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Abstracts*)

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P1

LEVELS OF SERUM M30 AND M65 PROTEINS AS BIOMARKERS OF APOPTOSIS IN CHILDREN EXPOSED TO PASSIVE SMOKING

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Key Words: child, cotinine, apoptosis, M30, M65
Background: Cigarette smoking and second exposure leads to DNA damage and change the natural apoptotic process of the cell. Unfinished repair process is highly significant factor in the pathogenesis of cancer. Cytokeratins (CK) are expressed from apoptotic cells. We aim to measure the cleaved fragment of CK-18 in aspartate 396, known as M30 antigen biomarker and M65 biomarker, usually related with both uncleaved and cleaved CK-18, as apoptosis biomarkers and to determine relation of passive smoking between the apoptosis.
Materials and Methods: A 79 children were included. We divided the children into 2 groups according to exposure to environmental smoke. 28 children [14(50%) girls and 14(50%) boys] included to the non-exposed group and the 51 children [26 (50.9%) girls and 25 (49.1%) boys] included to the group exposed one. Biomarkers M30 and M65 were measured in peripheral venous blood with PEVİVA/ALEXİS ELİSA kits.
Results: The mean urine cotinine level of the passive smoking group was higher than control group statistically ($p<0.05$). The mean M30 antibody levels of the passive smoking group were lower than control group statistically ($p< 0.05$). No statistically significant difference was determined between the groups in respect of the mean M65 antibody levels.
Conclusion: Until today, we know that our study is the first one, which evaluate the M30 and M65 biomarkers during apoptotic process in children exposed to tobacco smoke. According to results, it can be said that there was inhibition of the apoptosis (premalignancy) in those exposed to environmental tobacco smoke. There was no diffference between the M65 levels of the two groups, which suggests that exposure to environmental tobacco smoke changed the physiological process, but had no effect on the necrotic and autophagic death mode of the cells.

P2

SIGMA METRICS MODEL IN HbA1c DETERMINATION

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Key Words: sigma metrics, quality control, HbA1c
Background: It is essential to ensure the quality of results in clinical laboratories, since these results are going to be used in clinical practice. Most laboratories use Westgard Quality Control (QC) rules, but they have a high number of false negatives. Sigma Metrics is a tool for quality management based on the measure of the variability of a process. In clinical laboratories 3 sigma is considered the minimum acceptable quality, and 6 sigma is considered excellent.
Materials and Methods: We evaluated the internal quality control (at two levels) of two HbA1c analyzers: Capillarys 2 Flex Piercing (Sebia) based on capillary electrophoresis, and B-Analyst (Menarini Diagnostics), which is a POCT based on the principle of latex agglutination immunoturbidimetry. We used the data obtained during five months to estimate the mean, bias and CV (coefficient of variation). The aim of the study was to obtain Sigma values calculated as (TEa-bias)/CV [TEa: allowable total error] according to TEa specifications of 7% and 6% (in terms of NGSP certification), and quality specifications based on biological variability (BV).
Results: Sigma values for the Capillarys low QC and high QC were: 3.55, 3.78 (6% TEa), 4.14, 4.56 (7% TEa), 2.66, 2.59 (minimum BV), 1.78, 1.41 (desirable BV) and 0.89, 0.23 (optimum BV) respectively; For the B-Analyst, sigma values for the low QC and high QC were: 3.85, 2.69 (6% TEa), 4.20, 3.00 (7% TEa), 3.34, 2.22 (minimum BV), 2.82, 1.75 (desirable BV) and 2.30, 1.28 (optimum BV) respectively.
Conclusions: Sigma values for Capillarys presented the minimum acceptable quality when using TEa of 6% and 7%, the same as the low QC for B-Analyst. The high QC for the B-Analyst not presented an acceptable quality according to Sigma Metrics. BV specifications are more demanding, so none of the analyzers studied fulfilled the quality specifications.

