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Analytical technologies and applications

Cod: M027

COMPARISON OF TWO DIFFERENT METHODS (CHEMILUMINESCENCE AND FLUORESCENCE ENZYME IMMUNOASSAY) FOR DETERMINATION OF FAECAL CALPROTECTIN IN THE ASSESSMENT OF INFLAMATORY BOWEL DISEASE

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INTRODUCTION
Faecal calprotectin (FC) is becoming an essential non-invasive marker for inflammation bowel disease (IBD). It may be elevated in certain disorders such as ulcerative colitis and Crohn's disease, the active celiac disease, and colon cancer. The aim of our study was to compare three different FC assays and assess their performance and ability for diagnosis and follow up of IBD.
METHODS
FC of samples obtained from 61 symptomatic patients (28 with active IBD, 9 in clinical remission, 24 with other intestinal diseases) were analysed using: LIAISON® DiaSorin (chemiluminescence), EliA ThermoFisher Scientific, Phadia100 (FEIA), and Calprest® (ELISA), the actual method in use in our laboratory.
Correlation between assays was calculated by linear regression (Passing-Bablok) and difference plot (Bland-Altman) using R. The significance level was determined by the associated p-value set at <0.05. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative like-hood ratios (LR+ and LR-) were calculated.
RESULTS
Our results showed that both test for the measurement of FC showed a high sensitivity and non-significant difference between assays was observed. However, results showed a better concordance between Calprest and Liaison, compared to Calprest and EliA. Correlation coefficients ranged from 0,96 to 0,91, respectively, while the slopes and/or intercepts differed extensively, with up to 3.5-fold quantitative differences between assays.
Calculated sensitivity values were 100% and 96.3% for the Liaison and the EliA assay, respectively. Specificity of 73.5% and 61.8%, PPV of 0.81 and 0.70, NPV of 1.00 and 0.95, LR+ of 4.57 and 2.66, and LR- of 0.00 and 0.05 were obtained.
DISCUSSION
The results obtained showed a better correlation for the Liaison assay compared to the obtained for de FEIA-EliA. Furthermore, the Liaison test allows automation, a wider analytical range, and a 5-fold less time for the first result. However, with respect to specificity and the absolute values in the borderline and pathological range, we found major differences for the two assays investigated. Therefore, clinical interpretation of assay results should be made with caution depending on the assay used.

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Analytical technologies and applications

Cod: M094

SIMULTANEOUS MEASUREMENT OF WHOLE BLOOD VITAMIN B1 & VITAMIN B6 USING LC-ESI-MS/MS

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Background:
Vitamin B1 is used in several decarboxylation- and transketolase reactions and is also involved in the generation of ATP. Vitamin B6 is a cofactor in numerous biological processes that include gluconeogenesis, neurotransmitter synthesis and amino acid metabolism. Our aim was to develop a method to measure the concentration of the biologically active forms of vitamin B1 (thiamine pyrophosphate, TPP) and vitamin B6 (pyridoxal phosphate, PLP) in EDTA whole blood with LC-ESI-MS/MS and compare this new procedure with established homemade methods for total thiamine and PLP.

Methods:
A stable isotope (TPP-d3 & PLP-d3) was added to the samples, followed by deproteinization with 10% TCA. After centrifugation, 20 µl of the supernatant was injected into the LC-ESI-MS/MS. Reversed phase chromatography was performed on a UPLC system, using a Waters™ Symmetry C18 column, with a gradient of 0.1% formic acid in methanol. TPP and PLP were measured on a tandem MS with respective mass transitions of 425.1>121.85 and 247.9>149.9.

Results:
The chromatographic run lasts 2 minutes. The method is linear from 0-300 nmol/L. The intra-assay and inter-assay precision are 5.5% and 10.4% respectively for TPP and 3.8% and 5.5% for PLP. The matrix effect (absolute: TPP 107%, PLP 101% and relative: TPP 97%, PLP 93%), recovery (TPP 99%, PLP 94%) and lower limit of quantification (TPP 12 nmol/L, PLP 6 nmol/L) are acceptable.
The comparison of the new LC-ESI-MS/MS method for TPP with our current HPLC-Fl method for total thiamine yields the following equation: LC-MS/MS=0.97 [0.86-1.10] x HPLC - 10.61 [-27,77-2,70] (r2=0.94). The comparison of the new LC-ESI-MS/MS method for PLP with our current LC-ESI-MS/MS method results in LC-MS/MS new=1.01 [0.98-1.04] x LC-MS/MS old – 1.58 [-4.04-0.67] (r2=0.99).

Conclusion:
This LC-MS/MS based method is characterized by simple sample processing and a short run time. Comparison with the current methods is excellent. The new LC-MS/MS method is an appropriate method to determine TPP and PLP in whole blood for both clinical routine and research applications.

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Analytical technologies and applications

Cod: M097

ONE-TUBE SCREENING FOR PRIMARY IMMUNODEFICIENCIES

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Primary immunodeficiencies (PID) are rare diseases with a prevalence of 1 in 1000 for all currently recognized forms of such diseases. Most patients present with susceptibility to recurrent episodes of severe or unusual infection. A steadily increasing number of single gene mutations (to date over 300) have been described that explain defects in cell function and/or maturation. Established routine laboratory tests are generally not sufficient for detecting most of these entities because the numbers of basic cell populations in peripheral blood commonly remain within the wide reference ranges. The aim of our project was to set up a screening tube for most common immunodeficiencies, compatible with various systems, as far as possible standardized, and easy to use as routine test in most laboratories.

We designed an 11-parameter panel including absolute cell count which can be analyzed with common diagnostic flow cytometers with at least 8 color detectors. In a single step, with off-the-shelve reagent, using a simple lyse/no wash procedure, results were generated with previously established protocols in 5 different laboratories across Europe.

Our results show that the panel is easy to set up on FACS CANTO II (BD Biosciences) and NAVIOS or CYTOFLEX (both Beckman-Coulter) instruments. Results are comparable with previously used panels. The main immune cell populations can be detected and counted: CD4+ or CD8+ T cells, B and NK cells, monocytes, and PMNs. Furthermore, naïve and mature T cells (CD45RO/CD27), B cells (CD38/CD27/IgD), and functional entities of NK cells (CD16high CD56low) can be quantified as well. Finally, activated CD38+ T and NK cells can be counted. Instrument settings can be standardized based on target fluorescence provided for different systems. A typical template allows for additional drop-in parameters where appropriate lasers and detectors are available in the system.

In conclusion, such a panel can be easily implemented in non-specialized laboratories as a screening tool for detection of potential immunodeficiency and can overcome disadvantages of the common immune status tube in these patients. Following pathological findings, samples have to be further explored with detailed panels, functional tests, and genetic testing. Our novel panel can also be used for secondary immunodeficiencies as observed in chronic inflammatory diseases or sepsis.

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Autoimmune diseases - Allergy

Cod: M167

THE IMPORTANCE OF DETECTING ANTI-DFS70 IN ROUTINE CLINICAL PRACTICE

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BACKGROUND: Screening for antinuclear antibodies by indirect immunofluorescence (ANA-IIF) is considered mandatory in the diagnostic work-up of systemic rheumatic diseases (SRD). However, up to 20% of apparently healthy individuals may test positive, making the interpretation challenging, especially in the context of low pretest probability. Recent reports suggest that the detection of autoantibodies targeting the dense fine speckled 70 (DFS70) antigen may facilitate this challenge. Here, we present the data of 4 Belgian laboratories (1 primary, 2 secondary and 1 tertiary care) investigating their clinical importance.
METHODS: Consecutive routine serum samples with a homogeneous-like pattern on ANA-IIF (dilution 1 in 160) were collected at AML Antwerp (n=327), GZA Antwerp (n=106), OLVZ Aalst (n=211) and UZ Gent (n=50). All samples were tested with at least 1 specific DFS70 assay (DFS70 IgG ELISA and lineblot [Euroimmun, full length antigen], DFS70 IgG CLIA [Inova Diagnostics, truncated antigen]) and HEp-2 select (Inova Diagnostics), in case of discordant results. Anti-DFS70 positive samples were further characterized by documenting co-occurrence with SARD-specific ANA, demographics and clinical information.
RESULTS: In this multicenter study, up to 26% DFS70 positivity within the homogeneous-like population was found. We observed a trend towards higher anti-DFS70 frequencies in primary care (21%) compared to secondary (5,7%, p=0,001) and tertiary (1,9%, p=0,005) care, especially if only concordant samples and samples without co-occurrence of SRD-specific ANA were taken into account. Moreover, in this subpopulation of anti-DFS70 positive samples SRD was also less frequent, however, frequencies up to 50% in tertiary care were observed.
CONCLUSIONS: Anti-DFS70 are most prevalent in primary care setting. Our data do not support that anti-DFS70 is usable to exclude SRD. Nevertheless, anti-DFS70 may explain positive ANA-IIF results, contributing in the clarification of diagnostic challenges, especially if pretest probability for SRD is low. To avoid diagnostic confusion, we think that anti-DFS70 should not be reported in absence of a homogeneous-like pattern or presence of SRD-specific ANA.

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Biology of extreme ages - Pediatric and Geriatric laboratory medicine - Prenatal and postnatal testing

Cod: M188

PREGNANCY COMPLICATIONS IN WOMEN WITH LECTIN COMPLEMENT PATHWAY DEFICIENCY

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BACKGROUND The complement system is an important part of innate immunity which can be activated through three pathways: classical, alternative, and lectin. The lectin pathway is activated through mannose-binding lectin (MBL). MBL binds to carbohydrates found on the surfaces of many pathogens, and MBL deficiency may be associated with infections. Because inflammation and infection are risk factors that can threaten pregnancy throughout its duration, the aim of the study was to examine the serum levels of MBL in women with a risk of preterm labor.
METHODS A retrospective analysis of MBL levels in sera from 60 women in pregnancy weeks 24 to 28 was performed. All the women examined showed one or more clinical symptoms of preterm labor such as bleeding, contractions, or cervical-length shortening. Finally, 22 of them delivered preterm before the 34th week of pregnancy (W34). The remainder of them delivered after W34. Serum levels of MBL were examined by EIA (Human MBL Quantikine ELISA, R&D Systems, USA). Statistical analysis was performed using Statistica 12.0 CZ (StatSoft, USA).
RESULTS Serum MBL <100 ng/mL was found in 13/60 (21.6%) of the women. Significantly lower levels of MBL were present in those women who gave birth before W34 ($p < 0.0001$) and in those with cervical shortening below 15 mm ($p = 0.0006$). No significant differences were found according to bleeding complications ($p = 0.725$), and only a weak association with the occurrence of pathological uterine contractions ($p = 0.0472$). Mothers with low serum levels of MBL showed significantly more frequent histopathologically proven chorioamnionitis, and their newborns showed significantly more frequent FIRS (Fetal Inflammatory Response Syndrome), $p = 0.0001$.
CONCLUSIONS MBL deficiency may be a risk factor involved in septic premature births/miscarriages. For women with a history of chorioamnionitis and/or FIRS, MBL measurement before the subsequent pregnancy should be recommended. Causal treatment of MBL deficiency is not available currently; however, suitable immunomodulatory therapy may increase the chances of an uncomplicated course of pregnancy.
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Biomarkers in cancer

Cod: M214

NOVEL LECTIN-NANOPARTICLE CONCEPT TO SPECIFICALLY RECOGNIZE CANCEROUS ISOFORMS OF GLYCOPROTEINS BIOMARKERS OF DIFFERENT CANCERS

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BACKGROUND: The great majority of circulating cancer biomarkers are non-specific as substantial overlap in concentrations may be found with samples from healthy subjects and patients with benign diseases. Thus, most of them are used only for follow-up of the disease and monitoring the treatment. Cancer markers are mostly glycoproteins and altered glycosylation is a universal feature of cancer cells. Detection of cancer-related glycosylation changes in conventional biomarkers is highly attractive for early cancer detection. Lectins are carbohydrate-binding proteins and can be exploited for specific recognition of these changes.
METHODS: We have established a lectin library, where individual lectins with known glycostructure specificity are immobilized onto fluorescent Europium-chelate-doped 97 nm nanoparticles (Eu+3-NPs) making them multivalent and highly reactive toward the target. The library was used for screening of multiple glycoprotein markers isolated from benign and malignant sources.

