; p-value of 4.4E-4) for biomarker A.

Conclusion: Together, these RT-qPCR assays demonstrate the potential for robust performance and ease of use for companion diagnostics applications, such as identification or screening of tumor patients for personalized treatment.

A-041

Disease spectra and clinical characteristics of serum PIVKA-II-producing cancers other than hepatocellular carcinoma

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Background: The serum des-gamma-carboxyprothrombin (protein induced by vitamin K absence or antagonist-II, PIVKA-II) is a putative specific marker of hepatocellular carcinoma (HCC), but may also be produced by various tumors in the stomach, lung, colon, and pancreas. Although case reports of PIVKA-II-producing cancers other than HCC have been gradually increasing in number, their disease spectra and clinical characteristics remain unclear. In particular, there is no systematic study about clinicopathological features in PIVKA-II-producing cancers except HCC. The aim of this study was to identify clinical characteristics and diagnostic value of serum PIVKA-II in PIVKA-II-producing cancers excluding HCC.

Methods: We evaluated the serum PIVKA-II levels in 172 patients with various cancers (primary tumor sites: 59 stomach, 24 colon, 13 bile duct, 11 lung, 9 esophagus, 6 prostate gland, 4 rectum, 4 gall bladder, 20 etc.) excluding HCC in Chonnam National University Hwasun Hospital (Hwasun, Korea). The serum PIVKA-II level was determined using a chemiluminescent enzyme immunoassay system and an automated immunoassay analyzer (Lumipulse G1200; Fujirebio, Japan). In patients with more than 40 mAU/mL (cutoff value) of serum PIVKA-II, we investigated the clinicopathological characteristics of enrolled patients.

Results: Serum PIVKA-II levels in 172 patients with non-HCC cancers ranged from 10~110,179 mAU/mL (median, 24 mAU/mL). Of these patients, 22 patients (12.8%) showed PIVKA-II levels above 40 mAU/mL (median 102 mAU/mL). The most common type of cancers was gastric cancer (8 cases), followed by pancreatic cancer (4), cholangiocarcinoma (3), colon cancer (3), and renal cell carcinoma (2). Among 22 patients with PIVKA-II producing cancer, 8 patients (36%) had metastases to multiple organs, including 4 liver metastasis (50%). However, serum AFP levels (cutoff value, < 5.8 IU/mL) were abnormally high in 3 of all 22 patients, and in 1 of 4 patients with liver metastasis.

Conclusions: About 13% of various cancers excluding HCC showed elevated serum PIVKA-II level. Our results disclosed that serum PIVKA-II was not restricted in HCC and elevated serum PIVKA-II value was observed in mainly, gastrointestinal tract cancer. Additionally, when the serum PIVKA-II level is abnormally high in patient with gastric cancer, the possibility of liver metastasis should be considered.

A-042

Antibody-free microfluidics-based circulating tumor cell enrichment by Angle PLC Parsortix and downstream molecular characterization by Affymetrix branched DNA technology

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Background Enumeration of circulating tumor cells (CTC) in blood is a prognostic and predictive marker in metastatic breast cancer. However, enumeration of CTC by current approved methodology is of limited clinical utility and could be enhanced by molecular characterization. The unique feature of the Angle PLC Parsortix system that sets it apart from many other existing and nascent technologies is that it captures CTC without antibodies. It relies on the size and deformability of CTC with the advantage of harvesting them for subsequent downstream molecular characterization. The prime objective of this study is to validate the isolation of spiked breast cancer cell lines in healthy donor blood (HDB) with Parsortix followed by molecular characterization using Affymetrix QuantiGene Plex, a sensitive assay exploiting branch DNA technology.

Methods Four breast cancer cell lines (SUM190, MCF-7, MDA-MB-453 and MDA-MB-231) were separately spiked into 7.5 ml of HDB with EDTA anticoagulant and processed through Parsortix 10µm microfluidic cassettes for tumor cell enrichment. The harvested tumor cells were then suspended in 300µl of lysis buffer and analyzed by QuantiGene to detect the transcripts of 5 epithelial genes (*CDH1, EGFR, ERBB2, KRT18,* and *MUC1*) and 20 additional CTC and/or breast cancer-related genes. A gene was considered detectable if the transcript level was 2.5 standard deviations above the average transcript level of the gene in four unspiked HDB samples. Individual cell lines were similarly analyzed to determine the linearity and sensitivity of QuantiGene. Human Universal RNA was included as technical control for QuantiGene.

Results Tumor cells harvested by Parsortix were assayed for five epithelial genes customarily expressed by these cell lines. In terms of *specificity* of epithelial genes known to be expressed by these cells, 4 of 5 were detected in SUM190 and MCF-7 cells; 2 of 4 were detected in MDA-MB-453 cells; and 3 of 3 were detected by MDA-MB-231. In *linearity* studies, expression levels correlated well with the number of cells spiked into HDB and such a correlation was maintained ($R^2 > 0.9$) for most of the 25 genes tested. The *precision* of QuantiGene bead array assay for the molecular characterization of CTCs harvested by Parsortix was excellent with most CVs under 10%. In terms of *sensitivity*, dilution of the harvested cell extracts suggested that highly expressed genes such as KRT18 could be detected in as few as 20 SUM190 cells. KRT18 gene expression was detected when as few as 50 SUM190 (basal) cells or MCF-7 (luminal) cells were spiked into HDB; several genes were expressed when >50 cells were spiked. None of the 25 genes were detected in MDA-MB-453 (Her2-positive) when <500 cells were spiked. Gene expression was detected in the highly mesenchymal cell line MDA-MB-231 only when >500 cells were spiked.