P3

A COMPARATIVE STUDY BETWEEN IMMUNOTURBIDIMETRY AND CHEMILUMINESCENCE METHODS FOR MEASURING URINARY ALBUMIN

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Key Words: urinary albumin, immunoturbidimetry, chemiluminescence
Background: Several Immunoassays are used in medical laboratories for determination urinary Albumin. There are wide variations between the performances of these methods.,Therefore it is difficult for referral physicians to select appropriate laboratories for diagnosing and monitoring patients.
Materials and Methods: The present work is a comparative study between Immunoturbidimetry and Chemiluminescence methods used to assay urinary Albumin. The comparison was conducted on 108 random urinary samples. Both methods were verified first for precision, accuracy, linearity and analytical sensitivity.
Results: The precision of both methods was tested by repeatability and reproducibility studies, using control materials and patient samples. All calculated coefficients of variation (CVs), for both methods, in all levels, were compared to Westgard desirable specifications for precision and they were all accepted. Accuracy was tested by calculating the mean percentage recovery for both methods and by calculating the bias percentage after testing a reference material. Both Immunoturbidimetry bias (7.4%), and Chemiluminescence bias (14.8%), were accepted based on the desirable bias% recommended by Westgard. The analytical measuring range of Immunoturbidimetry (0.9-130 mg / L) was better than that of Chemiluminescence (2.5-60 mg / L). One hundred and eight random urinary samples were tested using both methods, and their results were compared using Wilcoxon Signed Ranks test, Bland Altman Plot and MCNemar test, and all showed p-values > 0.05, for grouped samples and for samples divided based on a medical decision level of 30 mg / L. Regression line analysis showed a significant strong positive correlation between the two methods with 91% agreement (for 95% CI). (P value: <0.001, R Sq Linear: 0.91).
Conclusions: Both Immunoturbidimetry and Chemiluminescence methods are comparable in measuring urinary Albumin. Immunoturbidimetry had shown better analytical measurement range and accuracy.

P4

MEASUREMENT UNCERTAINTY: ISO 15189 OR SUPPLEMENTARY STANDARDS AND GUIDELINES

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Key Words: measurement uncertainty, ISO 15189, quality management
Background: Measurement uncertainty (MU) is one of the major potential contributors to the uncertainty of result interpretation. The aim of this study was to analyze MU references and to present MU evaluation in tertiary health care institution, Center for Medical Biochemistry, Clinical Center of Serbia.
Materials and Methods:We reviewed 8 readily available relevant MU references published by responsible bodies: 1) Joint Committee for Guides in Metrology WG1, 2) Standards Council of Canada, 3) National Pathology Accreditation Advisory Council Australia, 4) International Laboratory Accreditation, 5) United Kingdom Accreditation Service, 6) Food and Drug Administration, 7) Clinical Laboratory Standard Institute and 8) Accreditation Body of Serbia. In addition, we present MU evaluation scheme that is designed in Department of polyclinic laboratory diagnostic, Center for Medical Biochemistry, Clinical Center of Serbia.
Results: In conformance with generally accepted standards, MU evaluation concept implies: specification of the measurand, identification of the limitations and interferences as uncertainty sources, calculation and notification of the standard deviation of the mean with presented level of confidence, indication of the calibrator traceability if possible and presentation of the relevant MU information. Based on the generally accepted recommendations for the MU concept, our laboratory point to the listed items: the name of the analyte, test principle, measuring technique, specification of equipment, units, reference intervals, test limitations and interferences, calibrator traceability uncertainty (coverage factor is 2), analytical imprecision (CVa) with internal quality control details for the specified measuring period, analytical goal and MU data for clinical user information.
Conclusion: Although, ISO 15189 is a “top level” standard, it does not provide detailed information for MU evaluation. However, all the analyzed MU references satisfy ISO 15189 principles. Still, good professional practice and understanding of the analytical aspects of assays is required for particular laboratory to define appropriate MU evaluation for specific laboratory test.