RESULTS: Using lectin-coated Eu+3-NPs, analytically sensitive cancer associated glycoprotein-lectin assays were developed that specifically recognize the isoforms of cancer biomarkers produced by cancer cells, whereas the detection of glycoproteins from benign conditions was reduced. This approach has been applied for CA125, PSA, CA15-3 and CEA derived from ovarian, prostate, breast and colon cancer specific cell line, respectively.

CONCLUSIONS: The improved analytical specificity of this test approach is dependent on a discriminating lectin immobilized in large numbers on Eu+3-NPs, providing both an avidity effect and signal amplification. The novel Lectin-Nanoparticle concept could be a trend-setting opportunity for early diagnostics in parallel with other timely approaches: circulating nucleic acid, exosomes (liquid biopsy) and play a part in determining cancer glycomics for “big data” approaches in the field of cancer omics. Finally, using appropriate combinations of lectins and antibodies, applying the lectin NPs concept can also be explored for other diagnostic targets, where changes in glycosylation are indicative of an ongoing disease process.

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Biomarkers in cancer

Cod: M231

ESR1 METHYLATION IN CIRCULATING TUMOR CELLS OF PATIENTS WITH BREAST CANCER

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BACKGROUND: DNA methylation is an epigenetic alteration which plays a decisive role in the regulation of signal translation processes. In our lab, we have demonstrated for the first time the epigenetic silencing of tumor and metastasis suppressor genes in CTCs through their promoter methylation. Estrogen receptor (ER) is an important prognostic biomarker and is predictive of response to endocrine therapy in breast cancer. In this study, we evaluated for the first time ESR1 methylation in CTCs of breast cancer patients.

METHODS: We developed and validated a novel highly sensitive and specific qMSP assay for ESR1 methylation using commercially available DNA methylation controls and the MDA-MB-231 cell line. We further examined its performance in EpCAM-positive immune-magnetically isolated CTC fractions, followed by DNA isolation and sodium bisulfite (SB) treatment from: a) 74 operable, b) 48 metastasis- verified breast cancer patients and c) 30 healthy donors (control group).

RESULTS: The developed assay is highly specific and sensitive since it can detect 0.1% methylated ESR1 sequences in the presence of 99.9% un-methylated. ESR1 was found to be methylated in 16/74 (21.6%) operable breast cancer patients, in 10/48 (20.8%) patients with verified metastasis, but only in 1/30 (3.3%) healthy donors (EpCAM-positive CTC fraction).

CONCLUSIONS: The EpCAM-positive CTC fraction was found to be methylated for ESR1 in about 20% of patients with breast cancer. We will further evaluate these findings in respect to the clinical outcome of these patients, since the epigenetic silencing of ESR1 could be of important clinical significance especially for its impact on the efficacy of treatment.

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Biomarkers in cancer

Cod: M276

THE CLINICAL UTILITY OF mir-125B AND mir-221/222 FOR BLADDER CANCER PROGNOSIS AND PATIENTS SURVIVAL OUTCOME FOLLOWING TREATMENT

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Background: Bladder cancer (BlCa) remains the fourth most common type of malignancy in the male populations of the developed countries. MicroRNAs (miRNAs or miRs) are small (21-25 nt) non-coding RNAs able to regulate gene expression and thus to control cellular homeostasis, including cell growth, proliferation, migration and apoptosis. Recent studies have highlighted the implication of miR-125b and miR-221/222 in human malignancies, including urothelial carcinoma, however, their clinical significance for disease outcome is unknown. In the present study, we have analysed the expression of miR-125b, miR-221 and miR-222 in bladder tumors and adjacent normal urothelium in order to evaluate their clinical significance as novel biomarkers and possible therapeutic targets for bladder cancer.
Methods: Bladder tumor and adjacent normal urothelium tissue specimens were obtained from 165 surgically treated patients. Total RNA was extracted following pulverization and was polyadenylated at the 3'-end by E. coli Poly(A) polymerase. Thereafter, poly(A) RNA was reversed transcribed to cDNA using a poly(T) primer. SYBR-Green based qPCRassays were developed, validated and applied for the quantification of miR125b, miR-221 and miR-222 levels using the comparative CT method $2^{-\Delta\Delta CT}$. Extensive statistical analysis was finally performed for the evaluation of miRNAs clinical significance for BlCa patients.
Results: The levels of miR-125b, miR-221/222 were significantly downregulated in bladder tumors compared to their normal counterparts. However, increased expression of miR-125b, miR-221/222 observed in muscle-invasive (T2-T4) compared to superficial (Ta, T1). Focusing on non muscle-invasive tumors (Ta, T1), loss of miR-125b and miR-221/222 was correlated high grade TaT1 tumors and higher EORTC-risk group patients. Kaplan-Meier survival curves and Cox regression analysis revealed the significant and independent clinical value of miR-125b and miR-221/222 for the prediction of non muscle-invasive bladder cancer (NMIBC) patients' risk for disease relapse and progression to muscle invasion stages, as well as for the estimation of muscle-invasive (MIBC) patients overall survival expectancy.
Conclusions: Our data clearly demonstrate the clinical utility of miR-125b, miR-221 and miR-222 to serve as novel biomarkers for bladder cancer prognosis and patients' survival outcome following surgically treatment.

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Biomarkers in cancer

Cod: M284

K-RAS MUTATIONS DETECTION IN CIRCULATING EXOSOMES OF PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA: A STUDY ON ANALYTICAL FEASIBILITY.

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Background: early diagnosis of pancreatic ductal adenocarcinoma (PDAC) is a key element to improve patients prognosis. k-ras gene mutations establish early in carcinogenesis and are present in almost 90% of PDACs. Exosomes shed in the extracellular compartment also by cancer cells are a non invasive and enriched source of circulating tumour DNA (exoDNA). Aim of this project is to verify whether the recognition of k-ras gene mutations in exoDNA is analytically feasible.
Methods: We verified pre-analytical processing (sample type, temperature, duration of storage, centrifugation protocol and exosome isolation procedures) on exoDNA. Blood from 5 donors was collected in serum and EDTA tubes and kept at room temperature (RT) or refrigerated (COLD). Samples were centrifuged one or two times after 30 minutes (30min) and 3 hours (3h). Aliquots (-80°C) were used for exosome isolation by two commercial kits: Total Exosome Isolation Kit (Life Technologies) (A) and ExoQuick precipitation solution Kit (System Biosciences) (B). DNA was extracted and quantified (fluorimetric assay).
exoDNA was obtained from sera of 11 PDAC patients (T2-3, N0, M0). Gly12Asp and Gly12Val k-ras mutations were analyzed by CAST PCR (Life Technologies). Results were compared with those from matched neoplastic and normal adjacent tissues.
Results: Mean efficiency of exoDNA isolation (ng DNA/mL of sample) was significantly higher in serum (15,94±11,51ng/mL) than in plasma (5,13±2,27ng/mL) ($p<0,001$) being independent on the number of centrifugations. Among sera mean efficiency of exoDNA isolation was significantly higher in (RT, 3h) samples (kit A=27,75±9,03ng/mL and kit B=30,61±7,72ng/mL) than in (COLD, 30min) samples (kit A=13,10±8,07ng/mL and kit B=14,22±7,75ng/mL) ($p<0,05$). k-ras mutations were detected in 10/11 tumour samples (7/11 Gly12Val and 3/11 Gly12Asp). No mutation was detected in normal adjacent tissues. Mean concentrations of exoDNA extracted from PDAC patients' sera was (57,74±51,20 pg/μL) and no k-ras mutation was detected.
Conclusions: the overall low concentration of exoDNA extracted from peripheral blood hampers K-ras mutation detection due to a low probability of sampling tumour exoDNA in the analytical phase.

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Pharmacogenetics - Personalized Medicine

Cod: M315

PHARMACOGENETICS OF STATINS RESPONSE: PRELIMINARY RESULTS OF A MULTICENTRIC STUDY.

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BACKGROUND
The response to statin therapy has a large interindividual variability (20-60%) in terms of reducing LDL-Cholesterol (LDL-C). For this reason, the study of the effect in the statin therapy response of genetic variants involved in lipid and statin metabolism can provide relevant insights to understand and explain this interindividual variability.

Our aim is to study the effect of 8 polymorphisms (TaqIB and I405V polymorphisms of the CETP gene, R219K of ABCA1 gene, CYP2D6 * 3, CYP2D6 * 4 and CYP2D6 * 6 of the gene CYP2D6, and CYP2C9 * 2 and CYP2C9 * 3 of the gene CYP2C9) in the reduction of LDL-C after treatment with simvastatin, atorvastatin or rosuvastatin in a Spanish population.

METHODS
171 patients were included in a prospectively way during two years. Clinical data and LDL-C were collected in the first and second visit (before and after treatment). The patients were genotyped by real time PCR and the method was validated by Sanger sequencing.
Multivariable linear regression models were used to explain the LDL-C variation between the first and the second visit, in each treatment group.

RESULTS
The most relevant results of the linear models explaining LDL-C variation with and without including genetic polymorphisms were the following: the inclusion of TaqIB genotypes significantly improves the variance explained by the model in rosuvastatin patients, from R2= 0,420 to R2= 0,720, (p value = 0,04 Regression coefficient (B) + confidence interval (IC) (95%)=29,05 (1,14-56,96)).
The inclusion of R219K polymorphism in the models explaining LDL-C variation within simvastatin (from R2= 0,066 to R2 0,097, p value= 0,11; B + IC (95%) =4,98 (-13,26-4,99) and atorvastatin patients (from R2= 0,162 to R2 0,223, p value= 0,06; B + IC (95%) =15,09 (-1,18-31,33)) is approaching statistical significance.