Conclusion Molecular characterization of cells harvested by Parsortix is more informative and clinically useful than the enumeration of CTC alone. As liquid biopsy provides repeated blood sampling, such characterization may provide a novel avenue for personalized noninvasive assessment of therapy response and warrants further exploration in controlled cohort studies.

A-045

Development and Initial Evaluation of a Multi-Protein Biomarker Blood Test for Organ Confined Prostate Cancer Diagnosis (OCProDx)

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Background: About one in six men will get a diagnosis of prostate cancer during their lives. Generally, prostate cancer is treated effectively, but for many men the disease is not life threatening and they will die with prostate cancer rather than because of it. Too many men are treated unnecessarily. For them active surveillance of the disease would be a better option. Unfortunately, the existing readily available tools for disease diagnosis (PSA test, digital rectal examination and trans-rectal ultrasound guided biopsy), do not adequately guide this key decision of whether to pursue active surveillance or invasive treatment. Through analysis of the key decisions in prostate cancer patient management we highlighted that establishing whether the disease is organ confined (localized, OC) or has spread beyond the extracellular capsule of the organ (non-organ confined, NOC) would provide important information to guide this decision [Oon SF, Pennington SR, Fitzpatrick JM, Watson RW. Nature Reviews Urololgy (2011) 8:131-8.]. Our objective was to identify serum protein biomarkers to determine disease status in terms of organ confinement.

Methods: We undertook unbiased protein discovery experiments using gel and LC-MS based proteomics. Discovery was undertaken with affinity depleted (MARS14) serum samples (n>50 for gel and n=30 for LC-MS) taken from patients at time of diagnosis and for whom OC or NOC status was determined following radical prostatectomy. Statistical analysis of differentially expressed proteins was undertaken at univariate (Student t-test) and multivariate levels to assemble a panel of 59 candidate proteins. We supplemented this panel of 59 proteins with 5 proteins identified from the literature and developed a multiplexed MRM assay to support the simultaneous measurement of 63 of the proteins. The protein panel was evaluated its by undertaking two initial validation studies in which first 31/63 and then 63/64 of the candidate proteins were measured using patient samples distinct from those used for the discovery experiments. Serum samples were from the Irish Prostate Cancer Research Consortium.

Results: Initially, the relative abundance of the highest MRM transition from 50 peptides was used to measure 31 proteins in 63 clinical samples. The data, extracted using Skyline, were fitted into a PLS-DA model and the predicted performance was assessed through 200 times bootstrapping. The predictions in the out-of-bag samples were compared with the true group information and ROC curves were generated. The AUC for differentiating between OC and NOC was 0.824. Subsequently, 63 candidate proteins were evaluated with total of 116 patient samples and data analysed using a range of different statistical approaches. The AUC values for distinguishing organ confined from non-organ confined disease were >0.8. It was notable that proteins within the second phase of MRM development (n=32) made a contribution to these AUC values.

Conclusions: This initial evaluation data clearly demonstrates the potential of the 63 protein multiplexed MRM assay to discriminate OC from NOC prostate cancer. With incorporation of appropriate QC methods we suggest the OCProDx MRM assay may be capable of translation to diagnostic use to support the discrimination between OC and NOC prostate cancer.

A-046

USING HEVYLITE OVERCOMES PROBLEMS WITH THE MONITORING OF MONOCLONAL PROTEINS DIFFICULT TO MEASURE BY CONVENTIONAL TECHNIQUES

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Multiple Myeloma (MM) monitoring is most frequently done by quantifying serum monoclonal immunoglobulins (MP) in agarose electrophoresis gels (SPE). This procedure is often complicated when the MP migration pattern overlaps with normal serum proteins, appear as broad band, multiple peaks or small peaks, which may occur in up to 40% of IgA type MM. Thus, the follow-up of these MP may result less accurate, require additional techniques and ultimately result in equivocal evaluation of patients' response to treatment. The Hevylite® immunoassay for the determination of immunoglobulins' specific heavy/light chains pair has been developed, which allows the exact quantification of the MP without the over- or underestimation that may occur when monitoring with SPE, mainly in IgA MM patients.

Objective: utility of Hevylite® versus SPE quantification in the follow-up of IgA MM patients.

Methods: Hevylite® measured by turbidimetry on a SPA_{PLUS} (Binding Site); SPE on a Capillarys Hydrasys Focusing device (Sebia). Population: 335 samples from 36 IgA MM patients followed at our center between 2012-2015.

Results: A high correlation was found between the MP quantification by SPE and Hevylite (iHLC=-0.203+1.15 SPE; r=0.928; p<0.0001) and between total IgA and the sum of Hevylite IgAk+IgAlambda (∑HLC=-1,63+1,12 totIgA; r=0.912).

Analyzing 21 patients with a medium of 13 (range:5-26) follow-up samples we found that when the MP is clearly distinguishable by SPE the evolution of the MP during follow-up by either SPE or HLC is virtually superimposable, validating the role of HLC as monitoring tool. In turn, some patients with complicated MP migrating patterns benefited from the use of HLC (see table).

