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

S. Kim, S. Cho, H. Yang, S. Kim, H. Lee, T. Park, Y. Kim. Kyung Hee University Medical Center, Seoul, Korea, Republic of

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, Nasushibara, Japan). The monoclonal components were detected in capillary electrophoresis (CE) via capillary 2 (Sefia, Lyse, France) and reconfirmed through a conventional gel electrophoresis (EP) with high-resolution gel EP in a Hydrazsys analyzer (Sefia) using Hydrazgel 15 HR gel (Sefia). 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, supravclavicular, 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 monocellular narrow 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 immunoabsorbtion 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.
Cancer/Tumor Markers

A-007

Urinary cell-free microRNA expression signatures serve as novel noninvasive biomarkers for diagnosis and recurrence prediction of bladder cancer
L. Du, X. Jiang, C. Wang, Qilu Hospital, Shandong University, Jinan, China

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: Misseq 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, urinary 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 p < 0.05).

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
L. Cao, C. Lee, J. Ning, B. Handy, E. Wagard, Q. Meng, MD Anderson Cancer Center, Houston, TX

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 miRNA 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 TOSSH 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.

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 model 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%.

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
H. A. Hagner1, T. Soliman2. 1Faculty of medicine Zagazig University, Zagazig, Egypt, 2Faculty of medicine Sohag University, Sohag, Egypt

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 HCV 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 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: 0.880 - 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 that 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
A. Ohuka1, H. Takeamura1, T. Takahashi2, K. Kimura2, A. Konishi1, K. Ichishishi1, T. Hori1, T. Mida1, Y. Tabee1, 1Juntendo University Hospital, Bunkyo-ku, Tokyo, Japan, 2Juntendo University, Bunkyo-ku, Tokyo, Japan

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 hematolgy analyser XN-1000 (SN 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 histioctye fractions. As a reference method, morphological manual differential (200 cells counts) was performed by two experienced technologists using cytopsin slides stained with the May-Grumwald 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 scattergrams compared to 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.

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

T. M. McFadden1, E. C. Harte1, L. C. Kelly1, C. Richardson1, P. Hendolin1, L. Palohimo1, R. I. McConnell1, J. V. Lamont1, S. P. FitzGerald1, Randox Laboratories Ltd., Crumlin, United Kingdom

**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 PGII 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 atrophic gastritis the levels of G17 are ultimately decreased. These serum biomarkers therefore, are valuable in the screening of atrophic gastritis 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.

**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 PGII, 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 (r²) and slope: PGII assay r²=0.826, slope 0.7267; PGII assay r²=0.9439, slope 0.9297 and G17 assay r²=0.8816, slope 1.068.

**Conclusions:** The results of this collaborative study indicate applicability of BAT to the simultaneous measurement of PGII, 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 for testing of patients.
**A-013**

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


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, during follow-up 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 cDNA 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 cDNA 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 cDNA in stage 1 patients were lower than in stage IV (1 000 copies/mL versus 3 523 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 3 462 copies/mL. Although we observed a decrease in EGFR activating mutations levels for both baseline and best response, this decrease did not reach significance during follow-up, T790M was detected in 53% of patients.

Conclusion: EGFR cDNA analysis by ddPCR seems a relevant tool for clinical management of NSCLC patients.

**A-014**

**A Laboratory Validation of the Dual Method of hCG and AFP in CSF**

Z. Shahani-Yi1, J. W. Martin1, A. A. Brunelle1, M. A. Cervinski1,1 The Geisel School of Medicine at Dartmouth, Dartmouth-Hitchcock Medical Center, Hanover, NH, 1Dartmouth-Hitchcock Medical Center, Lebanon, NH

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 sample 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 118.5mIU/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 LOD, LOQ 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 LOD, LOQ 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 10%; 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**


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: Resident 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. The results of this study show that MALDI-TOF MS can be used to detect and characterize serum free light chains in IFE-negative patient sera with abnormal K/L FLC ratios.