CONCLUSIONS

TaqIB variant (CETP gene) influences in rosuvastatin response in a statistically significant way. Furthermore, the presence of R219K variant (ABCA1) seems to influence in simvastatin and atorvastatin response, but no statistical significance was achieved. These are preliminary results and a larger number of patients are necessary to complete the study.

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Pharmacogenetics - Personalized Medicine

Cod: M316

CLOPIDOGREL-PATHWAY GENE POLYMORPHISMS AND CLINICAL RISK-STRATIFICATION OF PATIENTS WITH STEMI UNDERGOING PRIMARY PCI

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BACKGROUND: Accurate risk stratification has an important role in the management of patients with ST-elevation acute myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PCI). The incidence of major adverse cardiovascular events (MACE) in this population is about 10%. The MACE occurence can be partially explained by variability of clopidogrel response attributed to pharmacogenetics of clopidogrel metabolism or residual platelet reactivity in peripheral whole blood. The aim of the study was to investigate the association between clopidogrel-pathway gene polymorphisms and the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score on risk prediction of 30 day MACE in patients with STEMI treated by primary PCI.

METHODS: This prospective study included 140 consecutive patients referred to primary PCI for STEMI. The clopidogrel-metabolizing pathway SNPs used were: ABCB1 (rs1045642), CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560), P2RY12 (rs2046934), and PON1 (rs854560, rs662). The primary end point MACE was defined as death, nonfatal infarction or immediate target vessel revascularization. Patients were followed-up at 30 days after primary PCI.

RESULTS: Thirty-day MACE was 4.3%. All SNPs tested were in Hardy-Weinberg equilibrium ($p > 0.05$). Among the SNPs tested, only CYP2C19*17 was significantly associated with MACE. Addition of CYP2C19*17 T allele to CADILLAC score increased the area under the ROC (0.700 vs. 0.832). The addition of CYP2C19*17 T allele to CADILLAC score enhanced net reclassification improvement and integrated discrimination improvement, suggesting effective discrimination and reclassification.

CONCLUSIONS: These data revealed the combination of the established CADILLAC score and CYP2C19*17 could derive a more accurate prediction for clinical outcomes in STEMI patients.

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Cardiovascular diseases - Cardiac markers

Cod: T042

SURVEY OF CURRENT LABORATORY AND CLINICAL PRACTICES FOR CARDIAC TROPONIN TESTING IN AUSTRALIA AND NEW ZEALAND (PART 1)

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Introduction:
Recent international surveys have reported on the lack of harmonisation of cardiac troponin (cTn) measurement in the routine laboratory and at the point-of-care (POC). The Australasian Association of Clinical Biochemists (AACB) conducted a recent survey in April/May of 2016 to assess the current laboratory and clinical practices for cTn testing.
Methods:
Participants were asked about pre-analytical, analytical and post-analytical issues relating to cTn measurement in automated laboratories and at the POC. They were asked to tick all answers that applied to their location or to provide text, e.g. cTn decision cut-off value.
Results:
The 177 respondents represented largely Australia (75.4%), New Zealand (13.5%), and South East Asia (7.9%). Of the 11.9% of quantitative POC assays being used, the predominant assay was Abbott i-STAT cTnI. Automated laboratory cTn assays included the highly sensitive assays Abbott Architect STAT hs-cTnI (16.8%) and Roche Elecsys / cobas hs-TnT (32.3%), with other sensitive assays comprising 38.4%. The cTn decision cut-off was defined as the concentration above the 99th percentile by 34.4%, 10% CV by 7.2%, 20% CV by 2.4%, WHO cut-off by 4.8%, a cut-off from the reagent supplier by 19.6%, a cut-off derived and validated locally by 12.4%, and 15.8% did not know. Male and female cut-offs were used by 31 laboratories and age-specific cut-offs by 7 laboratories. Troponin decision cut-offs for hs-TnT ranged from 3-30 ng/L (57.9% used 14 ng/L), and for hs-TnI, from 23-50 ng/L (63.6% used a male cut-off of 26 ng/L and 95.5% a female cut-off of 16 ng/L). Troponin units were mainly µg/L (29.9%) and ng/L (57.1%).
Conclusions:
While decision cut-offs for hs-TnI and hs-TnT assays are fairly well harmonised between the 49% of laboratories using them, cut-offs used for sensitive and POC assays are less well harmonised. Units require improved harmonisation to ng/L in whole numbers.

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Clinical Studies - Outcomes

Cod: T058

AN IMMUNOHISTOCHEMICAL, HISTOPATHOLOGICAL AND BIOCHEMICAL ANALYSIS OF THE NEUROPROTECTIVE EFFECTS OF MEMANTINE, AND CURCUMIN AFTER CEREBRAL ISCHEMIA-REPERFUSION INJURY IN ELDERLY RATS

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Backroud: Cerebral ischemic stroke is one of the leading causes of human death and disability in the world. The aim of our study was to investigate the protective effects of curcumin(CUR) and memantine(MEM) on cerebral ischemia/reperfusion (I/R) in elderly rats.

Materials and Methods: The experiments were designed in 40 Wistar Hannover rats, randomly allotted into one of five groups(n=8): CUR(Group I), MEM(Group II), CUR+MEM(Group III), ischemia(Group IV), and sham-operated(Group V). The model adopted was that of surgically-induced cerebral ischemia, performed by means of bilateral common carotid artery occlusion for 30 minute, followed by reperfusion for another 72 hour. Biochemical parameters such as tumor necrosis factor-α(TNF-α), interleukin-6(IL-6), lactate dehydrogenase(LDH), catalase(CAT), glutathione peroxidase(GSPx), xanthine dehydrogenase(XDH), superoxide dismutase(SOD), and malondialdehyde(MDA) were investigated in brain tissue and serum of the rats in all groups. Brain tissues were evaluated immunohistochemically and Caspase-3 antibody. Apoptotic Index(AI) was used as a measure of the extent of apoptosis to detect neuronal loss in the brain tissue samples.

Results: Tissue and serum IL-6, TNF-α, MDA, LDH levels were found statistically significantly lower in the treated group compared to the untreated group ($p < 0.001$). Tissue and serum GSPx, SOD and CAT levels were found statistically significantly higher in the treated group compared to the untreated group ($p < 0.001$). Histopathologically, no statistically difference was detected between the untreated and treated groups. Statistical difference was not detected when comparing control and treated groups with positive apoptotic index ($p > 0.05$).

Conclusion: In conclusion, in this study, although CUR, MEM and CUR+MEM is effective in preventing oxidative damage following cerebral ischemia, they were found to be ineffective in preventing tissue damage.

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Clinical Studies - Outcomes

Cod: T063

MODELS 1B AND 2 ACCORDING TO EFLM CONSENSUS CONFERENCE GIVE THE SAME SPECIFICATION FOR ALLOWABLE TOTAL ERROR (TEA) OF PLASMA GLUCOSE MEASUREMENT

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BACKGROUND: The 2014 EFLM Consensus Conference (CC) identified outcome- and biological variation (BV)-based as the highest hierarchical models for defining analytical performance specifications (APS) of a measurand. Fasting plasma glucose (PG) plays a central role in diagnosis of diabetes mellitus (DM) and decision limits for the definition of glycaemic-related conditions have been established. As direct studies investigating the impact of performance of PG measurement on clinical outcome are not available, we aimed to apply an indirect outcome model (1b according to CC) to derive APS for TEa, by investigating the impact of performance of PG measurement on clinical classifications of fasting subjects. The 1b-TEa was validated by comparison with TEa obtained using PG BV data (CC model 2). Since PG is under strict homeostatic control, robust BV data can be derived. In particular, we employed data from the study by Carlsen et al. (CCLM 2011;49:1501) (CVI, 5.4% and CVG, 5.6%), which totally fulfilled the EFLM checklist for BV study appraisal.
METHODS: The decision limits defining impaired fasting PG (IFG) concentrations (110 to 125 mg/dL) were considered and 1b-TEa was derived by assuming that a subject with a fasting PG of 117.5 mg/dL should be differentiate from healthy condition from one side (<110 mg/dL) and a frank DM from the other side (>125 mg/dL). Model 2-TEa was estimated according to the equation: TEa = [1.65 x 0.5CVI + 0.25(CVI2 + CVG2)0.5].
RESULTS: A subject with fasting PG of 117.5 mg/dL will not be misclassified as diabetic or healthy if TE of PG measurement is <7.5/117.5 = <6.38%. The corresponding TEa derived from PG biological variability was ±6.4%.
CONCLUSIONS: The described model 1b, based on assumptions drawn from what evidence there is about the definition of glycaemic-related conditions and thereby on the probability of subject outcome, is applicable for the definition of TEa of PG measurement. The similarity with TEa derived from model 2 confirms the equivalence of the two models advocated in the EFLM CC in case of measurands with well-defined biological and clinical characteristics, as PG. Additional studies are necessary to determine the clinical impact of the TEa.

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Clinical Studies - Outcomes

Cod: T064

IMPACT OF MEASUREMENT ERROR OF PLASMA GLUCOSE ON CLINICAL CLASSIFICATION: A SIMULATION ANALYSIS

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BACKGROUND: According to EFLM Consensus Conference criteria on allocation of laboratory tests to the proper model to derive analytical performance specifications, the outcome-based model is well suited for fasting plasma glucose (PG), since the test is central to diagnose diabetes mellitus (DM) and to define glycaemic-related conditions. We previously described an indirect outcome model for the definition of allowable total error (TEa) of PG measurement. Here, we performed a simulation analysis to investigate the impact of derived TEa (±6.38%) on the clinical classification of the outpatient population served by our institution.

METHODS: We retrospectively retrieved PG results from outpatients for a 6-month period. PG was measured by a well-standardized and precise hexokinase assay on the Abbott Architect c16000 platform [average CV <1.3% and virtually unbiased (+0.2%) when compared with the CDC reference procedure]. The rate of subjects with impaired fasting PG (IFG), misclassified as frank DM or normoglycaemic if PG would be measured with a TEa of ±6.38%, was investigated.

RESULTS: The clinical classification of retrieved subjects [n=6537; median PG, 109 mg/dL; interquartile range (IQR): 99-128)] was 51.6% as healthy, 21.6% as IFG and 26.8% as DM. A +6.38% TE in PG measurement (median PG, 116 mg/dL; IQR: 105-136) resulted in +7.7% of subjects misdiagnosed as DM and +18.1% of healthy individuals classified as IFG. Conversely, a –6.38% TE (median PG, 102 mg/dL; IQR: 93-120) implied the shift of 6.2% DM to IFG category and of 12.6% IFG to the healthy group.

CONCLUSIONS: IFG represents a category at increased risk to develop DM. In this condition, the prevention of DM onset as well as of vascular hyperglycaemia-related complications is accomplished with interventions lowering PG over time. False negatives, i.e., IFG subjects misclassified as normoglycaemic, are therefore the most impacting results. In our served population, measuring PG with a TEa of ±6.38% theoretically implies that 12.6% of individuals would miss interventions necessary to stop the progression to DM and the worsening of related outcomes. Further clinical and economical evaluation is required to show if this misclassification rate is acceptable or a more stringent TEa should be applied.