Patient	MM Isotype	SPE M-spike migration pattern	Hevylite contribution
1	AL	Alpha 2	HLC allows to continue monitoring MP even after SPE becomes negative due to total overlap with normal serum proteins
2	AL	Beta	HLC identifies relapse earlier than to SPE. Also, HLC never normalizes in contrast to SPE which remains non-quantifiable for 11 months
3	AK	Small Beta	Very small M-spike by SPE, hard to identify and quantify. HLC allows an easy and accurate follow-up of the MP
4	AL	Split peak in Beta	SPE probably underestimating the amount of MP due to gross interference from other serum proteins
5	AK	Triplet spanning Beta and gamma	Hevylite confirms disappearance of MP while SPE shows a peak later identified by IFx as oligoclonal IgGL band
6	AK	Broad beta	Allows follow-up up to 4 months after the last positive SPE and IFx. Identifies relapse 3 months before SPE

Conclusion:

-Hevylite is an alternative method for MP quantification , adding value to the follow-up of MM patients particularly when SPE shows limitations.

- Additional Hevylite value might come from early indication of relapse. However this observation lacks confirmation from larger studies.

A-047

Precision profile of a second-generation multivariate index assay for malignancy risk assessment of adnexal masses

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Objective: A second generation multivariate index assay (MIA2G) has been developed for improved detection of ovarian cancer among women undergoing removal of adnexal masses. This test combines the levels of serum proteins apolipoprotein A-1, CA 125, HE4, FSH and transferrin using ensembles of classification models integrated into a 0-10 risk score. The analytical precision can be estimated empirically using specimens spanning the range of risk scores, but there are currently no methods that account for imprecision from the possibly large combination of biomarker values that can result in any one score. The objective of this study was to determine precision across the range of risk scores resulting from all possible combinations of biomarker concentrations.

Relevance: Multivariate index assays have multiple sources of imprecision. A complete precision profile could help determine the allowable error that would permit reliable assessment of risk of malignancy.

Methodology: Single-site precision and multi-site reproducibility studies were performed on pooled patient serum samples that spanned the range of risk scores. Biomarkers levels were determined using the Roche cobas® 6000 clinical analyzer. Variance components were determined from analysis of variance using a restricted maximum likelihood method. Monte Carlo (MC) simulations of all possible combinations of biomarker concentrations from the studies -- resampled from their empirical standard deviations (SD) -- were used to generate MIA2G scores, assuming biomarker values varied independently. A second MC simulation used data from intended use clinical studies, which retained the true correlation structure of biomarkers in the benign and malignant conditions. The precision profile is a graph of the coefficient of variation of the sampled MIA2G scores as a function of the median of the score

Validation: Repeatability (within run) of MIA2G ranged from a SD of 0.000 to 0.130 risk score units (CV of 0.00% – 2.57%) and reproducibility (total of all components) ranged from a SD of 0.000 to 0.175 risk score units (CV of 0.00% - 3.43%), depending on the pool tested. The CV of individual biomarkers never exceeded 2.69% for any component. MC simulations assuming independence of biomarkers showed decreasing CVs with increasing risk scores, as well as higher variabilities of CVs at lower scores. The highest CVs were never found at the cut off value. None of these simulations exceeded a 5% CV. Simulation of within run precision using data from clinical samples resulted in about 1% of the simulations showing a CV of ≥5%. MC simulations of repeatability using data from clinical samples resulted in <2% of the cases where the 2.5%-97.5% quantiles crossed the cut-off. Estimates of the sensitivity and specificity of MIA2G within the 2.5% - 97.5% quantiles of values obtained from the simulation resulted in no significance change in test performance.

Conclusions: MIA2G was implemented on high-quality clinical instrumentation using well-controlled assays. The MIA2G risk score imprecision was generally lower than the component assays, resulting in reliable and robust outputs. The novel application of MC simulations demonstrated that the algorithms were robust to random individual biomarker perturbations over the range of risk scores.

A-048

Hyperhomocysteinemia results from and promotes hepatocellular carcinoma via CYP450 metabolism

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Background & Aims: Hyperhomocysteinemia (HHcy) can result from liver cancer or dysfunction and further alters intracellular lipid metabolism. Cytochrome P450 (CYP) arachidonic acid epoxygenases are expressed in human cancers and promote human cancer metastasis. This study explored the cross-talk of homocysteine (Hcy) and CYP450 metabolism in hepatocellular carcinoma (HCC). Methods: We first screened arachidonic acid and Hcy metabolism by liquid chromatography-mass spectrometry, Meta-analysis, and ELISA. Hcy regulation of CYP450 enzymes was verified by ELISA, immunostaining, and quantitative PCR in 42 tissue samples of human HCC and their adjacent non-tumor tissue, as well as in an HepG2-cell orthotopic-injected model of HCC in BALB/C nude mice. The bioluminescence imaging system was used for sensitive detection of tumor growth in the mice. Results: Arachidonic acid was the most abundant in tumor tissue, about 721.04±358.32 ng/mg. Importantly, the accumulation of metabolites in the CYP450 pathway (5,6-EET, 8,9-EET, 11,12-