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 118.5mIU/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 LOD, LOQ 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 LOD, LOQ 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 10%; 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.
**Cancer/Tumor Markers**

**A-016**

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

M. A. Pereira¹, M. C. M. Freire², G. T. Torreznán², E. Mateo³, A. C. S. Ferreira¹, M. G. Zalis¹. ¹Hermes Pardini Institute (Research and Development Sector), Respavino, Brazil, ²Hermes Pardini Institute (Progenética), Rio de Janeiro, Brazil

**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, XPC and XRCC2. Two heterozygous mutations were identified: a known benign mutation in exon nine of the PALB2 gene (rs45551636: c.2993G>A; p.G996R) 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. Computer prediction 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. **Results:** 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’s well-being and survival.

**A-018**

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

J. R. Tate¹, C. Pretorius¹, S. Klingberg¹, A. Kennard¹, C. Hawley³, J. Campbell¹, M. Drayson², P. Mollee². ¹Pathology Queensland, Royal Brisbane and Women’s Hospital, Brisbane, Australia, ²Department of Renal Medicine, Princess Alexandra Hospital, Brisbane, Australia, ³Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom.

**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<sup>TM</sup> 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<sup>TM</sup> and N Latex 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<sup>TM</sup>, 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<sup>TM</sup> (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<sup>TM</sup> (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<sup>TM</sup> (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<sup>TM</sup> 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% CI. 128-152) to 21 mg/L (95% CI. 14.8-27.3) for Freelite<sup>TM</sup> versus N Latex, from 156 mg/L (95% CI. 142-170) to 59.5 mg/L (95% CI. 51.6-67.4) for Luminex versus N Latex, and increased from 16.1 mg/L (95% CI. 8.0-24.2) to 38.5 mg/L (95% CI. 31.7-45.2) for Luminex versus Freelite<sup>TM</sup> 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<sup>TM</sup>, and 66% for N Latex. Median lambda FLC decreased 63% for Luminex, 36% for Freelite<sup>TM</sup>, 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<sup>TM</sup>. 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<sup>TM</sup>. This difference in clearance of lambda FLC by Freelite<sup>TM</sup> possibly reflects a difference in antibody reactivity of various molecular forms of lambda FLC that may be present in renal disease.

**A-017**

**Evaluation of the Immunoassay Reagent Kit for PIVKA-II (ARCHITECT<sup>®</sup> PIVKA-II) for the Fully-Automated Chemiluminescent ARCHITECT Analyzer**

I. Tanaka¹, K. Fujita¹, M. Kano², Y. Itoh², K. Ohno², H. Saegusa², H. Kinukawa¹, T. Yoshimura¹, ¹Abbott Japan Co., Ltd., Chiba, Japan, ²Denka Seiken Co., Ltd., Nigata, Japan

**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 mg/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) were performed based on guidance from CLSI Document EP17-A2. Linearity was performed based on guidance from NCCLS Document EP6-A. Potential interferences were performed based on guidance from CLSI Document EP7-A2. Correlations to Picolumi and Luminex 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 mg/mL. The LoB, LoD and LoQ ranged from 0.45 to 0.64, from 0.15 to 1.45 and from 4.93 to 5.06 mg/mL. The assay is linear up to 30,000.00 mg/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 Luminex 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.
Circulating Free DNA Assessment of Prognostic Biomarkers in Prostate Cancer

A. Tomasi, G. Ponti, M. Maccaferri, M. Mandrioli, G. Bianchi, S. Micali, T. Ozben. UNIMORE, Modena, Italy, 'A'deniz University, Antalya, Turkey.

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 Qagen 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 carcinoma biomarkers including prostatic specific 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.

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

S. A. K. Saleh, H. M. Adly, A. M. Nassir. Umm Al Qura University, Faculty of Medicine, Makkaah, Saudi Arabia

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. Results: Serum levels of hK2, CCL11, f/tPSA and t/PSA 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 93% 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 t/PSA significantly differentiated between early and advanced PCa stages (p<0.05) with a sensitivity 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.