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Critical care, Emergency medicine, Blood gases, POCT

Cod: T079

CONTRIBUTION OF INTERLEUKIN-6 (IL6) IN THE DIAGNOSIS OF SEPSIS IN THE EMERGENCY DEPARTMENT

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Background: Sepsis, a common condition handled in the Emergency Department (ED), is a potentially life-threatening complication of an infection and it causes millions of deaths globally each year. The research on accurate and timely diagnosis or exclude of suspected sepsis is vital to patient, which can reduce morbidity, reduces cost, and improves patient outcome. Procalcitonin (PCT) is the best investigated biomarker for this purpose, and together with C-reactive protein (CRP), are the most frequently used biomarkers in clinical practice. Interleukin-6 (IL6) is widely investigated for its fast response to the infectious stimulus, but conclusive data for the application of this biomarker are missing. The aim of this study was to investigate the diagnostic value of IL6 compared to PCT.

Methods: 50 patients presenting at the ED with suspected sepsis were included. Blood samples were collected at first medical evaluation and PCT, CRP and IL6 were analyzed. After diagnosis, the patients were divided is two groups: A (non-infectious etiology, localized infection or SIRS) and B (sepsis, severe sepsis or septic shock).

Results: The three biomarkers showed significant differences between groups ($p=0.000$ for IL6 and PCT; $p=0.036$ for CRP). IL6 were significantly correlated with PCT ($r=0.470$; $p=0.000$), but not with CRP.
We developed a logistic regression model including CRP, PCT and IL6, and the AUC (0.940) was significantly higher compared to the use of biomarkers alone (AUCs = 0.897, 0.838 and 0.668 for IL6, PCT and CRP respectively). The model AUC was also significantly superior to the combined use of CRP and PCT (AUC=0.845), the biomarkers currently used in our hospital.

Conclusion: The diagnostic accuracy of IL6 is equivalent to the combined use of CRP and PCT, in both cases suitable for diagnosis of sepsis. The addition of IL6 to the biomarkers already used, PCT and CRP, imply a significant improvement and represents the best diagnostic performance. Therefore, we recommend to include IL6 in the diagnostic algorithm of sepsis management in ED because it may assist clinicians in their decision making for early antimicrobial administration, enable risk stratification and expedite the execution of sepsis bundle.

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Critical care, Emergency medicine, Blood gases, POCT

Cod: T100

COMPARISON OF LACTATE, PRESEPSIN AND PROCALCITONIN WITH THE NEW QSOFA (QUICK SOFA) SCORE FOR SEVERITY ASSESSMENT AND MORTALITY PREDICTION IN PATIENTS WITH INITIAL SEPSIS

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Background
The SOFA score is associated with an increased probability of mortality in sepsis. For assessment at admission in the ED several laboratory variables are necessary. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) defined a new qSOFA score, which does not require laboratory tests and can be easily assessed at patient admission.

Objective
To compare established sepsis biomarkers with SOFA and qSOFA to discriminate uncomplicated sepsis, severe sepsis or septic shock and to evaluate the association with poor outcome and increased risk of mortality.

Methods
66 Patients admitted to the emergency department with clear signs of sepsis and > 2 SIRS-criteria were included. Severe sepsis and septic shock were defined according to current guidelines. qSOFA score was calculated using the recommended thresholds: respiratory rate ≥ 22/min, GCS score <15, stystolic blood pressure ≤ 100mmHG. Presepsin and procalcitonin were determined on admittance using the POC assay PATHFAST Presepsin (PSEP), LSI Medience, Japan and the BRAHMS luminescence immune assay (PCT).

Results
Both scores, SOFA and qSOFA, differentiated significantly between patients with uncomplicated sepsis (n=30, mortality=6.6%) and the high-risk group with severe sepsis or septic shock (n=36, mortality=36.1%), p-values: 0.0003 and 0.0007. Discrimination between the groups revealed AUC values of 0.621, 0.627, 0.731, 0.740 and 0.781 for lactate, PCT, qSOFA, PSEP, and the combination qSOFA+PSEP, respectively. 15 patients died during hospitalization. AUC values of mortality prediction were 0.715, 0.558, 0.734, 0.758 and 0.803 for lactate, PCT, qSOFA, PSEP and the combination qSOFA +PSEP, respectively. qSOFA scores ≥2 should identify greater risk of death or prolonged ICU stay. Discrimination between qSOFA < 2 and ≥ 2 revealed AUC values of 0.756, 0.669 and 0.606 for PSEP, lactate and PCT, respectively.
Using the threshold ≥ 2 of qSOFA and ≥ 500 ng/L of PSEP, the combination qSOFA+PSEP detected 14 of 15 non-survivors (93%) and 33 (92%) patients of the high-risk group (n=36), whereas qSOFA alone detected only 10 non-survivors (67%) and 21 patients of the high-risk group (58%).

Conclusion
The results demonstrated that the qSOFA score is not a standalone criterion for risk stratification of sepsis at admission. Simultaneous assessment by combining qSOFA and PSEP improved the validity significantly. The POC assay PATHFAST Presepsin showed superior performance compared to lactate and PCT.

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Critical care, Emergency medicine, Blood gases, POCT

Cod: T101

ESTIMATED GLOMERULAR FILTRATION RATE (GFR) USING A POINT OF CARE (POC) MEASURE OF CREATININE IN PATIENTS WITH IOHEXOL DETERMINATE GFR

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Background

There is a significant risk to develop acute kidney failure after injection of an iodine contrast media for diagnostic purpose. The risk is even higher if the patient already suffers from chronic kidney failure. Determination of creatinine levels and GFR estimation before injection allows early detection of high-risk patients. However, because of organization issues, this analysis is rarely realized. A quick determination of creatinine by a POC device would facilitate the analysis. We recently evaluated the POC creatinine measured with the StatSensor (Nova Biomedical), which allows an enzymatic determination in 30 seconds.

Methods

56 participants (27 women and 29 men; mean age 56.63±2.53 and 49.89±2.34 respectively) for whom GFR was measured with the gold standard HPLC-iohexol method ($GFR_{(IOHEXOL)}$) were enrolled in this analysis. For each patient, enzymatic creatinine was determinate with two different devices: the routine Roche Cobas analyzer and the POC StatSensor. Both values of creatinine levels were used in the CKD-EPI equation for GFR estimation (GFRe).

Results

Passing Bablok regression gave the following equation: $GFRe_{(POC)}$ = -0,66 (95% CI : -6,00 to 5,62) + 1,04 (95% CI : 0,96 to 1,13) x $GFRe_{(COBAS)}$. The Bland–Altman plot showed a mean bias of -2±10 mL/min/1.73m^2. Compared to the iohexol GFR determination, we observed the following Passing Bablok equation: $GFRe_{(POC)}$ = -15,8 (95% CI : -29,5 to -4,99) + 1,15 (95% CI : 1,01 to 1,33) x $GFR_{(IOHEXOL)}$. Mean biais ($GFRe-GFR_{(IOHEXOL)}$) was estimated at -6±15 mL/min/1.73m^2 for the Roche Cobas and -4±15 mL/min/1.73m^2 for the POC.

Conclusion

The POC StatSensor (Nova Biomedical) is a reliable device in the GFR screening before contrast media injection.

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Endocrinology

Cod: T134

VALIDATION OF SERUM ANDROSTANEDIOL GLUCURONIDE BY LC-MS/MS

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Background: Plasma androstanediol-glucuronide (ADG) is considered to be a highly marker of peripheral androgenicity. The quantification of steroidal glucuronide conjugates by immunoassays may underestimate some conjugates since hydrolysis is needed in sample processing. To overcome these limitations, we have validated a LC-MS/MS method for ADG determination in plasma and serum and to compare it with our previously employed ELISA.

Methods: We used a HPLC system AD20XR Shimazu connected to triple quadrupole mass spectrometer TQ5500 (SCIEX, Framingham, Massachusetts, USA). 3 water and serum samples depleted in steroids were spiked with a known concentration (0.2, 1 and 5 ng/mL) of ADG; these samples were run on 3 different (n=3) days to evaluate within and between-run CV. With those samples, we evaluated also recovery and matrix effects. Linearity of the calibration curves(0.1, 0.5, 1, 5, 10 ng/mL) for serum was assessed by performing linear regression. The limit of detection (LOD) and limit of quantification (LOQ) were calculated with the lowest concentration that we tested. LOD and LOQ were respectively defined as 3:1 and 10:1 signal/noise ratio respectively. The e-noval software (Arlenda, Belgium) was used to perform the statistical calculations.

Results: The detection mode was MRM in negative mode. The intra-run precision (CV) was 2.5-6.3% and between-run precision (CV) was 4.7-7.4%. Recoveries were: into natural matrix (95%CI: 94.3-107.5) and water (95%CI: 101.2-111).Within the calibration ranges, the linear regression model is fitted ,the equation was: Y=0.03078+0.9867X. The LOD was 0.018 (+/-0.002) µg/L (n = 5) and the LOQ at 0.059(+/-0.006) µg/L (n = 5).For the comparison between LC-MS/MS(X) and ELISA(Y), the Passing-Bablok test gave the following regression equation: Y=1.14+1.31X. The average median was 2.57 µg/L (95% CI: 1.18-6.3) for LC-MS/MS and 4.33 µg/L (95% CI: 2.53-10.3) for ELISA. Between the serum (X) and plasma (Y) in LC-MS/MS, the regression equation was: Y=0.09+0.92X, the median average was 2.57 µg/L (95% CI: 1.18-6.3) in serum compared with a median average of 2.46 µg/L (95%CI:1.21-6.3) in plasma.

Conclusions: We have validated the method by LC-MS/MS. We noted a significant bias between ELISA and LC-MS/MS. Finally, we urge the Clinical Chemistry community to develop an international standard reference material for steroids and a candidate reference method for LC-MS/MS.

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Gastrointestinal diseases

Cod: T191

ANALYTICAL PERFORMANCE AND DIAGNOSTIC ACCURACY OF SIX DIFFERENT FAECAL CALPROTECTIN ASSAYS IN INFLAMMATORY BOWEL DISEASE

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Background
We evaluated the analytical performance of six different faecal calprotectin immunoassays together with their diagnostic accuracy in the discrimination between functional and organic bowel disorders.

Materials and methods
Measurement of faecal calprotectin was performed using Thermo Fischer EliA Calprotectin 2 assay on the ImmunoCAP 250, Diasorin Calprotectin assay on the Liaison, Inova QUANTA Flash® Calprotectin on the Inova BIO-FLASH instrument, Bühlmann fCAL Turbo on the Roche Cobas c501, Euroimmun Calprotectin assay and Orgentec Calprotectin assay on the Allegria instrument.
The faecal samples were obtained from Inflammatory Bowel Disease patients (n=27) at the time of diagnosis (Crohn's Disease (n=15), Colitis Ulcerosa (n=12)), gastroenterologic disease control patients (n=52) and rheumatologic disease control patients (n=26). All individuals included in the study underwent a concurrent ileocolonoscopy. Analytical performance (imprecision, accuracy, carry-over, correlation and agreement) and diagnostic accuracy (sensitivity, specificity, likelihood ratios) of the different assays were evaluated.

