Conclusions: Multiplex biochip array technology offers an innovative and patient-friendly approach to colorectal cancer screening. The diagnostic value of identifying further serum biomarkers and the potential advantage of combining biochip analysis with fecal occult blood has the potential to improve the performance of colorectal cancer screening and warrants further investigation.

**P17-10**

Identification of novel cancer biomarkers using the Randox-QuantiPlasm69 monoclonal antibody chip

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Background: Recently a novel monoclonal antibody based protein chip – QuantiPlasm69 (QP69) – has been introduced by Randox Laboratories. This system uses 69 monoclonal antibodies (mAbs) – developed by BioSystems International – that are immobilized on 9x9 mm ceramic chips. The QP69 assay can recognize concentration changes of several human plasma proteins simultaneously and in this way can identify novel plasma markers in a wide variety of diseases.

Materials and methods: Plasma samples and clinical data of 150-150 patients with prostate and lung cancer and 300 healthy controls were collected. Individual and pooled samples of the patients and controls were evaluated by the QP69 system. The plasma pools were created from the individual samples based on clinical, histopathological and laboratory data. Other biochemical parameters and the classical tumormarkers were also measured. To find the most predictive parameters principal component, binary logistic regression and ROC analysis was performed beside the classical statistics.

Results: A set of mAbs (three antibodies) present on the QP69 chip were able to discriminate between healthy controls and lung cancer patients, and a different set of mAbs (three antibodies) were able to distinguish samples of healthy controls from those of prostate cancer patients. These differences were independent of age and smoking habits. Combination of these antibodies in themselves or with classical tumormarkers could further improve their efficacy.

Conclusions: The QP69 kit can be an effective tool in biomarkers’ search and discovery. This work was supported by the National Office for Research and Technology of Hungary (TECH-09-A1-2009-0113; mAB-CHIC).

**P18 - Oncology - Tumor marker 2**

P18-01

Evaluation of tumor marker HE4 assay on the Elecsys 2010 analyzer

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Background: Whey-acidic protein human epididymis protein4 (HE4), a new promising biomarker for epithelial ovarian cancer (EOC). The measured HE4 value of patients sample can depending on the testing procedure use.

Methods: We evaluated a HE4 method on Elecsys 2010 analyzer. The method for quantitative determination of HE4 is direct, competitive electrochemiluminescence immunoassay. For quality control we use Elecsys PreciControl HE4 1 and 2. HE 4 was measure on sera obtained from 96 women (40 healthy and 56 with epithelial ovarian cancer).
Results: The Roche HE 4 assays showed a good linearity \((r = 0.99)\) and precision (intrasay and total CV < 5%). The median HE4 serum concentrations was significantly higher among EOC patients than healthy females \((P < 0.05)\). As a single marker, HE4 had a sensitivity of 78.4 % with a specificity of 95 %.

Conclusions: The presented results of the analytical evaluation methods for the determination of HE 4 on the Elecsys 2010 analyzer showed an acceptable accuracy and precision.

P18-02
CYFRA 21-1 new tumor marker in Abbott iArchitect family

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Introduction: CYFRA 21-1 is a tumor marker that measures fragments of Cytokeratins 19 using two monoclonal antibodies KS 19-1 and BM 19-21, but its main role is to monitor the course and success of the therapy of NSCLC.

Objective: Our goal was to investigate the acceptability of Abbott reagents for use in daily routine on immunochemical analyzer Architect i2000SR to ensure traceability of our results. Validation of immunoassays was performed using CLSI / NCCLS procedure EP 15-A2.

Materials and methods: The following parameters were assessed: Precision (random error) in which we determined repeatability, interprecision and overall laboratory precision by determination of a small group \((N = 30)\) and systematic error (deviation from expected values).