EET, 14,15-EET and their corresponding DHETs) but not COX or LOX pathways was higher in tumor versus adjacent non-tumor tissue. Among three enzymes of EET synthesis: CYP2J2, but not CYP2C8 and CYP2C9, expression at mRNA and protein levels were higher in tumor versus non-tumor tissues. CYP2J2 protein levels were positively associated with poorly differentiated tumors, tumor sizes, and levels of alpha-fetoprotein. Meta-analysis of 13 eligible studies of 1,144 cases and 1,147 controls were performed and revealed that HCC risk associated with high serum Hcy levels (odds ratio [OR] 12.0; 95% confidence interval [CI] 9.13~14.88) and low folate levels (OR -8.3; 95% CI -12.9~ -3.71). Furthermore, intracellular Hcy levels were higher but folate levels were lower in HCC tumor versus non-tumor tissue. Importantly, high intracellular Hcy level was frequently found associated with high 11,12-EET and CYP2J2 protein levels, and positively correlated with CYP2J2 mRNA in HCC. A mouse model of HCC was generated orthotopically using HepG2-GFP cells and mediated by Hcy, with 2%L(wt/wt) L-methionine in a chow diet or not. The significant increase of tumor growth rate, size, and weight in the mice with methionine diet compared with the control diet, and the similar increase in CYP2J2 protein and mRNA level, as well as, poorly differentiated HCCs were observed. Moreover, the tumors became smaller in mice with CYP2J2 knockdown in both control and methionine diet groups. Conclusion: HHcy may result from but also promotes hepatocarcinogenesis via CYP450-EET metabolism through CYP2J2. The Hcy-CYP2J2-EETs pathway might be a target for the diagnosis and treatment of HCC.

Keywords: hepatocellular carcinoma, homocysteine, CYP2J2

A-049

Evaluation of a newly developed lateral flow system for kappa and lambda free light chains in urine

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Introduction: We describe the evaluation of a newly developed lateral flow device for the detection of kappa and lambda free light chains in human urine. This dual analyte, lateral flow immunoassay uses highly specific and characterised monoclonal antibodies (Campbell et al., 2013 JIM) in a competitive/inhibition format. This arrangement does not suffer from antigen excess (high does hook effect), making it suitable for the detection of elevated urinary free light chains in the clinical investigation of plasma cell dyscrasias. The system comprises of a lateral flow device, application buffer and a small portable reader, which, coupled with short incubations to provide rapid near patient results in the physicians office laboratory. **Method:** The monoclonal antibodies to either kappa or lambda free light chain were conjugated to colloidal gold and dried in a pad within the lateral flow device. The nitrocellulose strip has two test zones; comprising of either immobilised kappa or immobilised lambda light chain. Urine is prepared in an application buffer and added to the device where the gold labelled monoclonal antibodies rehydrate and travel by capillary action along the nitrocellulose. In the presence of the specific light chain, inhibition of binding will occur during the incubation period. The device also has a third zone on the nitrocellulose that acts an independent immunoassay control. The reader performs the 10-minute incubation, interprets the line intensity and converts these into a concentration via a predetermined calibration (10 to 100 mg/L for each light chain) contained within a barcode. **Results:** Each assay demonstrated acceptable inter-assay precision; repeated analysis of a patient urine sample across 30 devices over several days yielded mean values of 35.2 mg/L (CV, 10.4%) and 22.4 mg/L (CV, 10.6%) for kappa and lambda free light chains respectively. A normal urine sample was analysed at the same time; all kappa and lambda values for this read less than 10mg/L. There was no influence of pH: Negative synthetic urine was tested at pH 5, 6, 7 and 8 and remained negative. Aliquots at each pH were fortified with kappa (concentration 28 mg/L) and lambda (concentration 14 mg/L) and tested and were also unaffected at these pH levels. No significant interference was seen from glucose tested at 2000 mg/dL, human albumin tested at 1000 mg/dL or hemoglobin tested at 0.67mg/dL (equivalent to 200 RBC/microlitre).

Clinical testing has started with the retrospective analysis of 71 pathological urine samples submitted for immunofixation electrophoresis. The limit of detection for this method is 10 mg/L). This urine population comprised of 31 samples that were negative for monoclonal free light chains, 18 samples where kappa light chains were present and 22 samples that had lambda light chains. The new lateral flow device correctly identified each sample versus its immunofixation status. Kappa results for the lateral flow device ranged from 22 to 3995 mg/L whilst lambda results ranged from 27 to 2484 mg/L. **Conclusion:** Further work is planned to expand the patient cohorts and to assess potential interference from other paraproteins.

A-050

EFFECT OF REAGENTLOT-TO-LOTVARIABILITY ON CARCINOEMBRYONICANTIGEN PATIENT´S RESULTS

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Background Carcinoembryonic Antigen (CEA)is widely recommended in the follow up of some types of epithelial cancers. There is evidence that the reagent lot-to-lot variability has influence on patient's result. CLSI recommends verifying this circumstance, especially valuable in immunoassay methods, having an impact in their clinical applicability and affecting the decision-making process. **Aim** To evaluate the effect of reagent lot-to-lot variability on CEA patient's results. **Patients and Methods** Patient results were retrospectively obtained from the Laboratory Information System over a 2 years period (2014-2015). Serum CEA was measured by chemiluminescence(Centaur XP®, Siemens HCD). Manufacturer traceability statement:Internal standard (highly purified material). We performed the Kolmogorov-Smirnoff test and calculated the median and interquartile ranges (IQR) for every group of results related to each reagent lot. Kruskall-Wallis and median test were used to evaluate if differences were statistically significant. To assess the clinical significance we applied two criteria: desirable biological variation (BV) specification for systematic error (14.3%) and NACB guideline criteria of clinical significance (30%). **Results** 45,987CEA results from 20,871 patients were recruited. Kruskall-Wallis and median test showed statistical significant differences between serum CEA patient results grouped by reagent lot ($p<0.01$). Table shows median and IQR, relative difference related to the previous lot (RelDif), maximum difference between each lot and the rest (MaxDif),expressed as percentage and the number of results obtained with each lot.