Results
All methods demonstrated acceptable analytical sensitivity: within-run and total imprecision varied from 0.6% to 19.7% and 1.5 to 23.3%, respectively, and no significant carry-over was detected (<0.03% for all methods). The results of the eQC-samples (Instand® Quality control scheme) were qualitatively correctly interpreted (i.e. positive or negative) by all the assays and at the manufacturer's cut-offs. Using Passing and Bablok and Bland-Altman analyses, low quantitative agreement was observed between the assays.
All the assays showed excellent diagnostic accuracy, with areas under the receiver operating curves ranging from 0.974 to 0.998. The areas were not statistically significantly different among the assays ($P > 0.05$). Diagnostic sensitivity at the cut-off at a fixed specificity of 75% ranged from 95.2-100%.
Introduction of positive likelihood ratio's for IBD for multiple test result intervals (<1x Upper Limit of Normal (ULN), 1-3 x ULN, 3-10 x ULN and >10 x ULN) increased the clinical interpretation of all the assays.

Conclusion
Analytical and diagnostic performance of the evaluated faecal calprotectin assays is good, but numerical values differ substantially between the assays. Introduction of multiple test result intervals increased the diagnostic performance of all the assays, aiding in clinical decision-making.

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Hematology - Hemostasis

Cod: T195

ABERRANT EXPRESSION OF MYELOID SPECIFIC MARKERS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background and Purpose:
Documentation of aberrant antigen expression is essential in depicting the neoplastic process and the detection of minimal residual disease. (MRD). Flow cytometry is a significant tool in recognizing aberrant phenotypes. Frequency of aberrant phenotypes varies noticeably in various investigations and its relationship with prognostic issues is quiet controversial. The present study was done to find the frequency of aberrant phenotypes on immunophenotyping in a large series of de novo acute lymphoblastic leukemia (all) and to assess any relationship with initial clinical and hematological features.
Methods:
In the present study, 280 patients of de novo ALL cases were included from two Iranian Immunophenotyping centers during January 2011 to December 2012. The immunophenotype of all cases of ALL was studied using FACSCalibur (BD Biosciences, San Jose, USA).
Results:
Unusual myeloid antigen expression was seen in 38.5% of cases. Most frequent aberrant myeloid antigen was CD13 (31.1%), followed by CD33 (32.2%) and CD117 (24.3%). The expression of CD117 was relatively common in comparison to previous reports which designate its rare expression. Adult T- ALL showed higher expression of CD117 and CD33 than pediatric T-ALL ($p = 0.02$ and 0.04, respectively). Myeloid antigen expression in all cases was associated with lower WBC count ($p<0.05$) and lower number of peripheral blasts ($p<0.05$).
Conclusions:
In summary, CD117 is a fairly commonly expressed myeloid marker contrary to previous reports which denote its rare expression. ALL cases with low blast count and CD34 positivity are more likely to express abnormal myeloid markers.

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Hematology - Hemostasis

Cod: T223

IDENTIFICATION AND QUANTIFICATION OF URINARY MONOCLONAL PROTEINS BY CAPILLARY ELECTROPHORESIS IN AL AMYLOIDOSIS

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Identification and quantification of urinary monoclonal proteins (uMPs) is fundamental in the diagnosis and monitoring of monoclonal gammopathies (Kyle et al. Leukemia 2010). We prospectively assessed the performance of the Sebia Capillarys urine protein capillary electrophoresis (UPCE) and immunotyping in 75 patients with AL amyloidosis. Samples were tested with: homemade high-resolution agarose gel immunofixation electrophoresis (hr-IFE) of serum and concentrated (10 times) urine; commercial semi-automated agarose gel immunofixation of urine (Sebia Hydragel BJ on Hydrasys 2, SHBJ); UPCE and immunotyping (Sebia Capillarys 2 Flex Piercing Urine); quantification of circulating free light chains (FLC) by Freelite and N latex FLC. Urinary MPs were quantified using Sebia Phoresis software tools. Sixty-eight patients in whom uMPs were detected by hr-IFE were included in the study. A uMP was detected by UPCE in 62 cases (91%), and was quantifiable in 55 (81%). The median uMP excretion was 130 mg/24h (range 10-1610). Nine of the 12 patients with dFLC<50 mg/L (Freelite) had a quantifiable uMP (median 90 mg/24h). The uMP was also quantifiable on SHBJ in 51 patients (75%). There was a good correlation between measurements of uMP excretion on UPCE and SHBJ (Pearson's r = 0.87, 95%CI 0.78-0.92). So far, 16 patients with quantifiable uMP and dFLC (Freelite) >50 mg/L were treated and had response data at 3 months. Five subjects responded with a median 69% dFLC decrease (range 51-90%). In all of them uMP excretion decreased (median 100%, range 30-100%). Among non-responders, only one had a relevant reduction in uMP (from 740 to 250 mg/24h, dFLC from 746 to 619 mg/L) with stable renal function. Post-treatment UPCE was also available in 5 cases with baseline dFLC (Freelite) <50 mg/L. In 2 of them the uMP was still visible but was no longer quantifiable, in 2 it remained stable and in one uMP increased from 20 to 40 mg/24h. UPCE can identify uMPs in patients with AL amyloidosis with a good sensitivity, and can quantify uMP excretion as low as 10 mg/24h. Changes in uMP excretion can be monitored during therapy, including patients with low FLC disease. Further studies are warranted to evaluate the response assessment.

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Inflammation, Vascular biology, Endothelium, Oxidative stress

Cod: T253

SERUM CONCENTRATIONS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS – ASSOCIATION WITH AUTOANTIBODY PROFILE AND CARDIOVASCULAR INVOLVEMENT

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Background and objectives: Angiogenesis plays a significant role in the pathogenesis of systemic lupus erythematosus (SLE). Vascular endothelial growth factor (VEGF) is a potent stimulator of angio- and vasculogenesis. The study was designed to evaluate the association between VEGF concentrations and laboratory parameters, classical atherosclerosis risk factors and vascular disorders in SLE patients.

Materials and methods: The study was performed in 83 patients with SLE and 20 matched controls.
The concentrations of VEGF were determined with ELISA test (R&D Systems). There were taken into account more than 100 variables including inflammatory and immunologic markers as well as classical risk factors for atherosclerosis and organ manifestations. Vascular involvement was assessed using B-mode ultrasound, bilateral transcranial Doppler, MRI scans of the brain and 3D contrast-enhanced MR angiography.
Statistical analysis was performed with chi2 Yates, chi2 Pearson, rank Spearman correlations tests, logistic regression analysis and multivariate stepwise analysis.

Results: High VEGF levels were significantly associated with the elongation of activated partial thromboplastin time (OR= 22,8; 95% CI: 2,3-230,6) and the presence of anti-phospholipid antibodies (aPLs) (OR= 10,7; 95% CI: 2,1-53,4). Myocardial relaxation disorders were significantly more frequent in patients with high concentration of VEGF (OR= 8,0; 95%CI: 1,6-39,5). The low concentrations of VEGF significantly decreased the risk of the existence of selected autoantibodies: aPLs (OR= 0,18; 95% CI: 0,0-0,72), anti-double stranded DNA (OR=0,31; 95% CI: 0,11-0,91) and anti-endothelial (OR= 0,30; 95%CI: 0,11-0,85). Furthermore, they were associated with reduction of the risk of atherosclerotic lesions in lower limb arteries (OR= 0,24; 95% CI: 0,0-0,99) and vasculitis development (OR= 0,17; 95% CI= 0,03-0,91).

Conclusions: High VEGF levels are significantly associated with the presence of aPLs and may increase the prothrombotic risk in SLE patients. Lower concentrations of VEGF seem to be protective as they are significantly related to the decreased risk of immunologic disorders, atherosclerosis as well as vasculitis development in SLE patients.

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Inflammation, Vascular biology, Endothelium, Oxidative stress

Cod: T274

PREDOMINATION OF ANGIOGENESIS IN FEMALES AND TISSUE REMODELLING IN MALES – A GENDER-SPECIFIC DYSREGULATION OF CYTOKINES IN EARLY KNEE OSTEOARTHRITIS

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Background. Investigation of the pathogenesis of osteoarthritis (OA) is particularly difficult due to myriad of processes involved. The aim of the present study was to investigate shifts in the profile of cytokines in early knee OA (KOA).
Methods. We assessed 60 cytokines, by xMAP technology (R&D Systems), in EDTA plasma of a subset (61 subjects) of an initial Estonian population-based middle-aged KOA cohort (mean age 50±7.3). The stage of KOA was assessed by grading system (grades 0–3) of Nagaosa-Doherty (2000).
Results. The prevalence of KOA (grade 1–3) in the investigated population was 65%. The proportion of early cases of OA (grade 1) was relatively high (32%) and that of advanced cases (grade 3) comparatively low (10%) as might be expected in a middle-age population. Osteophytosis was the prevailing diagnostic feature in both early and in more advanced stages (grades 2 and 3).
We found that KOA associated ($p<0.05$) with angiogenesis markers (ANG1, FGFacidic, PDGF-BB and TSP2) and proinflammatory markers (RANTES, MIP1b, IP10) in females. In males, KOA associated with proinflammatory IP10 and MIC1 and tissue remodelling biomarkers (TIMP2 and TIMP4). In females, MMPs or TIMPs did not associate with KOA. Dysregulation of cytokines took place already in early stage (grade 1) of KOA. So, RANTES was significantly upregulated but IP10 was downregulated in grade 1 of KOA in the whole group ($p<0.02$). We also demonstrated that leptin associated significantly with KOA in the whole group ($p = 0.0017$) and definitely in females ($p = 0.0006$). In addition, BMI correlated positively with FactorD in females but inversely with adiponectin in males. Of note, inflammatory markers IL6, IL1R4 or TNF-RII did not associate with BMI in our study.
Conclusions. The present study in a middle-aged population demonstrated that several biochemical processes proceed in parallel in the early phases of knee OA. However, remarkable gender-differences take place. In females, KOA associated more prominently with inflammation and angiogenesis. In males, KOA is characterised by a shift in the plasma level of tissue remodelling biomarkers as MMPs and TIMPs. The xMAP technology is an excellent tool to study OA mechanisms and to identify biomarkers in a cost-effective manner.