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

K. Turner, M. Kalafatis. Cleveland State University, Cleveland, OH

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 understanding of death 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 BD Bioscience. 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 9.9%±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.
**Cancer/Tumor Markers**

**A-025**

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


1University of Louisville, Louisville, KY; 2First Urology, Jeffersonville, IN; 3University of Arizona, Tucson, AZ

**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 transposons. DNA hyper-activation of the LINE1 promoter regulates its activity 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 proteins. The assay was performed in cases with biopsy-proven cancer with biopsy and 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 ORF1 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 ORF1 protein concentration in biopsy-confirmed prostate cancer subjects (n = 8) was 33.7 (SD=15) ng/ml, and 23.7 (SD=10) in biopsy-normal 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**

M. C. M. Freire1, G. T. Torrezan2, M. A. Pereira3, M. G. Zalis4, A. C. S. Ferreira5, E. Mateo6,7, Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil, 2Hermes Pardini Institute (Progenética), Rio de Janeiro, Brazil

**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 genomic alterations that can lead to malignant transformation and cancer. Around 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 BRCA1 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 PGMTM 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 deleterions resulting in frame shifts: a BRCA1 mutation in exon 11 (c.7987,799delATT:p.Y266fs/ rs80357724) and a BRCA2 mutation in exon 11 (c.2008_2011delACACA/p.K663fs/ rs80359542) for a 58-year-old woman, we identified a BRCA1 mutation in intron 13 (c.3478+1G>A/IVS13+1G>A/ rs80358027) and a BRCA2 mutation in exon 11 (c.6402_6406delTAACAT:p.N2134fs/ rs08359548). 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 causes 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.
Prevalence of EGFR mutation in non-small cell lung cancer patients from Brazil: A personalized medicine for EGFR-TKI test

G. T. Torrezan¹, A. S. Matos², M. C. M. Freire², M. A. Pereira³, E. Mateo², M. G. Zalis¹, A. C. S. Ferreira¹, C. G. Ferreira¹. Hermes Pardini Institute (Progenetica), Rio de Janeiro, Brazil; Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil, ¹Instituto Nacional de Cáncer, INCA, Rio de Janeiro, Brazil

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’ 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 Progenetica 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), with 53% women and 47% men. Ten percent of the patients showed invalid results and these samples were associated with poor DNA quality and/or quantification (p<0.01). From the remaining patients, we identified mutations at 432(24%). A total of 416 patients presented only one mutation in EGFR gene and 16 were double mutant. Mutations in exon 19 (96%) and exon 21 (LS858) 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. Another 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 & 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.

Improving quantification of M-protein Using Capillary Electrophoresis Immunosubtraction

L. F. Schroeder, S. Li, D. F. Keren. University of Michigan, Ann Arbor, MI

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 immunosubtraction (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 Capillars 2™. Capillars 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.

Light Chain Escape: An infrequent relapse in Multiple Myeloma

J. L. García de Veas Silva¹, T. De Haro Muñoz², R. Escobar Conesa³, R. Rios Tamayo¹, M. Lopez Velez³, J. Garcia Lario³. Complejo Hospitalario Universitario de Granada, Granada, Spain, ¹Hospital Comarcal de Jarrio, Asturias, Spain

Background: Free light chain escape (FLC escape) is defined as an increase in monoclonal serum light chain (sFLC) without a corresponding increase in the concentration of the non-mutated monoclonal free light chain (FL), and can be observed in patients with multiple myeloma (MM) who had previously achieved a complete response with negative SPE, and remains a challenge for the detection of minimal residual disease in patients who have undergone 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.
Cancer/Tumor Markers

J. L. García de Veas Silva¹, T. De Haro Muñoz¹, A. Espuch Oliver¹, R. Escobar Conesa¹, M. López Velez², J. García Larío¹. ¹Complejo Hospitalario Universitario de Granada, Granada, Spain, ²Hospital Comarcal de Jarrio, Asturias, Spain.

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, neuroblastoma and sarcoma. The aim of this study is to show the clinical 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.

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

Conclusion: The presence of anti-Hu antibodies was associated with lung cancer in three patients while in the remaining patient was not found a tumour. In these three patients, 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.

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A-031

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

M. C. Cárdenas¹, C. Benavente¹, A. Iglesias¹, W. Torres¹, L. Campos¹, R. Martínez¹, M. Arroyo¹.¹Hospital Clínico San Carlos, Madrid, Spain, The Binding Site, Madrid, Spain

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 (HLC) 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 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 HLC, 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 HLC, 83.5 % presented increased iHLC and 80.7 % decreased uHLC. Moreover, HLC 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 HLC and uHLC suppression (kappa coefficient, 0.510 and 0.439, respectively).