Results:
- Control Level 1: 5 ng / mL (3.5 to 6.5) (Repeatability \(x = 5.23, Sr = 0.22, KV% = 4.2\%\))
- (Interprecision \(x = 5.23, Sb = 0.123, KV% = 2.4\%\))
- (Overall laboratory precision SL = 0.218, KV% = 4.2\%)
- (Systematic error is 0.23 (4.6\%))
- Control Level 2: 35 ng / mL (24.5 to 45.5) (Repeatability \(x = 34.922 / ml, Sr = 0.44, KV% = 1.3\%\))
- (Interprecision \(x = 34.922, Sb = 0.524, KV% = 1.5\%\))
- (Overall laboratory precision SL = 0.635, KV% = 1.8\%)
- (Bias -0.08 (-0.23\%))

Conclusion: Based on the results of this study, we can conclude that the tested reagent CYFRA 21-1 Abbott is acceptable and we recommend its use on automated immunochemical analyzer iArchitect Abbott.

P18-03
Analytical performance of tumor markers on UniCel Dxl 600 using Sigma metrics

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Background: The Sigma concept of a tolerance limit provides guidance for defining intended medical use in the form of an allowable total error. Performance is characterized on a sigma scale. In terms of Sigma, if a method has a value less than three is considered to be unreliable and should not be used in routine laboratory practice. The aim of this study was to assess the performance of automated immunodiagnostic chemiluminescence system UniCel Dxl 600 (Beckman-Coulter, Tokyo, Japan) for qualitative detection of tumor markers.

Materials and methods: For CA19-9, CA15-3, CA125, and PSA method validation Lypochek Tumor Marker (Bio-Rad Laboratories, Marnes-la-Coquette, France; Control lot 54530- Level 1 and 3) control samples were obtained. All immunoassays were performed on the UniCel Dxl 600 analyzer
according to the manufacturer instructions. Control samples were tested in triplicate every day during the 5 days according CLSI/NCLLS protocol EP15-A2. Bias was calculated using method comparison. Sigma was calculated using Ricos quality requirements.

**Results:** Calculated sigma metrics for CA15-3 were 2.5 and 1.4; for CA125 6.5 and 5.9; for CA19-9 6.4 and 9.2; and for PSA-1.0 and 2.2.

**Conclusion:** Analytical performance for CA125 and CA19-9 is world class. On the other hand, CA15-3 and PSA analytical performance is poor or even unacceptable. Using graphic tool it becomes apparent that bias is the main problem with CA15-3 and PSA performance. With lower bias CA15-3 assay performance might even reach Six Sigma zone. Therefore, it is necessary to use the same method for individual patient. In that case, even these two methods became of excellent quality.

**P18-04**

**Prognostic value of Cyfra 21-1, CEA and NSE in patients with NSCLC**

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**Background:** The aims of this study were to evaluate prognostic effects, sensitivity and specificity of Cyfra 21-1 in detecting non-small cell lung cancer.

**Subjects and methods:** The study included 118 randomly selected patients with NSCLC compared with control group of 30 patients with nonmalignant pulmonary disease. Histology tumor diagnosis was based on biopsy specimens obtained at bronchoscopy, lymphode biopsy or thoracotomy. Tumor markers (Cyfra 21-1, CEA and NSE) were assayed in Immunological Abbot’s Axsym System analyzers. Chemotherapy included cisplatin and carbocisplatin were aplicated in doses (80-100 mg/m² day 1.3 wk cycle and 200-300 mg/m² day 1.3 wk cycle for six cycles).

**Results:** The level of tumor markers was determined in both population in different stage of age before and after chemotherapy. Median level of Cyfra 21-1 at diagnosis was 36.2 ng/mL with range 2.8-215.0 and decreased significantly after second cycles of chemotherapy 24.3 ng/mL (range 3.4-145.0; P < 0.01). Same results we obtained in the NSE concentration (23.4 μg/mL before and 14.2 μg/mL after therapy, but CEA shows not significantly changes. Cyfra 21-1 were elevated in 22.3%, NSE in 10.8% and CEA in 16.5% of patients respectively.

**Conclusion:** Cyfra 21-1 reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC. Combining Cyfra 21-1, NSE and CEA correlated with prognosis in a significant and independent manner.