P5

SERUM MONOCYTE CHEMOATTRACTANT PROTEİN 1 (MCP-1) AS POTENTIAL BIOMARKER FOR THE DIAGNOSIS OF PANCREATIC ADENOCARCINOMA

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Key Words: MCP-1, diagnostic, serum, pancreatic adenocancer
Background: Chemokines may control the macrophage infiltrate found in many solid tumors. Monocyte chemotactic protein-1 (MCP-1/CCL2) plays a key role in the recruitment and activation of monocytes during inflammation. MCP-1 is small chemotactic protein that has been found in several kinds of tumor tissue samples and functions as key regulator of cancer progression. This study was conducted to investigate the serum levels of MCP-1 in patients with pancreatic adenocarcinoma (PA) and the relationship with tumor progression and known prognostic parameters.
Materials and Methods: Thirty-five patients with PA were investigated. Serum samples were obtained on first admission before treatment and follow-up. Both serum MCP-1 levels were determined using enzyme-linked immunosorbent assay (ELISA). Age and sex matched 32 healthy controls were included in the analysis.
Results: The median age at diagnosis was 61 years, range 38–84 years; 21 (60%) patients were men. The tumor was located in the head of pancreas in 24 (69%) patients. The most common metastatic site was liver in 20 patients with metastasis (n=18, 90%). Forty-four percent of 18 metastatic patients who received palliative chemotherapy were chemotherapy-responsive. The median follow-up time was 24.0 weeks (range: 1.0-191.0 weeks). At the end of the observation period, while twelve (34%) patients experienced disease progression and twenty-three patients (66%) were dead. Median progression-free survival (PFS) and overall survival (OS) of the whole group were 13.7±2.3 weeks (95% CI=9-18 weeks) and 48.0±12.8 weeks (95% CI=23-73 weeks), respectively. The baseline serum MCP-1 levels were significantly higher in patients with PA than in the control group (p=0.02). Moreover, serum MCP-1 levels were significantly higher in the patients with low albumin and platelet levels (p=0.04 and p=0.05, respectively). However, serum MCP-1 had significantly affect on neither PFS nor OS survival (p=0.20, and p=0.49, respectively).
Conclusions: Although serum levels of MCP-1 assays were found to be diagnostic value, no predictive and prognostic value was determine in PA patients.

P6

INTERCHANGEABILITY OF BIOCHEMISTRY TEST RESULTS IN THE CLINICAL LABORATORY

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Key Words: interchangeability, autoanalyzers, patient safety, CLSI EP-31-A-IR
Background: According to ISO 15189:2003, clinical laboratories must periodically verify the interchangeability of test results among autoanalyzers at clinical decision levels, aiming at an improvement in patient safety. In this study, we applied the CLSI EP-31-A-IR protocol to check the interchangeability of general biochemistry tests, and thus establish appropriate corrective actions.
Materials and Methods: In our laboratory, we use 4 different Architect c16000 autoanalyzers (Abbott Diagnostics), where 45 general biochemistry magnitudes are assayed (30 in serum, 15 in urine). The control materials used were Assayed Chemistry, Urine Chemistry and Immunology Plus (BioRad Laboratories). Approximate analyte concentration was estimated on the basis of clinical decision levels and known imprecision; the latter obtained from the intraserial standard deviation (RS, n=20) and the total standard deviation (TS, n=number of controls assayed in a 6-month period). We established the maximal allowed difference (MAD,%) among the results of different analyzers, according to quality specifications in our laboratory. From these, we calculated the critical difference (CD=MAD·analyte concentration), the ratios CD/TS and RS/ST to determine the number of sample series needed and the replicate number in every series, the mean of the results in each autoanalyzer and the difference between means (DbM). Limit of acceptability was defined as LoA=CD·L, where L is the rejection range. Magnitudes were defined as interchangeable if DbM<LoA.
Results: From a total of 45 assayed magnitudes, only 2 were shown to be not interchangeable: serum alanine-aminotransferase (DbM=3.8; LoA <1.7%) and serum urea (DbM=2.84; LoA <1.16%). In both cases, the corrective measure was to cease biochemical assaying at the non-interchangeable analyzers.
Conclusions: The clinical laboratory must periodically verify the interchangeability of the results from its autoanalyzers, establish pertinent corrective measures to improve result quality and hence ensure patient safety.