Conclusion: These results confirm HLC 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.
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 endogeneous substances (human anti-mouse antibody, chemulades 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 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 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) ranging from 33.4 - 4620.0 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 Risk of Ovarian Malignancy Algorithm (ROMA) Derived from Lumipulse G HE4 and Lumipulse CA125II Assays


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 and 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 = -8.89 + 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 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.

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

J. Detmer, M. Ray, M. Baumeister, X. Qiu, M. Matias, Z. Wang, A. Uzgiriz, Siemens Healthcare, Berkeley, CA

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-qPCR, 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.
Cancer/Tumor Markers

**Cancer/Tumor Markers**

H. Choi1, M. Shin1, S. Cho1, S. Kim1, S. Kee1, J. Shin1, B. Park2, S. P. Suh1, 1Department of Laboratory Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of, 2Department of Internal Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of. 3Department of Food Science & Nutrition, Mokpo National University, Mokpo, Korea, Republic of.

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 U/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.

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

C. Rooney1, Y. Fari1, R. Inzitari1, B. Hernandez1, A. Parnell2, P. J. Twomey2, S. R. Pennington1, 1University College Dublin, Dublin, Ireland, 2St. Vincent’s University Hospital, Dublin, Ireland

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 these 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 Urology (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 performance 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.

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

E. N. Cohen1, G. Jayachandran1, H. Gao1, S. Jellbauer2, J. D. Khoury1, J. M. Reubén1, 1The University of Texas MD Anderson Cancer Center, Houston, TX, 2Affymetrix eBioscience, Inc., San Diego, CA

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 that it captures CTC and predictive marker in metastatic breast cancer. However, enumeration of CTC by Angle PLC Parsortix and downstream molecular characterization sets it apart from many other existing and nascent technologies is that it captures CTC and predictive marker in metastatic breast cancer. However, enumeration of CTC by Angle PLC Parsortix and downstream molecular characterization.
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

R. Pérez Garay1, I. Ventura1, A. G. Melendez1, L. Campos2, E. A. Diez1, M. V. Pampiega1. 1Hospital Universitario Cruces, Bilbao, Spain, 2The Binding Site, Barcelona, Spain

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 SP®2002 (Binding Site); SPE on a Capillarlys Hydramys 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® (HLC=0.203+1.15 SPE; r=0.928; p=0.0001) and between total IgA and the sum of Hevylite IgAκ/IgAλ (∑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 IgGλ 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 adenal masses

T. Pappas1, V. Bonato2, A. Smith2, Z. Zhang1. 1Vermillion, Inc, Austin, TX, 2ACI Clinical, Bala Cynwyd, PA, The Johns Hopkins University School of Medicine, Baltimore, MD

Objective: A second generation multivariate index assay (MIA2G) has been developed for improved detection of ovarian cancer among women undergoing removal of adenal 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 <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

D. Zhang, W. Cui. Peking Union Medical College Hospital, Beijing, China

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-

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**A-050**

**EFFECT OF REAGENT LOT-TO-LOT VARIABILITY ON CARCINOEMBRYONIC ANTIGEN PATIENT’S RESULTS**


**Background**
Carcinogenic properties of 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-Smirnov 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
45,987 CEA 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 (RelDi), maximum difference between each lot and the rest (MaxDi), expressed as percentage and the number of results obtained with each lot.

**A-049**

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

M. Xaviger, D. Baldwin, M. Shemar, A. Din, C. Hand. Abingdon Health Ltd, Birmingham, United Kingdom

**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 immunassay 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 this 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 0.7mg/dL (equivalent to 200 RBC/microlitre).

Clinical testing has started with the retrospective analysis of 71 pathological urine samples for urothelial cancer. The system shows high sensitivity with 55% for kappa (95% CI, 41.7-68.3) and 46% for lambda (95% CI, 32.7-60.7). The system shows a specificity of 95% for kappa (95% CI, 90.4-98.8) and 96% for lambda (95% CI, 93.2-98.9). The system shows an area under the curve of 0.98 for kappa (95% CI, 0.95-1.00) and 0.93 for lambda (95% CI, 0.89-0.97).