Lot number	Median (ng/mL) (IQR)	RelDif(%) (CI 95%)	MaxDif(%) (CI 95%)	N
152	1.14 (0.54-2.20)	(First Lot)	22.8 (21.56-24.04)*	4,561
154	1.36 (0.78-2.29)	19.3 (18.13-20.47)*	19.1 (17.93-20.27)*	4,479
155	1.40 (0.78-2.40)	2.9(2.40-3.40)	21.4 (20.19-22.61)*	4,564
156	1.20 (0.6-2.3)	-14.3(-15.34- -15.34)*	16.7 (15.14-18.26)*	2,291
157	1.10 (0.5-2.1)	- 8.3(-9.45- -7.15)	27.3 (26.30-28.30)*	7,995
158	1.30 (0.6-2.3)	18.2(17.34-19.06)*	15.4 (14.38-16.42)*	5,041
159	1.30 (0.7-2.3)	No Difference	15.4 (14.44-16.36)*	5,601
160	1.10 (0.5-2.20)	- 15.4(-16.36 - -14.44)*	27.3 (26.08-28.52)*	5,377
161	1.10 (0.5-2.1)	No Difference	27.3 (25.62-28.98)*	2,813
162	1.40 (0.7-2.4)	27.3(25.62-28.98)*	21.4(19.96-22.84)*	3,265
Total	1.25 (0.6-2.28)	-	-	45,987
*Desirable SE -BV (%) No one exceeded NACB criteria (30%)				

Conclusions Differences observed in CEA results in this study based on BV, although do not exceed the NACB criteria, reinforce the need of performing an evaluation of new reagent lots prior to analyze patient samples. This could be a source of analytical error so laboratories should apply protocols for detecting them in order to avoid erroneous clinical decisions.

A-051

Biomarker Discovery by Proteomic Analysis of Ubiquitin Modification in Ovarian Cancer Cells

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Background: Cancer biomarkers are essential for screening, diagnosis, treatment assessment and monitoring disease recurrence in patients. Preliminary identification of biomarkers is challenging and initial screening is often conducted in cell models where the protein profile is examined with respect to expression, mutations and abnormal post-translational modification. In this study, ubiquitin post-translational modification is examined in an ovarian carcinoma cell line with the aim of establishing a ubiquitination profile and identifying alterations in ubiquitin signaling that can be translated to disease presentation. Ubiquitination is primarily associated with degradation by the 26S proteasome. However, ubiquitination is also involved in other signaling mechanisms that impact protein function in a degradation-independent manner. The objective of this project is to utilize stable isotope labeling by amino acids in cell culture (SILAC) and LC-MS/MS as a preliminary biomarker screen through the identification of ubiquitinated proteins in SKOV3 ovarian cancer cells. This approach assesses ubiquitin-dependent changes in protein levels, while differentiating between proteins targeted for degradation and those ubiquitinated for degradation-independent signaling.

Methods: SKOV3 cells are cultured in RPMI media with 10% FBS (Light) and SILAC RPMI media containing $^{13}C_6$-L-lysine and $^{13}C_6{}^{15}N_4$-L-arginine (Heavy) media supplemented with 10% FBS. Light cells are treated with MG132 proteasome inhibitor, a combination of MG132 and tunicamycin (N-glycosylation inhibitor), as well as DMSO (control). Both Light and Heavy cells are harvested 48 hours post-treatment and lysed in urea buffer. Protein concentration is determined for each sample and the Heavy and Light lysates are combined at a 1:1 ratio for each treatment. The samples then undergo reduction and alkylation, followed by trypsin digestion, and offline basic reversed phase (bRP) fractionation (Global samples). A sub-faction of the peptides undergoes further K-ε-GG ubiquitin remnant motif peptide enrichment, following the initial bRP fractionation step (Ubiquitin-enriched samples). Samples are subjected to LC-MS/MS using an Orbitrap mass spectrometer, and protein identification and quantification are conducted using MaxQuant. To validate the reproducibility and precision of this preliminary screen, the experiment is repeated and varying MG132 concentrations and treatment times are tested for optimization.

Results: Proteasome inhibition by MG132, shows overall enhanced protein up-regulation in Global and Ubiquitin-enriched samples compared to DMSO and MG132/tunicamycin treated cells. Ubiquitin-enriched samples represent the ubiquitinated portion of proteins, where MG132 treatment results in up-regulation of 46% of proteins compared to 0% in the DMSO control. Only 12% of proteins are up-regulated in the MG132/tunicamycin treated Ubiquitin-enriched sample, suggesting an effect of glycosylation on ubiquitin modification. Proteins identified in the Ubiquitin-enriched samples, especially ones up-regulated with MG132, are assessed in the Global samples to determine if increased protein levels are due to stabilization by proteasome inhibition or degradation-independent ubiquitination. This approach enables differentiation between protein targets modified by ubiquitin for turnover and those ubiquitinated for trafficking or other non-proteasome signaling.