P7

ULTRASENSITIVE THYROGLOBULIN: ANALYTICAL EVALUATION AND CLINICAL IMPACT

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Key Words: thyroglobulin, ultrasensitive assay, differentiated thyroid carcinoma, analytical performance
Background: Thyroglobulin (Tg) concentration in serum depends on the mass of thyroid tissue and could reflect damage at the thyroid gland. It is used as clinical biomarker for the monitoring of differentiated thyroid carcinoma (DTC), also in fine-needle aspiration biopsies (FNAB). Therefore, an increase in sensitivity would yield an improvement in DTC patient management. In this study, we assessed the performance of two Tg assays and evaluated their clinical impact.
Materials and Methods: A conventional Tg assay (cTg;Immulite2000,Siemens), and a new ultrasensitive method (TgII;Cobas,Roche) were compared. A Passing-Bablok correlation was performed by using 29 serum samples; intraserial imprecision of TgII was calculated by assaying one sample 10 times; and interserial imprecision by assaying three control materials for 20 days (Roche). Results were compared with quality specifications in our laboratory. Further patient samples (413 serum and 46 FNAB) were also analyzed using both methods, to assess clinical impact in terms of the limits of detection: LoD(cTg)=0.20ng/mL, and LoD(TgII)=0.04ng/mL.
Results: Passing-Bablok regression yielded: slope 0.872(IC95%: 0.767–0.917), y-intercept 0.091(IC95%: -0.204–0.509), r=0.990. For TgII, intraserial imprecision was 1.27%; interserial imprecisions were <3.70% in all three controls; systematic errors (SE)<6.00% and total errors (TE)<12.00%. Laboratory quality specifications were fulfilled (imprecision<7%, SE<10.36%, TE<21.91%). A total of 31 serum samples(6.9%) and 4 FNAB samples(6.1%) had Tg concentrations between 0.04–0.20ng/mL, thus being only detectable with the TgII assay.
Conclusions: Both methods are not interchangeable. As the new TgII method fulfilled the quality requirements in our laboratory, it could be used for healthcare, although reference values needed to be changed. The implementation of the new TgII assay leaded to an improvement in analytical sensitivity; now being able to quantify Tg in samples previously classified as undetectable. This analytical advance will certainly help in the management of DTC patients by improving monitoring and making early treatment possible.

P8

VALIDATION OF A METHOD FOR THE GENOTYPING OF THROMBOSIS AND HEMOCHROMATOSIS-RELATED GENES WITHOUT DNA EXTRACTION

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Key Words: multiplex PCR, extraction-free, melting analysis, thrombosis, hemochromatosis
Background: Daily practice in a clinical hematology laboratory involves nucleic acid amplification-based techniques. Current methods for PCR use purified genomic DNA, mostly isolated from white blood cells (WBCs). We previously developed a new genotyping method based on a multiplex asymmetric PCR without DNA extraction. This new method consisted of red blood cell selective lysis, followed by WBC washing to remove the heme group (a well-known PCR inhibitor) and subsequent DNA amplification using the intact WBCs (direct addition of PCR reagents to the cell suspension). Following this breakthrough, we performed a validation study to assess its possible introduction in the clinical setting.
Materials and Methods: We evaluated the optimum WBC number for the optimization of PCR parameters (melting analysis), with concentrations ranging from $0.2 \cdot 10^6$/ml to $60 \cdot 10^6$/ml. After that, we processed in parallel 34 patient samples with the traditional method (DNA amplification, with prior DNA extraction/purification), using a DNA concentration of 20ng/µl, and with our new developed method, with a concentration of $5 \cdot 10^6$WBC/ml. We compared the clinical performance between both strategies by assessing patient classification for F2, F5, F12, HFE and MTHFR genes.
Results: Of all WBC concentrations studied, the one yielding highest fluorescence signal was $5 \cdot 10^6$WBC/ml. Amplification curves from WBCs only suffer a small delay of 4 cycles in the melting analysis versus purified DNA. This delay does not affect subsequent analyses and patient classification. All patients matched in their gene mutation classification; fluorescence values were high enough, thus allowing robust genotyping without any previous DNA purification/extraction.
Conclusions: The present protocol represents a rapid DNA extraction-free method for the genotyping, based on melting curves, of different genes associated with thrombotic events and hemochromatosis. Moreover, we show that the results are good enough to withstand the current genotyping of several genes, demonstrating the suitability of the results for patient healthcare.