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Kidney diseases

Cod: T284

ARE LABORATORY CREATININE METHODS TRACEABLE? – COMPARISON WITH ID-GCMS REFERENCE METHOD

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Traceability of laboratory results is a recognised need within quality standards based on ISO15189 and ISO17025. Where possible higher order reference methods are used for comparison of participant results with traceable values in the Weqas EQA schemes. With the drive for more traceable creatinine measurements, there is now a trend to the use of more specific enzymatic methods.
8 Pools of human serum covering a creatinine range of approximately 25 to 600µmol/l were distributed on 4 occasions to all Weqas participants. Each had associated creatinine reference target values using a JCTLM listed ID-GCMS Reference Method. Returned results showed an increase in laboratories using enzymatic methods from 18% in 2012 to 37% in 2016.
The Jaffe ID-GCMS traceable group showed good correlation with the reference method above 150µmolL but exhibited a negative bias below this value. The Vitros ID-GCMS traceable group showed good correlation above 150µmol/L with a positive bias at lower concentration. The enzymatic methods showed good agreement with the ID-GCMS target value across the range of distributed samples. Using the MAPS (minimum analytical performance specification) bias criteria for creatinine, only the enzymatic method group is within the specified 3.8% desirable limit or 5% achievable limit. However within the enzymatic group the overall mean was influenced by the Roche and Olympus methods, which showed a marked positive bias. The Abbott and Siemens Dimension enzymatic methods showed closer agreement with the ID-GCMS target values. Within each enzymatic method group a large variation was also observed with substantial overlap of the data seen between groups.
Whilst traceability of creatinine methods has improved, even within the ID-GCMS traceable methods, variability has been observed as highlighted by comparison of EQA returns with the ID-GCMS reference method. The use of ID-GCMS reference targets as opposed to comparison with mean data eloquently highlights the variability.

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Laboratory and Sports medicine

Cod: T310

COMPARISON OF CARDIAC BIOMARKERS FLUCTUATION IN RUNNERS OF MARATHONS, SEMI-MARATHONS AND UNTRAINED RUNNERS

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Background: Regular exercise is important for the prevention program of cardiovascular disease. There are several studies on biomarker changes during marathons especially cardiac biomarkers have been studied and mild to moderate elevations have been described as a results of a running exercise Exact underlying mechanism for these elevations reflecting physiological or even pathobiological changes is unknown and less trained athletes might exhibit a higher risk compared to well trained The aim of our study was to compare three cardiac biomarkers for ischemic condition (Troponin), cardiac stretch (natriuretic peptides) and fibrosis(Galectin-3) in different type of runners, trained marathon and semi-marathon runners and untrained runners at 3 different time.

Methods: 23 (mean age 41± 8.8 yo) marathon runners, 15 semi-marathon runners(44.1±8.4yo) and 17 healthy sedentary subjects (37± 4.4 yo) (race of 10 km, <2h of sport/week).
Blood samples were taken just before (T0), just after (T1) and 3 hours (T3) after the race,, centrifuged , aliquoted and stored frozen at -80C before further analysis. The study was approved by the Ethical Committee of our University Hospital.The analyses were performed on the Abbott ARCHITECT i2000SR (Abbott Laboratories, Germany) for the hs cTnI, BNP and Gal-3 and on the C8000 (Roche Diagnostics,Switzerland) for hs-cTnT and NT-proBNP according to the manufacturer's instructions for use.

Results: In all 3 running groups there is an increase of cardiac biomarkers Troponin I, BNP, Galectin-3 and NT-ProBNP after completion of the physical exercise. Biomarkers increase is depending on the intensity and duration of the exercise and is higher in long distance marathon and semi-marathon runners compared to the control group with a 1 hour run. Cardiac biomarker levels between trained marathon and semi-marathon runners were not statistically different in the pre-exercise baseline samples for BNP, NT-Pro-BNP and Galectin-3. Compared to untrained runners only Troponin I levels were higher in baseline sample of marathon runners (hs-cTnI, $p<0.03$) when compared to controls, cardiac Troponin T (hs-cTnT, $p<0.29$) was less significant.

Conclusions: Our study demonstrates that exercises of different intensity can be associated with biochemical abnormalities that may reflect adverse consequences on cardiac structure as fibrosis and biology. With exeption of Troponin I there was no difference in the pre-exercise baselines samples.

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Evidence based medicine - Guidelines

Cod: W005

SAMPLE SIZE GUIDANCE AND JUSTIFICATION FOR STUDIES OF BIOLOGICAL VARIATION

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BACKGROUND

Biological variability studies have major importance in providing researchers with estimates of test variability allowing tests to be selected for further evaluation and used in diagnosis and monitoring of patients.

This work provides researchers with a tool to enable sample sizes for biological variability studies to be planned and justified prior to recruitment of participants. This tool will enable researchers to understand the likely estimates producible from the studies they plan and if necessary modify the sample size to achieve the required accuracy.

METHODS

Test data were simulated using the model $y_{ijk}=\mu+\alpha_i+\beta_{ij}+\varepsilon_{ijk}$, where μ is the mean value of the measure, $\alpha_i \sim N(0,\sigma_G^2)$, $\beta\_{ij} \sim N(0,\sigma_I^2)$, $\varepsilon_{ijk} \sim N(0,\sigma_A^2)$ and i=1,..,n1, j=1,..,n2 and k=1,..,n3. This gave n1×n2×n3 observations; n3 assessments of n2 observations for n1 participants.

The generated test data was analysed using a linear model with random effects for participants and observation points within participants. Fitting the model allowed estimates of σ_A, σ_I and σ_G to be obtained. In addition to standard deviations, other measures of variability were produced (coefficient of variation (CV), index of individuality (II) and reference change values (RCV)) using the estimates from the model.

Comparison of the pre-specified variability measures and those calculated for a given sample size indicated how close estimates obtained from a study of that size would be to the true variability. Repeated simulations were carried out with bootstrapping to estimate the variability of results.

RESULTS

Using repeated simulations and varying the sample size at each level (number of participants, observations and assessments) showed clear trends. Increases in the number of participants decreases the variability around estimates of CV_A, CV_I and CV_G; increases in the number of observations decreases variability around estimates of CV_A and CV_I; and increases in the number of assessments decreases variability around estimated CV_A.

CONCLUSIONS

The simulation model can be used to estimate the variability in results for a given sample size and is intended for use by researchers when planning biological variability studies.

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Laboratory Management, Accreditation, Quality Assurance

Cod: W027

DEVELOPMENT OF AN EXTERNAL QUALITY ASSESSMENT SCHEME FOR URINE DRUGS OF ABUSE

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Introduction
External Quality Assessment (EQA) is an essential part of assuring the quality of laboratory diagnostic services, and participation in EQA is a requirement for laboratory accreditation to ISO15189 and Point of Care (PoCT) accreditation to ISO22870. Drugs of Abuse testing is most commonly performed on urine as most drugs and their metabolites are excreted in urine. Urine testing for both prescribed and illicit drugs is increasingly used in both Laboratory and PoCT settings.

The Weqas Urine Drugs of Abuse EQA Scheme caters for both urine screening and confirmatory testing and allows both qualitative and quantitative reporting.

Aim
To develop and validate material for use in an EQA scheme for Urine Drugs of Abuse testing and to assess the stability and commutability of the material.

Method
Urine pools spiked with drugs or their metabolites were assessed for their short-term stability at 4°C and long term stability at -20°C. Comparability of the urine was assessed from EQA returns across Laboratory, PoCT and Mass Spectrometry methods.

Over 60 sites were recruited to take part in the study. Each site was sent 3 samples per month with negative and positive samples covering 16 drugs / metabolites. The majority of users were using Laboratory or PoCT screening methods reporting qualitative results.

Results & Discussion
The majority of spiked drugs appear stable for 14 days at 4°C and for 4 months at -20°C.

Qualitative interpretation was based on the gravimetric value for each drug according to pre-determined cut points, with the gravimetric value provided for quantitative reporting. For most drugs the qualitative results show over 90% positivity for samples, with spiked values above the cut point. For some drugs, specific performance characteristics have been identified where certain methodologies are unable to measure specific metabolites.

Conclusion
An EQA scheme for Urine Drugs of Abuse has been established using stable, comparable material suitable for Laboratory and PoCT methods, for screening and confirmation testing with quantitative and qualitative reporting available.

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Laboratory Management, Accreditation, Quality Assurance

Cod: W047

EUROPEAN HPV DNA TEST EXTERNAL QUALITY ASSURANCE SCHEME (EHEQAS)

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Background / Objective
To improve the quality of laboratories in HPV detection and typing we run the program of external quality control - EHEQAS.

Methods
EHEQAS was founded in 2006 and by 2016 already 25 laboratories from 8 European countries are participating. Batches of 5-7 samples are sent from the coordinator to participants 1-2 times per year. Samples are either real patient samples (including cervical cell pellets) or prepared from international standards as standalone dilutions or mixtures with real patient samples. Samples that are not international standards are pre-tested by reference laboratories and only samples for which there is a high level of agreement between reference laboratories are used. To test for reproducibility, samples are used in duplicate in the same and in different rounds. Linearity is evaluated by different dilutions of the same sample in the same and in different rounds. Results are evaluated and consensus results are issued and announced to participants in a confidential way. Marks are awarded to participants based on defined rules that reflect the clinical value of the result (e.g. higher penalty for errors regarding types 16 and 18). Certificates of competence that reflect the performance of a laboratory during the past 4 years are issued.

Results
Until now 219 samples have been tested in 22 rounds: 60 negative, 62 single infections, 97 co-infections. 31 different types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 68, 70, 73, 81, 82, 83, 84) were detected during the period 2006-10 and the same 31 types were also fully represented during the period 2011-15. Laboratories using IVD tests made significant errors in HPV detection and typing, depending on the skills of laboratory personnel and on whether they correctly followed manufacturer's instructions.

Conclusions
There is a gradual increase in the number of participants and in the quality of their performance. EHEQAS improves quality with the coordinating team providing feedback to participants on how to improve their methodology. EHEQAS assesses the quality of laboratories in: (a) detecting a shift in sensitivity and specificity in time. (b) HPV typing (high- or low-resolution). Successful participation in EHEQAS is extremely helpful to high-quality HPV labs that also verify or validate their methods: success in an EQA is a prerequisite for granting ISO15189 accreditation.

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Laboratory Management, Accreditation, Quality Assurance

Cod: W058

A PROPOSAL FOR ESTIMATING MEASUREMENT UNCERTAINTY USING QUALITY CONTROL DATA AND EXTERNAL QUALITY ASSESSMENT SCHEMES

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Background:
In order to comply with the accreditation process, ISO 15189:2012 requires that medical laboratories estimate the measurement uncertainty (MU) of their measurement procedure (MP). However, ISO 15189 does not specify a method for deriving the estimation of MU. Aim of this study is to describe the strategy used for MU estimation of tests of different areas of our laboratory, including clinical biochemistry, haematology and coagulation, clinical molecular biology and diagnostic immunology.

Methods:
To define the model for MU estimation, several MPs were evaluated for: a) classifying test in relation to the clinical purpose, b) identifying criteria for calculating the MU component. The imprecision and the bias and bias uncertainty were calculated by using the long-term internal quality control (IQC) results and external quality assessment schemes (EQAs) results, respectively. Several guidelines and manuscripts were studied before selecting MU models.

Results:
For a total of 263 MPs, two MU models estimation were identified: a) the first model including only the imprecision component, which has been calculated for the test results principally used in monitoring patient's results, (e.g. tumour markers) (51 MPs); b) second model, MU was calculated by combining imprecision, bias and bias uncertainty (190 MPs). For a total of 28 MPs, those in which a significant trend was observed between imprecision and/or bias and analyte concentrations, MU was calculated on different levels of analytes concentration. For 22 MPs, EQAs statistics were not feasible for MU calculation.