Conclusion: Protein ubiquitination in SKOV3 ovarian carcinoma cells can be classified into degradation and non-degradation signaling functions. The ability to distinguish these proteins by SILAC-based quantitative proteomics allows screening for cancer biomarkers whose functions are altered in ovarian cells due to abnormal non-degradation mediating ubiquitination.

A-055

Frequency of Somatic TP53 Mutations in Combination with Known Pathogenic Mutations In Non-Small Cell Lung Carcinoma as Identified by Next-Generation Sequencing

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Background: The tumor suppressor gene TP53 is the most frequently mutated gene in human cancer and encodes p53, a DNA-binding transcription factor that regulates multiple genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis and senescence. TP53 is associated with human cancer by either frameshift or nonsense mutations that lead to a loss of wild-type p53 function or by missense mutations that confer alternate oncogenic functions (gain of function) that enable them to promote invasion, metastasis, proliferation and cell survival. Identifying TP53 mutations in tumor cells may help direct more effective therapies for treating cancer; gene therapies to restore the function of TP53 are currently being evaluated. In this study, we identified which TP53 somatic mutations predominated in non-small cell lung carcinoma (NSCLC) using Next Generation Sequencing (NGS) technology. We also identified somatic mutations in numerous actionable genes including *BRAF*, *EGFR*, *KRAS*, *and PIK3CA* that occurred concurrently with these TP53 variants.

Methods: DNA was extracted from 592 NSCLC tumors from formalin-fixed-paraffin-embedded sections and used to prepare barcoded libraries using the Ion Torrent Cancer 50 gene Hotspot Panel v2. Samples were multiplexed and sequenced using Ion Torrent 318v2 chips on the PGM Sequencing Platform. Variants were identified using the Variant Caller Plugin (v4.0.2) available in the Torrent Suite and Golden Helix SVS (v7.7.8) was used to assess quality and functional predictions.

Results: 215 of 592 (36.3%) of NSCLC cases were found to have one or more mutations in the TP53 gene. The two most common P53 mutations in our patient population were V157F and R158L; however, both mutations were present in only 6/215 patients (2.8%). Pathogenic variants were observed in 100 (46.5%) of tumors, including *KRAS* (59/215, 27.5%) *EGFR* (25/215, 11.6%), *PIK3CA* (9/215, 4.2%) and *BRAF* (7/215, 3.3%).

Conclusion: Intense efforts to develop drugs that could activate or restore the original p53 pathway have reached clinical trials. For this reason, the identification of both the particular TP53 mutation and concurrent known actionable genes present in the tumor could lead to appropriate treatment and improved clinical outcomes for the patient.

A-056

Comparison of two methods for determination of squamous cell carcinoma antigen in serum: electrochemiluminescence immunoassay (ECLIA) and chemiluminescent microparticle immunoassay (CMIA).

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Background: Squamous cell carcinoma antigen (SCC) is a serum tumor marker for the diagnosis and management of squamous cell carcinoma. Squamous epithelial cells are the main part of the epidermis, but are also present in the lining of the digestive tract, lungs, and other areas of the body. SCC occurs as a form of cancer in diverse tissues, mainly the lung, uterine cervix, vagina as well as lips, mouth and esophagus. The aim of this study was the comparison of two methods for determination of serum SCC: ECLIA and CMIA. **Methods:** We studied samples of patients who were required to determine serum SCC. Serum SCC were analyzed by two methods: 1. ECLIA: electrochemiluminescence immunoassay in Modular E-170 (Roche diagnostic®), with reference range < 2.3 ng/mL. 2. CMIA: chemiluminescent microparticle immunoassay in Architect i 2000SR (Abbott®), with reference range < 1.5 ng/mL. Statistical analysis was performed by Bland and Altman test and Passing and Bablock regression using the software MedCalc®. **Results:** We analyzed 56 samples. Descriptive statistics are showed in following table (CI: confidence interval; IR: interquartile range):

	Lowest	Highest	Median (95% CI)	IR
ECLIA (ng/mL)	0.66	7.13	1.55 (1.24-1.87)	0.9
CMIA (ng/mL)	0.7	8.1	1.30 (1.00-1.80)	0.8

Spearman's coefficient of rank correlation (rho) was 0.911 ($p<0.0001$). The mean of differences between ECLIA and CMI using Bland and Altman test was 0.15 ng/mL. The Passing and Bablock regression was: CMIA = 0.0254 + 0.8972 ECLIA. **Conclusions:** The serum SCC values were highers using ECLIA than CMIA. The serum SCC determined on samples by different assay methods cannot be used interchangeably.

A-057

Elevation of serum human chorionic gonadotropin level in a patient with giant cell tumor of the bone

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Background: Human chorionic gonadotropin (hCG) is a glycoprotein that is predominately secreted by the trophoblastic cells of the placenta after implantation of the fertilized ovum. hCG test on urine and serum is the most popular and reliable approach for early detection or serial monitoring of a pregnancy. Moreover, serum hCG elevation has been well documented as a tumor marker in a number of gestational trophoblastic and non-trophoblastic neoplasms. However, the alteration of hCG level in patients with giant cell tumor of bone (GCTB) is still largely unknown. The objective of this study is to report a case of GCTB with a concomitant elevated serum hCG, highlighting the variability based on the specific hCG isoforms.