**P18-05**

**Computational screening for non-small cell lung carcinoma biomarker candidates**

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**Introduction:** Comparison of gene expressions obtained on control and patient samples based on computational methods allows selection of relevant genes and their protein products which may serve as biomarker candidates. For the purpose of non-small cell lung carcinoma biomarker screening a multivariate classification method known as k-nearest neighbors has been selected in this study.

**Materials and methods:** The study was conducted on a publicly available set of gene expressions
(Showe MK et al. Cancer Res 2009;69:9202-10) which consists of 291 gene expression profiles (137 non-small cell lung carcinoma patient samples and 91 control samples), each containing 15 227 gene expressions. k-nearest neighbors method was applied to all pairs of gene expressions. t-test with Benjamini-Hochberg correction for multiple testing has been used as a univariate filter.

**Results:** Application of k-nearest neighbors resulted in the test set accuracy of 81.6-86.8% which corresponds to 1000 top ranking gene pairs. Less than a half of highly ranked genes were statistically significant according to the corrected t-test. The majority of the top ranking genes are responsible for the processing of gene information and for the regulation of signaling pathways.

**Conclusion:** k-nearest neighbors method provides insight into the relevance of single genes and gene interactions for differentiation of patient and control samples. Low correspondence between univariate results and the results of k-nearest neighbors underlines importance of the latter. This might influence the quality of candidates for non-small cell lung carcinoma biomarkers and corresponding diagnostic approaches which laboratory evaluation is underway.

**P18-06**

**Prognostic and predictive significance of ERβ1 in primary breast cancer**

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**Background:** Adjuvant endocrine therapy is effective treatment in breast cancer. Patients receive endocrine treatment according to steroid receptor positivity (ERα and PgR). Estrogen receptor β (ERβ) is a second estrogen receptor and its role in endocrine treatment is not fully elucidated. The aim of this research was to determine prognostic and predictive value of isoform EBβ1 in breast cancer.

**Materials and methods:** In the study were included 150 consecutive primary breast cancer cases operated at University hospital Zagreb (year 2002-2003). Overall and disease free survival were recorded until January 1st, 2011. Immunohistochemistry was used for EBβ1 determination (anti-ERβ1 clone PPG5/10, Dako, Denmark).

**Results:** ERβ1 and ERα expressions were not correlated (P = 0.178, r = 0.123). There was no association of ERβ1 with age, menopausal status, lymph node status, tumor size, histological grade, histological type, nuclear grade, Nottingham prognostic index, proliferation index Ki67 expression, steroid receptor status and HER-2/neu status. In univariate Cox-regression analysis, ERβ1 was associated with overall survival (P = 0.026; HR = 0.46; 95%CI 0.24-0.92) but there was no association with disease free survival (P = 0.054; HR = 0.51; 95%CI 0.25-1.04). Kaplan-Meyer survival curve analysis confirmed prognostic significance of ERβ1 for overall survival. In the subgroup of patients that received endocrine treatment (N = 93), there was no statistical difference in survival between patients with high and those with low ERβ1 [overall survival (P = 0.27; HR = 1.73; 95%CI 0.65-4.7), disease free survival (P = 0.08; HR = 2.25; 95%CI 0.91-4.94)].

**Conclusions:** Cancer tissue expression of ERβ1 is a prognostic, but not a predictive marker in primary breast cancer.
**P18-07**

**Comparative study of two chemiluminescent methods for determining free PSA**


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**Background:** The % free PSA (fPSA) is, on average, lower in prostate cancer than the benign prostatic hyperplasia and has been commonly used as an aid in the diagnosis of prostate cancer when PSA is between 4-10 ng/mL.

**Materials and methods:** In our study, fPSA were analyzed in 50 serum samples of patients with total PSA between 4-10 ng/mL. The results obtained using the methods ADVIA Centaur® fPSA test (Siemens) and fPSA Immulite2000 (Siemens), were compared in order to ensure transferability of results between both methods. Total PSA was measured by ADVIA Centaur® method. Analysis of data was performed using the MedCalc®, using Passing-Bablok nonparametric regression test, Pearson’s correlation and agreement between methods was evaluated using the Kappa statistics, according to the following groups: positive > 15% and negative < 15% fPSA.