P9

ANALYTICAL ASSESSMENT OF A NEW CAPILLARY ELECTROPHORESIS SYSTEM FOR SERUM PROTEIN ANALYSIS

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Key Words: protein electrophoresis, methodology change, Passing-Bablok, quality specifications
Background: Protein electrophoresis is an analytical technique which allows the examination of five fractions of serum protein (albumin, α_1, α_2, β, γ), thanks to charge and mass-based separations. Relative quantification of every fraction is expressed as a percentage, and absolute quantification is calculated by considering the total protein concentration in serum. Despite the development of new diagnostic more-specific methodologies, proteinograms remain a fundamental tool in clinical biochemistry for the assessment of monoclonal components. In this study, we evaluated the analytical performance of a new electrophoresis system and checked whether it fulfills the analytical specifications in our laboratory. We also performed a correlation study by comparing it with our previous analyzer.
Materials and Methods: Two analyzers were compared: the new Capillarys™ Protein(e)6C2 (Sebia) and the V8™ Helena Biosciences (Izasa). Intraserial imprecision was assessed by 24 consecutive measurements of one serum sample, and expressed as coefficient of variation (CVi). Systematic error (SE) was calculated from 10-times measurements over 3 days of a single control material, and expressed as percentage of deviation from manufacturer's reported value. For the further correlation study, a total of 85 samples spanning over all the analytical range were analyzed by both analyzers. A non-parametric Passing-Bablok regression was performed. SPSS v17.0 was used for statistical studies. Signification level was fixed at 0.05.
Results: For each fraction, both CVi and SE fulfilled quality specifications in our laboratory. By calculating slopes and y-intercepts, Passing-Bablok correlations showed constant systematic differences in both β and γ fractions, and a proportional bias for the α_1 fraction. A good correlation (r^2=0.993; ref>0.975) was only achieved by the γ fraction.
Conclusions: The new analyzer Capillarys™ 6C2 fulfills quality requirements to be used for health care in our laboratory, although it would be essential to modify the reference intervals of every fraction for a successful and accurate interpretation of the proteinogram.

P10

UNCERTAINTY OF MEASUREMENT IN THALASSEMIA SCREENING: HbA2

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Key Words: thalassemia screening, HbA2, measurement uncertainty
Background: Quantitative determination of HbA2 is criticial in screening for β-thalassemia carriers. The aim of this study was to calculate uncertainty of HbA2 measurement on Primus Ultra[2] analyzer by using internal and external quality control data as described in Nordtest manuals.
Materials and Methods: In calculation of measurement uncertainty 'six step' approach defined in Nordtest guide was used. HbA2 analysis was performed using Primus Ultra[2] analyzer HPLC system, in Aydın Public Healty Laboratory.
Results: Combined standard uncertainty of HbA2 measurement was 2.7% and expanded uncertainty value was ±5.4% at a 95% confidence interval. Accordingly value of HbA2 measured at 3.5% -cutoff used for β-thalassemia carriers- was expected to lie between 3.5±0.2 at a 95% confidence interval.
Conclusions: Measurement uncertainty of HbA2 was found to be acceptable comparing with total allowable error values defined in previous studies performed in 2005 and 2013. However measurement uncertainty of HbA2 could not meet the desirable specification value of 2.5% for total allowable error in the 2014 update of biological variation database. This may be due to the lack of primary reference material and reference measurement procedure for HbA2 yet. Different approaches used in these studies in order to determine measurement uncertainty may also be contributing to the variations met among studies. In conclusion we would like to emphasize the need for evaluation of HbA2 measurement uncertainty with regular intervals in Thalassemia screening.