Conclusions:
The models proposed appeared to be suitable for MU estimation in medical laboratories, thanks to the availability of IQC and EQA data. Further, these models were developed accounting the different test purpose and the clinical needs to produce effective information in order to improve patients' outcome.

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Laboratory Management, Accreditation, Quality Assurance

Cod: W060

HOW EFFECTIVE IS ELECTRONIC GATE-KEEPING IN INFLUENCING TEST REQUESTING BEHAVIOUR AND COST SAVING: A RE-EVALUATION

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Introduction: Helath care budgets are under increasing pressure and clinical laboratories have therefore developed strategies to adapt to the increase in laboratory workload. Unnecessary repeat testing can contribute up to 25% of a laboratory's total workload. Electronic gatekeeping (EGK) has been implemented at selected laboratories in South Africa, as a demand management strategy to limit unnecessary repeat testing. Here, we performed an audit of chemistry tests subjected to EGK to determine its merit and effectiveness as a sustainable demand management tool.

Method: A retrospective observational audit of all chemistry test requests at the Pretoria NHLS academic laboratory over a period of 27 months was performed. EGK rules were programmed into the laboratory information system. Tests violating the programmed EGK rules are rejected upon registration, prior to analysis. Cost savings were computed from the number of tests subjected to EGK. The impact of EGK on the test requesting pattern of clinicians was deduced from the percentage trend of EGK-held tests following the implementation of the demand management strategy. Trend analysis for the urea and electrolytes test profile, liver function test profile and thyroid function test profile was also executed.

Results: The total savings generated from EGK test rejections amounted to (currency ZAR) R2 222 966 (2.4% of billed tests – R92 417 278). Greatest savings were generated from high cost tests: glycated haemoglobin (R 279 379), urea (R276 739) and thyroid stimulating hormone (R225 394). The average number of EGK-held tests as a percentage of their total requested number over the 27 month period was 2.80% (Becomes 3.10% if I exclude December 2013 – April 2014). Test profile trends mirrored the pattern of the total number of EGK-held tests.

Discussion: The total savings from EGK-held tests were not as effective as anticipated and were only moderate. This may have some impact on in a cost-constrained setting. The monthly percentage of EGK-held tests and test profiles were largely unchanged therefore EGK was concluded not to have a substantial effect on the clinician test requesting pattern. On its own, EGK does not appear to as effective a strategy as anticipated or demonstrated in other studies.

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Laboratory Management, Accreditation, Quality Assurance

Cod: W061

BENEFITS OF AUTOVERIFICATION IMPLEMENTATION AT THE LARGEST UNIVERSITY HOSPITAL IN THAILAND

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Background: Autoverification (AV) is the process in clinical laboratories to report results that are automated actions performed by a computer system using criteria, logic established and tested by the medical staff of the laboratories. The number of test orders is increasing every year; therefore, AV was considered to implement in the laboratory in order to increase productivity. The purposes of this study are to study the accuracy and efficiency of the implementation of AV system. **Methods:** AV rules we set in the laboratory information system (LIS) consist of validation ranges, critical values, delta check, and complex rules which established by brainstorming among clinical pathologists and medical technologists. We used 500 retrospective data to determine the accuracy of AV rules by comparing with manual verification by five experienced medical technologists. We studied the efficiency of the implementation of AV system by using data from one month of error reporting, calculating the decline of full time employee per day by using AV passing rate, using five-question questionnaire from 43 staff for their satisfaction and collecting data before and after implementation of AV from 20 official working days for turnaround time (TAT). **Results:** The accuracy of AV rules was 80.2%; no difference in billable test per hour during 24 h of 20 official working days while TAT was slightly reduced from 45 to 41 minutes (P = 0.166). The passing rates of chemistry, coagulation, hematology and microscopy were 95.0, 85.0, 39.0 and 43.0% respectively. The error report detected decreased from 0.0008 % to 0.0007%. Staff were reduced 0.12, 0.33, 0.05 FTEs for all days, peak hour, and non-peak hour, respectively. Staff satisfaction were increased from 65% to 89%. **Conclusion:** Our AV rules provide the high accuracy and the passing rate was quite similar to previous studies in chemistry, coagulation and urinalysis. Overall staff satisfaction was increased, while error rate and FTEs were to some extent reduced after the implementation of AV.

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Microbiology - Infectious diseases

Cod: W087

PREVALENCE OF ANTI-HCV AND ACTIVE HCV INFECTION IN AN ITALIAN HOSPITAL POPULATION

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BACKGROUND AND AIMS
The prevalence of hepatitis C virus (HCV) infection in Italy is estimated by reports of acute or chronic hepatitis and/or on surveys carried out in open populations from small towns. As both systems are not a reliable indicator of the actual spread of HCV in the general population we aimed to get a better picture by an observational study in a hospital population.
METHODS
We initiated a prospective, 6 months evaluation on routine specimens from in- and outpatients referred for anti-HCV testing to our Microbiology department that centralizes serological testing for the urban area of Bologna (around 1 million inhabitants). To avoid a selection bias, patients admitted to clinical wards dealing directly with HCV-related diseases or from groups at very high risk of infection, such as dialyzed patients, drug addicts or patients attending STD clinics, were excluded. All specimens positive for anti-HCV have been assayed for HCV core antigen (HCVAg) by a commercial immunoassay (Abbott Architect) and samples who resulted negative for HCVAg will be tested for HCV-RNA by a highly sensitive real-time PCR (Abbott).
RESULTS
Over the first three months of the study 10,849 patients were tested for anti-HCV and out of those 299 were positive (2.8%), with a mean age of 61.2+18.2 years (males: 59.5+17.1; females: 61.3.0+19.1; p= n.s.). HCVAg was positive in 152/299 cases (50.8%; males 46.5%, females 55.6%) and the positivity rate was higher in samples with a sample/cutoff ratio >5 compared to samples with a weaker reactivity (59.9% vs. 2.1%; $p<0.001$), the latter belonging to slightly older subjects (p= n.s.). So far, HCV-RNA was never positive on HCVAg negatives.
CONCLUSIONS
While the overall rate of anti-HCV positivity is lower than the one found in several open population studies carried on in the past, it largely exceeds the official data on acute and chronic hepatitis C reported to the European Centers for Disease Control (ECDC) for Italy. Most of the infections are then asymptomatic and will escape diagnosis until a clinical illness will develop, usually at a late stage. On the other side, almost 50% of anti-HCV positives do not appear to be carrying the infection. While this figure may show a slight decrease after testing for HCV-RNA on all HCVAg negative specimens, it stresses the need to test for markers of active viral replication and not only for specific antibodies when carrying out population surveys aimed to interventional or preventing strategies.

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Molecular diagnostics - Genetic testing

Cod: W150

NON INVASIVE PRENATAL FETAL BLOOD GROUP GENOTYPING IN THE MONITORING OF ALLO-IMMUNISED ANTI-RH4 PREGNANT WOMEN: EXPERIENCE OF THE FRENCH NATIONAL CENTER FOR PERINATAL HEMOBIOLOGY (CNRHP).

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Background:
Maternal-feto blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is characterised by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-KEL1 and anti-RH4. CNRHP provide non invasive fetal genotyping as a routine service to help the practitioners to improve the accuracy follow-up in pregnant women anti-RH1and KEL1 allo-immunised but this assistance are not yet provided to monitoring pregnant women RH4 allo-immunised. Among the 300 patients/year followed by CNRHP, about 100 have severe immunisation (RH4 antibody dosage higher than 500UCHP/ml) leading to specific antenatal monitoring if partner are RH4.
Aim:
Set up and evaluation of non invasive prenatal fetal genotyping to guide the follow-up of allo-immunised anti-RH4 pregnant women.
Methods:
To set up non invasive fetal RHc genotyping, DNA from 19 plasmas of RH:-4 women between 12 and 38 weeks amenorrhea were isolated using manual methods. Then RHc allele was detected by PCR using an adapted published method (Finning et al. Transfusion, 2007, 47: 2126-33) and compared to RHc genotype determined from amniotic cell or to red blood cells phenotype of the babies at birth.
Results:
Non invasive fetal RHc genotyping set up result show a sensibility, specificity and a positive and negative predictive value of 100%.
Since April 2016, CNRHP does non invasive fetal RHc genotyping from peripheral maternal blood. 30 non invasive fetal RHc genotyping from allo-immunised anti-RH4 women with RH: 2.4 partners were done to identify foetuses at risk for HDFN. For 24% of the allo-immunized women, the pregnancy was found compatible.
Conclusion:
Non invasive RHc fetal genotyping is a powerful tool to diagnose a feto-maternel red blood cells incompatibility and allows to legitimize a costly and heavy specific antenatal monitoring only to pregnant women carrying incompatible fetus with every two weeks an anti-RH4 dosage and weekly a search for signs of fetal anemia (Velocimetry Doppler).

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Neurological - Neurodegenerative diseases

Cod: W168

DO GENES CORRELATE WITH INTELLIGENCE ?

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BACKGROUND: Only 2% of the global population belong to those with elevated IQ. Research to date has had limited success in identifying DNA variants responsible for the heritability of intelligence. The aim of this study is to determine the effect of HTR2A/ rs1328674, FAAH/ rs324420, ANKK1/ rs1800497, SNAP25/ rs363050 and BDNF/ rs6265 on intelligence.
METHODS: 80 volunteers of Greek society of Mensa with known high IQ score (Figure reasoning test) and almost 900 volunteers of general population were examined. Following volunteer's informed consent undersigning, samples were de-identified and anonymized. DNA was isolated from epithelial cells collected from the oral cavity, using nucleic acid isolation columns. Genotypes were determined by real-time polymerase chain reaction using the Simple Probes commercial LightSnip kit. Logistic regression analysis was performed in all results.
RESULTS: rs1328674 G/G genotype is associated with High and very High IQ, with significant statistical differences ($p<0,05$) in all 5 different logistic regression models. Five genotype combinations of examined polymorphisms shown significant association with very high intelligence with $p<0,05$.
CONCLUSION: Preliminary data show that combined genotype determination of rs324420, rs1800497, rs363050 and rs6265 and rs1328674 polymorphisms have a close relationship to data derived from established IQ test. The rs1328674 polymorphism was statistically different between persons with very high IQ (>135) and the rest sample. High IQ score can be linked with the genetic background, as classified in this study.