**Results:** The following values of fPSA were obtained (range, median) 0.39-2.72, 1.191 (95% CI: 1.037 to 1.346) and 0.27-2.46, 1.135 (95% CI: 0.985 to 1.285) for Immulite2000 and ADVIA Centaur® respectively, yielding a correlation coefficient of 0.9519 between both methods (P < 0.001). The Passing-Bablok regression equation obtained had a Slope = 0.9583 (95% CI 0.8729 to 1.0596) and Intercept = 0.0196 (95% CI: -0.0837 to 0.1084). The concordance of % freePSA is good, yielding a Kappa index of 0.797 (95%CI: 0.628-0.966).

**Conclusions:** Both methods of fPSA show a good correlation (0.9 <= R <1) and concordance. Results between methods are transferable.

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**P18-08**

**Soluble urokinase plasminogen activator receptor (suPAR) in breast cancer**

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**Introduction:** The urokinase plasminogen activator system plays an important role in many processes involved with cancer invasion and metastasis. The elevated levels of uPAR as well as uPA and PAI-1 are associated with poor prognosis in cancer patients. Recently, similar suggestions are brought forward to soluble form of this receptor – suPAR. The aim of study was the evaluation of relationship between suPAR and selected biochemical and clinical parameters in breast cancer patients.

**Material and methods:** The study of suPAR, CA 15-3, CEA, CRP was performed in the group of 146 breast cancer patients before surgical treatment and in the reference group of 43 healthy women.

**Results:** In breast cancer patients in comparison with the reference group there were found significantly higher levels of suPAR, CA 15-3, CEA and CRP. Significantly higher levels of CA 15-3 had patients in more advanced stages of disease, with tumor greater than 2 cm, and CRP levels > 3 mg/L. There were no significant differences between analyzed biochemical factors between groups selected in respect to: lymph node status, histological grade, percentage of S-phase cells, steroids receptor status. In the group of breast cancer patients with HER2 overexpression significantly lower suPAR and significantly higher CA 15-3 levels and the percentage of S-phase cells there were observed.

**Conclusions:** In breast cancer patients suPAR level seems to be associated with tendency to inflammation. The reciprocal relationship between HER2 status and suPAR levels may indicate potential role of this receptor in prognosis of some breast cancer patients.
P18-09
BRAF: potential prognostic marker in colorectal cancer

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Introduction: Incidence and mortality of colorectal cancer (CRC) are on constant increase and represent one of the major health problems in Croatia. Activation of BRAF oncogene is implicated in colorectal carcinogenesis. BRAF protein is a serin/threonine kinase, component of a conserved signaling pathway that regulates cellular responses to extracellular signals. In human cancer, it is commonly activated by hotspot mutation of the BRAF gene which leads to a single aminoacid substitution p.V600E. The aim was to determine the incidence of BRAF gene mutations in CRC patients in Croatia and to assess whether they are linked with clinicopathological features of poor prognosis.

Materials and methods: Sections from 113 formalin-fixed paraffin-embedded tumor samples were evaluated for the BRAF mutation using LightCycler PCR with allele-specific fluorescent probe melting curve analysis. Obtained results were confirmed by sequencing method.

Results: Our results show that BRAF gene mutation p.V600E was detected in 8.8% (10/113) CRC samples. Statistical analysis revealed a significant association between the BRAF mutation and Dukes's stage (P = 0.04) where all mutations were found in tumors classified as Dukes' C. Incidence of mutation was higher in males, patients older than 60 years, tumors bigger than 5 cm, tumors with angioinvasion and poor differentiated tumors, but no significant association was found. All BRAF gene mutations were detected in colon cancers.

Conclusions: The incidence of BRAF gene mutation in CRC patients in Croatia is within commonly accepted limits. Higher incidence in tumors with poor prognostic markers shows that BRAF gene mutation play a role in the progression of CRC.