P11

COMPARISON OF DIFFERENT EQUATIONS FOR THE ASSESSMENT OF GLOMERULAR FILTRATION RATE

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Key Words: glomerular filtration, MDRD-IDMS, CDK-EPI, chronic kidney disease, cystatin C
Background: Estimation of glomerular filtration rate (GF) is routinely performed in clinical laboratories based on serum creatinine concentration. However, despite standardization of creatinine assays, these equations often fail in the correct classification of some patients. Inclusion of cystatin C (CysC) in such equations has been suggested to improve accuracy in GF calculations. In this study, GF was calculated using four different equations (CDK-EPI using creatinine alone, CysC alone or CysC+creatinine) and MDRD-IDMS, and were compared.
Materials and Methods: A total of 100 patients (67 male, 33 female) in different stages of chronic kidney disease (CKD 1-5) were included. Mean age was 65.9 (range: 18-89). The equations used were the internationally defined ones. Mean GF and standard deviation were calculated for every stage. To evaluate the differences between GF values using different equations, Bland-Altman analysis and Passing-Bablok regression were used. Percentage of reclassification was calculated to assess concordance between CKD stage classifications.
Results: GF means were: MDRD-IDMS: 43.4±27.9ml/min; CDK-EPI(creat): 43.9±28.4ml/min; CDK-EPI(CysC): 43.8±28.4ml/min; CDK-EPI(creat+CysC): 54.3±25.7ml/min. Although equations correlated successfully (CDK-EPI(creat+CysC) towards others yielded r=0.96;r=0.99;r=0.94), concordance tests showed significant differences: 11% patients were reclassified from stage 1 to stage 2 when CDK-EPI(creat+CysC) was used instead of CDK-EPI(creat) and, most importantly, up to 20% patients previously classified as stages 4 or 5 changed to stage 3.
Conclusions: According to K/DOQI guidelines, calculation of GF is essential for proper CKD classification, since most therapeutic strategies are based on CKD stage. Current guidelines accept MDRD-IDMS and CDK-EPI as most reliable equations, but accurate GF estimation is crucial. The equation CDK-EPI(creat+CysC) shows a significant discordance with the rest of equations. Patients' classification improves when both creatinine and CysC are included in the equation, rather than using only one magnitude. In this sense, CDK-EPI(creat+CysC) would yield a significant reduction in referrals to nephrologist, thus decongesting specialized consultation.

P12

ANALYTICAL ASSESSMENT OF THREE AUTOMATED IMMUNOASSAYS FOR THE QUANTIFICATION OF SERUM INSULIN-LIKE GROWTH FACTOR

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Keywords: insulin-like growth factor 1, growth hormone, IDS-iSYS, quality requirements, correlation
Background: Insulin-like growth factor-1 (IGF-1) is a liver-produced polypeptide under the stimulation of growth hormone. Although its serum concentrations remain constant throughout the day, variations are detected depending on age, gender and nutritional status. IGF-1 levels are used for the assessment of growth hormone status and pituitary gland dysfunction. Due to the recent acquisition of new analyzers in our laboratory, we aimed at the analytical evaluation of three different assays for IGF-1 quantification.
Materials and Methods: Up to 70 serum samples were analyzed using the following immunoassays: Immulite 2000(Siemens), IDS-iSYS(Vitro) and Liaison(DiaSorin). For the latest two, calibrators were traceable to the WHO Standard 02/254. Immulite used the WHO NIBSC IRR 87/518. For method comparison, the non-parametric Passing-Bablok regression was used. Method imprecision was assessed by 10 consecutive determinations of the same control material, and intraserial coefficient of variation (CVi,%) was calculated. Systematic error (SE,%) was estimated by measuring three different control materials on 10 non-consecutive days.
Results: After outlier removal, correlations were successful between pairs of analyzers (r^2>0.975). Passing-Bablok regressions yielded a proportional bias among IDS-iSYS–Liaison, while mixed systematic biases in other comparisons were found. The calculated CVi for IDS-iSYS was 1.69%. It matched the data provided by manufacturer (1.68-2.28%) and also fulfilled the quality specifications of the Spanish Society of Clinical Biochemistry, SEQC, (CVi<7.3%). Systematic errors were always below 6.6%, thus also fulfilling SEQC's quality specifications (<11.9%).
Conclusions: Good correlation among analyzers was obtained, especially between IDS-iSYS and Liaison, both of which use the WHO International Standard calibrator. Results are not, however, interchangeable between analyzers, since a systematic error was detected in each case. Therefore, it would be essential to use the reference values established by each manufacturer. Vitro's analyzer fulfills quality specifications in terms of imprecision and accuracy and may be used for health care.