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Neurological - Neurodegenerative diseases

Cod: W176

THE EMPIR PROJECT: INNOVATIVE MEASUREMENTS FOR IMPROVED DIAGNOSIS AND MANAGEMENT OF NEURODEGENERATIVE DISEASES (NEUROMET)

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BACKGROUND:
Neurodegeneration is an incurable, debilitating process which presents a growing global challenge. Alzheimer's and Parkinson's disease are two of the most common neurodegenerative diseases (NDD). Both involve the build-up of specific proteins in the brain and subsequent neurodegeneration leading to physical and mental impairment and ultimately dementia. Here we present the overview of the EMPIR NeuroMET project, which brings together the diverse expertise of National Measurement Institutes, clinicians and academics, to overcome the specific measurement issues currently constraining clinical innovation in NDD diagnosis and treatment.
Here we provide an overview of the progress made in
1. developing improved magnetic resonance imaging (MRI) methods
2. understanding bias and uncertainty of immunoassays and digital PCR methods for neurodegenerative disease protein and miRNA biomarkers
3. development of reference methods for tau and alpha synuclein
4. improving the assessment of cognitive performance for early diagnosis of Alzheimer's disease

METHODS:
Liquid Chromatography Mass Spectrometry, LC-MS (triple quadrupole and quadrupole time of flight and quadrupole-orbitrap), immunoassays (single, multiplex and ultra-sensitive), digital PCR and magnetic resonance imaging and spectroscopy 7T (MRI and MRS) and modern psychometric analysis techniques

RESULTS:
Protocols for high resolution MRI of the whole brain and hippocampus and MRS of posterior cingulum were developed on healthy individuals and the preliminary results of the application of those protocols on a cohort formed by healthy, Alzheimer's disease and mild cognitive impairment individuals are presented.
An initial assessment of the uncertainty of immunoassays for quantification of Alzheimer's biomarkers in plasma including tau and neurofilament light chain was performed and applied on a number of platforms commercially available by using plasma samples from the Alzheimer's patient cohort.
A primary calibrator to be used for the analysis of α-synuclein and tau protein by LC-MS and for implementation of reference methods was developed
Preliminary results from psychometric analyses on cognitive performance measures are presented, identified key area for improvement.

CONCLUSIONS:
The early findings from EMPIR NeuroMET are promising, and the project is currently on schedule to deliver findings across the multiple sub-studies, and subsequent recommendations, by end of 2018.

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Neurological - Neurodegenerative diseases

Cod: W177

THE ITALIAN PROGRAM FOR STANDARDIZATION OF CEREBROSPINAL FLUID BIOMARKERS AS DIAGNOSTIC TOOL IN LABORATORY AND CLINICAL SETTINGS

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BACKGROUND. The redefinition of mild cognitive impairment and Alzheimer's disease (AD) has considerably modified criteria for diagnosis of neurodegenerative dementia, providing an increased level of certainty by evaluating the biomarkers of AD pathology (McKhann 2011). However, standardization of procedures is still lacking.
In Italy, scientific societies are synergistically moving with the aim to standardize the analysis of cerebrospinal fluid (CSF) biomarkers, amyloid ß 1-42, tau and phosphorylated tau in the clinical and laboratory practice.
In the first phase, the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Society of Neurology and dementia (SINDem-ITALPLANED) have promoted a census of the laboratories and hospital which perform CSF analysis in a nationwide contest, focusing on critical issues as standardization of procedures, harmonization of ranges of normality and participation to quality control programs.
METHODS. Questionnaire was designed using SurveyMonkey and sent via email to the members of SIBioC, SINDem-ITALPLANED and to main Neurological Clinics all over Italy (n=1815).
RESULTS. We found an heterogeneous distribution of CSF laboratories along the territory, with several regions lacking of CSF biomarkers' evaluation (7/20). Some centralized laboratories (n=15) guarantee the biomarkers' analysis for neighbors memory clinics (n=15). Fifteen hospitals have an internal laboratory for CSF biomarkers. In sum, 40 neurological centers require CSF analyses. Both standardization and harmonization need a systematic organization. Only half of the laboratories (56.00%) participate in International Quality Control programs.
CONCLUSIONS. In conclusion, our data demonstrate that the use of CSF biomarkers is still limited in clinical practice and only a restricted number of patients receive an integrated clinico-biological diagnosis in Italy. In the next phase, the Scientific Societies will co-operate to define common national guidelines for both the standardization of CSF analysis in laboratory and the appropriate use of diagnostic tool in clinical setting, with the aim to allow a better harmonization of the CSF biomarkers along the territory.

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Patient sample management, (standardization, harmonization,reference ranges, etc)

Cod: W255

NUMBER: NATIONAL REFERENCE INTERVALS AND DECISION LIMITS IN THE NETHERLANDS USING A 'BIG DATA' APPROACH

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BACKGROUND
External Quality Assessment (EQA) programs for general chemistry tests have evolved from between laboratory comparison programs to trueness verification tools. In the Netherlands, the implementation of a type 1 EQA program 'SKML Combi New Style' in 2005 has been very effective in reducing inter-laboratory coefficients of variation for electrolytes, substrates and enzymes. However, universal and metrologically traceable reference intervals are still lacking, hindering universal use of guidelines and preventing adequate interpretation of test results. Under the umbrella of Calibration 2.000, we have initiated a national endeavour named NUMBER, to set up a sustainable system for the determination and long-term monitoring of traceable reference intervals in the Netherlands.

METHODS
We adjusted the evidence-based 'big-data' approach for deducing reference intervals from primary care data from Australia and New Zealand to the Dutch setting, using millions of test results readily available in clinical laboratory databases. Thirteen clinical laboratories across the country, covering all local IVD-manufacturers, have agreed to provide anonymous test results of primary care patients (July 2015-June 2016). Per laboratory, per test, outliers are excluded, data are transformed to a normal distribution (if necessary), means and standard deviations (SDs) are calculated. Then, means and SDs per test are combined using a random effects model to generate pooled reference intervals. Suitability of these reference intervals is evaluated using EQA-trueness and precision data. Flagging rates are tested and discussed with participating laboratories and manufacturers in expert meetings before national implementation.

RESULTS and DISCUSSION
A sustainable procedure for determining traceable reference intervals for general chemistry tests is set up using a 'big-data' approach that was validated and successfully implemented in Australia and New Zealand. To continuously support medical laboratories in the verification and long-term monitoring of reference intervals, as required by NEN-EN-ISO 15189:2012, the Calibration 2.000 steering committee and the Dutch EQA-organizer SKML will also set up a surveillance system to structurally monitor reference intervals in time and space.

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Patient sample management, (standardization, harmonization,reference ranges, etc)

Cod: W257

BIOLOGICAL VARIATION ESTIMATES OBTAINED FROM 91 HEALTHY SUBJECTS FOR SIX ELECTROLYTES IN SERUM. EBIOVAR STUDY OF THE EFLM WORKING-GROUP ON BIOLOGICAL VARIATION.

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Aim: EBioVar (European Biological Variation) study, an EFLM project, was established to deliver new biological variation (BV) data. The results of serum Sodium (Na), Potassium (K), Chloride (Cl), Calcium (Ca), Magnesium (Mg) and Phosphate (P) are presented.

Method: A cohort of 91 healthy subjects (38 male and 53 female, 21-69 years old) were phlebotomized for 10 consecutive weeks at six European laboratories. An equivalent and stringent pre-analytical protocol was followed at each center to deliver the blood samples. Separated sera were stored at -80°C prior to analysis in duplicate within a single run on ADVIA 2400 (Siemens Healthcare) at San Raffaele Hospital, Milan. The data were subject to outlier analysis prior to CV-ANOVA, to determine the BV estimates with confidence intervals (CI).

Results: CV_A calculated by ANOVA on sample's replicates for Na and Mg were higher than desirable analytical performance specification (APS) currently used (Na: 0.4% vs 0.3%; Mg 2.5% vs 1.8%) while for the others electrolytes CV_As obtained were always within their APS. No statistical differences between genders in within-subject estimates (CV_I(95%CI)) were found. CV_Is obtained were: Na (142.7 mmol/L): 0.53% (0.50-0.57); K (4.28 mmol): 3.92% (3.7-4.1); Cl (105.6 mmol/L): 0.98% (0.93-1.04); Ca (2.24 mmol/L): 1.81 (1.72-1.92); Mg (0.832 mmol/L): 2.88% (2.7-3.1); P (1.17 mmol/L): 7.67% (7.2-8.1). All CV_Is were significantly lower than the ones reported in Westgard database.

Between-subject biological variation estimates (CV_G) obtained were: Na: 1.21% (1.06-1.43); K: 4.08% (3.6-5.0); Cl: 1.34% (1.2-1.6); Ca: 2.73 (2.4- 3. 2); Mg: 5.79% (5.0-6.8); P: 10.5% (9.2-12.6). CV_G obtained are similar to those reported in Westgard database with the exception of Na (higher) and K (lower).

Conclusion: The new estimates of CV_Is obtained using a stringent protocol are statistically significantly lower than existing published data whereas CV_Gs are similar.

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Patient sample management, (standardization, harmonization,reference ranges, etc)

Cod: W280

DERIVATION OF RUSSIAN REFERENCE INTERVALS FOR IMMUNOCHEMISTRY ANALYTES MEASURED BY BECKMAN COULTER ANALYZER: A STUDY CONDUCTED AS A PART OF IFCC GLOBAL MULTICENTER STUDY ON REFERENCE VALUES.

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Background
A multicenter study was organized to explore sources of variation (SVs) of reference values (RV) for immunochemistry analytes and to determine reference intervals (RIs) specific for Russian population as a part of a global study coordinated by IFCC Committee on Reference Intervals and Decision Limits (C-RIDL).
Methods
By the use of C-RIDL protocol for the global study, 796 healthy volunteers 18-80 year old (yo) in 3 cities were recruited. 23 analytes were measured on the DxI 800 analyzer in Helix lab in Saint-Petersburg. To explore SVs, 3-level nested ANOVA and multiple regression analysis were used. Latent abnormal values exclusion method was applied to reduce influence of latent diseases on RVs of insulin, growth hormone (GH), testosterone and thyroid hormones. Individuals with high anti-thyroid antibodies or taking L-thyroxine were excluded from the analysis of thyroid panel. Pre-menopause females with total β human chorionic gonadotropin (TbHCG) > 2.9mIU/ml or those taking oral contraceptives were excluded from the analysis of reproductive panel. Volunteers with body mass index (BMI) >28 were excluded from deriving RIs for analytes with BMI-related changes in RVs. Partition by the status of menopause (MP) was done for reproductive panel, for other analytes with age-related changes in RVs were partitioned at 45 yo. RIs were computed by the parametric method.
Results
No between-city difference was observed in any analyte. Significant BMI-related changes in RVs were found for insulin, GH in both sexes, testosterone, sex hormone binding globulin (SHBG), free androgen index (FAI), progesterone in males, cortisol and parathyroid hormone in females. The upper limits (UL) of RIs for insulin, thyrotropin (TSH), and testosterone were lower than those of the manufacturer. The lower limit (LL) of testosterone decreased with age slightly, while UL of SHBG increased. Thus, the RI for FAI strongly shifted to the lower side with age. RIs were also partitioned by age for αfetoprotein, carcinoembrionic antigen. Median and LL of the RI for CA125 decreased with age in females, but partition by age was not performed because its UL was constant. The RI for TbHCG strongly shifted to the higher side after MP, especially the UL after MP, which strongly exceeded that of the manufacturer for non-pregnant women.
Conclusion
RIs for 23 immunochemistry analytes and FAI for Beckman Coulter analyzer were established in Russia by use of up-to-date methods proposed by C-RIDL.