P18-10
Assessment of serum CA 15-3 and CEA concentrations in patients with breast cancer

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Background: Breast cancer is the most common malignancy in women. The aim of this study was assessment of serum CA 15-3 and CEA concentrations in patients with breast cancer and monitoring of their status by these tumor markers.

Material and methods: Serum samples were acquired from female patients with breast cancer (N = 224) grouped according pTNM staging. Control samples (N = 44) were collected from healthy females. Concentrations of tumor markers were measured by the immunochemical analyzer Vitros ECI with enhanced chemiluminiscence.

Results: According obtained results concentrations of CA 15-3 in patients in grade IIIb (102.50 ± 10.61 U/mL) and grade IV (134.50 ± 179.74 U/mL) were 86.67% and 89.84% significantly higher (P < 0.01, P < 0.05 respectively) than in healthy women (13.66 ± 8.55 U/mL). Concentration of CEA in patients in grade IV (19.3 ± 17.4 ng/mL) was 92.64% higher (P < 0.01) compared with healthy women (1.42 ± 1.02 ng/mL). Three months after surgery in patients was noted increase of CA 15-3 values,
which after six months were 71.14% higher, despite CEA values that were in referent range.

Conclusions: Because of low sensitivity tumor markers CA 15-3 and CEA are more suitable for monitoring of state of patients after surgery than for early screening or diagnosis of breast cancer.

Results and conclusions: Total of 1459 samples from clinical routine with known outcome were examined in year 2011. 42 out of the 1459 samples were from pregnancies with confirmed Down’s syndrome. 8 out of the 1459 samples were positive for Edward’s or Patau’s syndrome. Cut-offs for Trisomy 21 and 18-13 were 1/250.

P19 - Pregnancy

P19-01
Results of first trimester screening for Down’s, Edward’s and Patau’s syn using SsdwLab5 software

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Background: First trimester screening is indispensable in early identification of Down’s, Edwards and Patau syndrome in fetuses. We performed combined protocol which included: maternal biochemistry (free beta-hCG and PAPP-A) and sonographic determination of nuchal translucency (NT) during year 2011. Screening was performed between 11+0 and 13+6 weeks of pregnancy. Risk calculation was performed using the software SsdwLab version 5.0. This software makes use of an algorithm described by Palomaki and is based on the mathematical calculation using Gaussian multivariate distribution. Risk analysis is based on maternal age, NT as well as on the results of biochemical parameters, corrected by different factors like e.g. maternal weight, smoking and ethnic background of the pregnant woman.

Materials and methods: Free beta-hCG and PAPP-A were performed by electrochemiluminescence immunoassay on COBAS E 411 immunoassay analyzer.

Results and conclusions: Total of 1459 samples from clinical routine with known outcome were examined in year 2011. 42 out of the 1459 samples were from pregnancies with confirmed Down’s syndrome. 8 out of the 1459 samples were positive for Edward’s or Patau’s syndrome. Cut-offs for Trisomy 21 and 18-13 were 1/250.

P19-02
The role of antenatal screening and amniocentesis on the Down’s syndrome diagnosis – our experiences

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Description: Antenatal screening identifies high risk pregnancies for Down’s Sy. The screen includes the risk assessment based on data processing of the maternal serum PAPP-A and Fβ-hCG and the NT. It is performed between 11w0d-13w6d of gestation. According to the risk assessment women are distinguished into the high risk group (> 1/250) screen positive, and low risk group (< 1/250) screen negative.

Aim: Assessment of correlation between the screening high risk pregnancies and the amniocentesis in our center.

Material and methods: Study included 472 pregnant women tested between 02/2011-04/2012, 22-42 years, gestation 11+0 to 13+6 weeks. The testing of the maternal biomarkers has been performed on Roche Elecsys2010. Measurement of the NT has been performed by Toshiba-XarioXG ultrasound. The risk has been calculated on FMF’s software, where the woman age, CRL, parity, smoking, BMI and ethnic origin has been evaluated too. Amniocentesis has been performed between 17w 1d-20w 6d weeks of gestation on ultrasound guided free hand technique using the 22G needle.