P13

E. COLI BACTERIOPHAGE MODIFICATION FOR DETECTION OF *E. COLI* BACTERIA BY CAPACITANCE METHOD

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Key Words: *E. coli* bacteria, bacteriophage, capacitance, biotin
Background: Escherichia coli (*E. coli*) bacteria are one of the dangerous human pathogens. The study and preparation of *E. coli* biosensors is very important and required for diagnostic laboratories. we propose label-free, inexpensive, cheap, rapid and renewable *E. coli* biosensors by immobilizing of modified bacteriophage on electrode by capacitance method.
Materials and Methods: In this research, *E. coli* bacteriophage was modified by biotin for oriented imobilization on electrodes surface. The electrodes were applied as indicator electrodes for capacitance determination of *E. coli* bacteria. *E. coli* bacteriophage modification was characterized by FT-IR and scanning electron micrograph (SEM).
Results: Oriented imobilization of *E. coli* bacteriophage on electrodes surface were confirmed by SEM. Through this method, *E. coli* bacteria was detected in a concentration range from 33 to 330 N/mL (number of *E. coli* per mL) with a correlation coefficient of 0.99 and a detection limit of 12 N/mL.
Conclusions: The proposed biosensor show good linear range, low detection limit and excellent selectivity over *E. coli* bacteria. Also, this method can be extension to detection of other biological agent.

P14

BLOOD GAS ANALYSIS ON GEM® PREMIER™ 4000: EVALUATION OF IMPRECISION AND ACCURACY

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Keywords: blood gas analysis, coefficient of variation, quality control, quality specifications
Background: Arterial blood gas analysis represents a usual practice in patient handling at the Emergency Department. The substitution of our current gas analyzers (RapidLab® 1265 Series, Siemens) by GEM® Premier™ 4000 (Abbott) made it necessary to evaluate the new model for healthcare purposes, and to compare both. The new analyzers have an integrated internal quality control material (IQM) and an automatic quality management system.
Materials and Methods: A total of 5 magnitudes were studied: pH, pCO_2, pO_2, potassium and ionized calcium. Patient samples were collected in Li^+-heparin syringes. GEM® Premier™ 4000 imprecision was assessed by calculating (1) intraserial coefficient of variation (CVi,%) of a single patient sample, analyzed 10 times in one day; and (2) interserial coefficient of variation (CVe,%) of two control materials analyzed in 10 different series. Accuracy was evaluated by calculating the total error (TE) after processing both internal control materials in 3 series. TE was also calculated using the external quality control material offered by the Spanish Society of Clinical Biochemistry (SEQC), in 3 different months. For the comparison of both analytical systems, 50 patient samples were processed. The non-parametric Passing-Bablok regression was used to assess the degree of correlation.
Results: All studied magnitudes achieved biological variation-based quality specifications in terms of CVi and CVe, except for pO_2, which had a CVi=6.4% (similar to RapidLab). Regarding TE, all magnitudes achieved manufacturer-declared quality specifications. Concerning SEQC external quality control, pCO_2 and potassium failed in 1 month. Passing-Bablok correlation showed no differences for pH and pCO_2, whereas pO_2 and ionized calcium presented proportional biases, and potassium had both a proportional and a constant bias. Correlation was r>0.98 in all magnitudes.
Conclusions: The GEM® Premier™ 4000 blood gas analyzer fulfilled quality requirements in terms of TE. Respecting CV, results were similar as with the current analyzer.