Method: Serum samples were collected from a patient with GCTB and a healthy pregnant woman as a positive control, and hCG level was tested by three immunoassays (Beckman, Abbott, and Roche)

Results: We presented a case of an 18 year old woman with a slow growing painful mass located at the proximal head of her left humerus. Radiologic imaging revealed a large lytic bone lesion which on core needle biopsy contained spindled and osteoclast type giant cells. Surgical excision was planned. On the day of her surgery, her urine and serum hCG were positive with the serum level at 38 IU/L (Beckman, reference interval: <5 IU/L). Since patient was not sexually active, a potential false positive result was further investigated. The elevated level of HCG was confirmed by our lab and other two local clinical labs (using immunoassays of Abbott or Roche). However, a significant inter-assay variation (ranging from 19 to 40 IU/L) was noticed among the three assays. Meanwhile, a serum sample of a known healthy pregnant patient also was tested concurrently in the three labs as the positive control, which showed the consistent hCG levels of 48 IU/L. Twenty four hours post tumor resection, the patient's serum hCG level dropped to 2 IU/L and remained at this level during her

hospital stay. The identical low serum hCG levels detected by the three labs after tumor resection, suggested that the variable results of tumor-derived hCG might be due to different isoforms. Moreover, post-surgical histopathology diagnosis of the lesion indicated GCTB with hCG expression in mononuclear cells, atypical giant cells, and S100-positive dendritic cells. Our results indicated that an elevated serum hCG level in this patient was primarily produced by GCTB. The different isoforms other than the intact form of hCG derived from GCTB contributed to the inter-assay variation of hCG results by the three immunoassays.

Conclusion: Since GCTB often occurs in younger females, it is important to be aware of the possibility of hCG expression by GCTB leading to a false positive pregnancy result. hCG may serve as an ancillary marker in diagnosis, post-treatment follow-up and monitoring for recurrence. Finally, our results also indicate a standardized assay targeting specific hCG isoforms is needed for the diagnostic test of GCTB.

A-060

Detection of non-innoculated fecal immunochemical test kits

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Introduction: Fecal immunochemical testing (FIT) is now considered a "gold standard" for colorectal bleeding and cancer screening programs. As with any patient collected specimen, FIT runs the risk of improper sample collection, including failure to collect a sample before the kit is returned to the lab. The spectrophotometers currently available for analysis of FIT kits cannot detect whether the kit is inoculated or not. Thus, a low absorbance can be due to lack of blood in the specimen or no specimen. This could pose a patient safety risk if an uninnoculated specimen was not caught and was incorrectly reported as a negative, especially for a large lab than runs several hundred specimens daily. We found that the problem also exists at other labs using the same kit.

Methods: FIT is performed at our lab using the OC-Sensor Diana system from Polymedco. The screening cutoff is 75 ng/mL that has been set by the provincial colorectal cancer screening program. Specimens are collected into FIT kits also from Polymedco. Kits are distributed through laboratory services to patients along with provincially standardized instructions on how to properly collect the test. The collection kits are often prelabelled with a laboratory information system patient identifier label to ease identification and sample return. Twenty non-innoculated FIT kits were run on the instrument to determine the absorbance for empty tubes.

Data pulls were made from the OC-Diana sensor instrument as well as from our laboratory information system (Cerner Millennium). All data was analyzed in Microsoft Excel.

Results: Results showed that non-innoculated FIT kits gave an average result of 15 ng/mL. We then reviewed over 140,000 patient results obtained over 16 months and found that over 70% of results had a reading of 15 ng/mL or lower indicating that using a cutoff was not a viable option. In addition, we investigated the use of administrative controls to prevent acceptance of non-innoculated specimens. Prelabelling of FIT kits was discontinued and patients returning a kit were required to log their specimen in with accession staff. This has been successful in reducing the number of non-innoculated FIT kits arriving at the laboratory from 1-2 a week to only 2 detected since the implementation of the administrative block. The manufacturers of the kits were also contacted for solutions, which has resulted in a short term solution of redesigning the FIT kit labels with a break-away seal to indicate that the kit has been opened.

Conclusion: Labs running FIT should evaluate their local process to ensure that non-innoculated FIT kits are detected prior to analysis.

A-061

BRCA1 and BRCA2 NGS Sequencing and Pathogenic Variants Prevalence in Female Patients in Brazil

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Background: Breast cancer is the leading cause of death from cancer in women in Brazil. About 10-15% of breast cancer cases present a heritability pattern and pathogenic mutations in BRCA1 (Breast Cancer 1) gene , located on chromosome 17 and in BRCA2 gene (Breast Cancer 2), located on chromosome 13, are responsible for half of this type of cancer and both are associated with predisposition to Hereditary Breast and Ovary Cancer Syndrome (HBOC). The *BRCA1* and *BRCA2* genes encode tumor suppressor proteins that act in DNA repair pathways and are important to maintain the stability of genomic DNA. Mutation in one of these genes, decreases cell repair effectiveness facilitating accumulation of mutations that can lead to cancer. The cumulative risk throughout life for a female who carries a germinative deleterious mutation in either of these genes is around 85% whereas it is about 12,5% in total population. Therefore, molecular testing to identify these mutations becomes a powerful tool that enables the identification of individuals at risk and initiate a surveillance and early prevention. Sanger sequencing was the established technique used to identify these mutations but with the advent of Next Generation Sequencing (NGS) we are able to sequence a larger amount of samples in a faster and cheaper way, increasing the availability of molecular tests to those eligible for screening. **Objective:** The aim of this study was to evaluate the prevalence of BRCA1 and BRCA2 genes mutation with NGS in female patients in a large Brazilian private laboratory.