**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**

V. Hristova, S. N. Thomas, S. Sun, D. W. Chan, H. Zhang. Johns Hopkins University School of Medicine, Baltimore, MD

**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 ubiquitinating signal 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.
**Conclusions:**
Protein ubiquitination in SKOV3 ovarian carcinoma cells can be classified into degradation and non-degradation signaling functions. The ability to distinguish these functions utilizing SILAC-based quantitative proteomics allows screening for cancer biomarkers whose functions are altered in ovarian cells due to abnormal non-degradation mediated ubiquitination.

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### 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**

Z. Shajani-Yi, F. B. de Abreu, J. D. Peterson, G. J. Tsongalis, Dartmouth Geisel School of Medicine/Dartmouth-Hitchcock Medical Center, Hanover, NH, Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH

**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 PI3KCA 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 318x2 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. The Ion Torrent DNA bases were called and variant allele frequencies were calculated using the software MedCalc®.

**Results:** We analyzed 56 samples. Descriptive statistics are shown in following table (CI: confidence interval; IR: interquartile range). Spearman’s coefficient of rank correlation (rho) was 0.911 (p<0.0001). The mean of differences between ECLIA and CMIA using Bland and Altman test was 0.15 ng/mL. The Passing and Bablok regression showed CMIA: 0.0254 ± 0.0872 ECLIA. The serum SCC values were highers using ECLIA than CMIA. The serum SCC determined on samples by different assay methods cannot be used interchangeably.

**Conclusion:** Serous ovarian carcinoma cell tumor markers can be classified into degradation and non-degradation signaling functions. The ability to distinguish these functions allows screening for cancer biomarkers whose functions are altered in ovarian cells due to abnormal non-degradation mediated ubiquitination.

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### A-056

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

C. Cañavate-Solano, J. D. Santotoribio, M. García-de la Torre, F. Arce-Matute, J. Cuadros-Muñoz, S. Pérez-Ramos, Puerto Real University Hospital, Cadiz, Spain, Virgen de la Victoria University Hospital, Malaga, Spain

**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 Bablok regression using the software MedCalc®. **Results:** We analyzed 56 samples. Descriptive statistics are showed in following table (CI: confidence interval; IR: interquartile range).

**Conclusion:** Serous ovarian carcinoma cell tumor markers can be classified into degradation and non-degradation signaling functions. The ability to distinguish these functions allows screening for cancer biomarkers whose functions are altered in ovarian cells due to abnormal non-degradation mediated ubiquitination.

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### A-057

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

H. Xu, M. Haroon Al Rasheed, R. Kazemirood, E. Wiley, M. Jin, University of Illinois at Chicago, Chicago, IL

**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 control. Serum hCG samples were analyzed by two methods: 1. Immunofluorescence (IF) and chemiluminescence microparticle immunoassay (CMIA) and chemiluminescent microparticle immunoassay (ECLIA). **Results:** We presented a case of a 18 year old woman with a slow growing mass located at the proximal head of her left humerus. Radiologic imaging revealed a large lytic bone lesion which on core needle biopsy contained spindle cell and osteoclast type giant cells. Surgical excision was planned. On the day of her surgery, her urine and serum hCGs 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 immunooassays 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...
Tuesday, August 2, 9:30 am – 5:00 pm

Cancer/Tumor Markers

A-060

Detection of non-innoculated fecal immunochemical test kits

J. M. Boyd, D. Orton, H. Sadrzadeh. Calgary Laboratory Services, Calgary, AB, Canada

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 uninoculated 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. Prelabeling 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-062

Genomic DNA Purification From Human Whole Blood and Buffy Coat

C. Newton, C. Cowan, A. Blatter, C. Moreland. University of Wisconsin, Madison, WI, University of Colorado Boulder, Boulder, CO, University of Wisconsin LaCrosse, Madison, WI, Texas State University, Madison, WI

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 benchtop 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 protocols. 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 blood 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 A260/A280 ranged from 1.85 to 1.91 while the purity ratios for A260/A230 ranged from 1.92 to 2.44. The resulting DNA performed well in qPCR amplification.

A-061

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

P. Y. Nishimura, L. T. Galindo, M. P. Migliavacca, O. Fernandes, L. C. Scarpelli. DASSA - DIAGNOSTICOS DA AMERICA, BARUERI, Brazil

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 IVG (Integrative Genome Viewer) analysis was performed and then additional biological annotation for each candidate variant was made considering 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/CHK2 (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.
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.