Methods: 104 DNA samples obtained from a female group of patients with breast and/or ovarian cancer were sequenced in the Ion PGM platform (Thermo Fisher). The region of interest was amplified using Ion AmpliSeq BRCA1 e BRCA2 Panel (Thermo Fisher) and the sequencing analysis was obtained using the Ion Torrent Browser. The medium coverage was 200X. After identification of candidate variants IGV (Integrative Genome Viewer) analysis was performed and then additional biological annotation for each candidate variant was made consulting the following data bases: ClinVar, Breast Cancer Information Core (BIC), Leiden Open variation Database (LOVD), ARUP and EVS (Exome Variant Server). Sanger sequencing then confirmed the variants classified as pathogenic. To identify intragenic deletion or duplication, MLPA (Multiplex Ligation Dependent Probe Amplification) was performed using the SALSA MLPA KIT P002 BRCA1 and the SALSA MLPA KIT P045 BRCA2/CHEK2 (MRC Holland Amsterdam, The Netherlands).

Results: From the 104 samples analyzed, 26 (25%) presented pathogenic variants, of which 12 were present in BRCA1 gene and 14 in BRCA2 gene. All 26 pathogenic variants were confirmed with Sanger Sequencing. MLPA was performed in all samples and no deletions or duplications were identified.

Conclusion: In conclusion, the results obtained with Sanger Sequencing were in accordance with NGS results, suggesting a prevalence of 25% of pathogenic variants in BRCA1 and BRCA2 genes among the patients analysed.

A-062

Genomic DNA Purification From Human Whole Blood and Buffy Coat

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Background: Whole blood and buffy coat are commonly used sample types for genomic analysis in applications such as qPCR, microarrays, and sequencing among others. The Maxwell® RSC Whole Blood DNA and Maxwell® RSC Buffy Coat DNA kits have been developed to purify genomic DNA from these sample types on the Maxwell® RSC instrument, a bench top personal magnetic particle handler. A user adds sample directly to the first well of the pre-dispensed cartridge and starts the purification protocol on the instrument. Whole blood collection tubes containing common anticoagulants (EDTA, heparin, and citrate) are compatible with these purification chemistries. The 45 minute purification protocol results in large molecular weight genomic DNA of high purity and concentration that is compatible with downstream amplification. The Maxwell® RSC Whole Blood DNA kit can process between 50-500µl of blood while the Maxwell® RSC Buffy Coat DNA kit can process between 50-250µl of buffy coat sample. **Method:** To demonstrate performance of the Maxwell® RSC Whole Blood DNA kit, whole blood was collected from six Individuals and used for purification. For each Individual, four replicate blood samples of 500µl volume were purified and analyzed using a Nanodrop spectrophotometer and agarose gel electrophoresis. Quadruplicate whole blood samples from two additional individuals were purified. Eluates from these samples were analyzed using a Taqman-based qPCR assay to assess quantitation and inhibition. For the Maxwell® RSC Buffy Coat DNA kit, whole blood samples were collected from six Individuals and the blood tubes were centrifuged to separate the blood into plasma, white blood cell, and red blood cell layers. Buffy coats were drawn from the white blood cell layers and quadruplicate 250µl buffy coat samples from each individual were used for purification. Samples were analyzed for using a Nanodrop spectrophotometer and agarose gel electrophoresis. An additional sample of buffy coat was purified in quadruplicate for analysis using a Taqman-based qPCR assay to assess quantitation and inhibition. **Results:** Using the Maxwell® RSC Whole Blood DNA kit, average DNA concentrations ranged from 70 to 370 ng/µl while average yields ranged from 4µg to 16µg depending on white blood cell count of the initial blood sample. The purity ratios for A_{260}/A_{280} ranged from 1.85 to 1.91 while the purity ratios for A_{260}/A_{230} ranged from 1.92 to 2.44. The resulting DNA performed well in qPCR amplification.

Using the Maxwell® RSC Buffy Coat DNA kit, average DNA concentrations ranged from 260 to 860 ng/µl while average yields ranged from 27µg to 53µg depending on the white blood cell count. The purity ratios for A_{260}/A_{280} ranged from 1.87 to 1.93 while the purity ratios for A_{260}/A_{230} ranged from 2.19 to 2.46. The resulting DNA performed well in qPCR amplification.**Conclusions:** The data generated from the Maxwell® RSC Whole Blood DNA kit produced highly intact, amplifiable DNA with excellent purity ratios from up to 500µl of human whole blood. The Maxwell® RSC Buffy Coat DNA kit can process up to 250µl of buffy coat from human whole blood producing highly intact, amplifiable DNA with excellent purity ratios.