P15

EVALUATION OF D-100 VS G8 HPLC ANALYZERS FOR HBA1C DETERMINATION

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Key Words: D-100, HbA1c, HPLC, diabetes, G8
Background: Glycated hemoglobin is an interesting alternative to glucose, not only for monitoring diabetes mellitus (DM), but also for diagnosis. HbA1c is proved to be much more stable and its determination less variable than other DM diagnosis tests. The gold standard method in HbA1c determination is ion exchange High Performance Liquid Chromatography (HPLC).
Materials and Methods: We randomly selected samples received in our routine laboratory for HbA1c determination. Samples were collected by venipuncture into EDTA tubes and analyzed in duplicate. We used G8 (Tosoh®) as a reference method and we evaluated D-100 (Bio Rad®). Both employ cationic exchange High Performance Liquid Chromatography (HPLC) method. In the comparison study we selected 100 samples divided in 4 HbA1c concentration ranges: 4-6%; 6-8%; 8-10% and more than 10%. In the inter-assay variability test, a pool of different samples was divided in aliquots, frozen at -80 °C and measured in 20 consecutive days. In intra-assay study a low and a high level HbA1c samples were chosen and measured 20 consecutive times. Passing-Bablok regression and Bland-Altman plot were used for statistical analysis of the results.
Results: Passing- Bablok: slope: 0,973, confidence interval (CI) (0,963-0,983), intercept: -0,007, CI (-0,07-0,069), (p=0,05), correlation coefficient (r): 0,992. Bland-Altman plot: bias mean difference: -0,229, CI (0,256--0,202) (p=0,05). Inter-assay CV: 0,81%. Intra-assay CV: 1,04% (low level), and 0,78% (high level).
Conclusions: The correlation between D-100 and our reference method is acceptable. The CI of the linear regression slope does not includes the value 1, thus we find a systematic error of 2,5% between D-100 and the reference method. This systematic error is also present in a small bias in the Bland-Altman plot. The small systematic error calculated and the variability (both, inter and intra-assay) fulfill the quality specifications of total admissible error in the determination of HbA1c (3%).

P16

EVALUATION OF MEASUREMENT UNCERTAINTY FOR ETHANOL ANALYSIS AND ITS EFFECT ON RESULTS CLOSE TO CLINICAL DECISION LEVELS

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Keywords: ethanol, uncertainty of measurement, “top-down” approach
Background: Uncertainty of measurement is a functional parameter which shows the deviation of measurement results from real values and reflects the quality and performance of the measurement method. The aim of this study is to calculate the measurement uncertainty value of ethanol analysis and reevaluate the patient results with respect to the calculated uncertainty value.
Materials and Methods: Measurement uncertainty of ethanol test which is measured by Abbott ARCHITECT c16000 autoanalyzer was calculated by a “top-down” approach. Components of uncertainty were determined as uncertainty on the within-lab reproducibility (uR_w) and measurement uncertainty on the bias (u_{bias}). uR_w component of uncertainty was calculated from annual internal quality control results. As no external control value was present for ethanol, u_{bias} was calculated with the relative difference between the certified value and the laboratory mean value of certificated reference material/ethanol calibrator (bias) and the standard deviation of the bias (S_{bias}). Using uR_w and u_{bias} components, combined and expanded measurement uncertainties (u_c and U) were generated. Retrospective test results which were close to decision limits were re-evaluated with regard to the calculated uncertainty. Microsoft Office Excel 2013 was utilized for calculations.
Results: The uncertainty of measurement value was calculated as 10.01% for ethanol test. Number of ethanol test results given by our laboratory last year was 2365. Application of measurement uncertainty value to the results led to a change in results of 20 patients.
Conclusions: A test which has a forensic significance like ethanol is reported as numerical value, but authorities use this result with regard to their predetermined cut-off levels. As the interpretation of results which are close to cut-off levels may change when assessed with measurement uncertainty, further confirmatory analyses may be required.

P17

EVALUATION OF COBAS B 101 (POCT) VS D-100 (HPLC) FOR HbA_1c DETERMINATION

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Keywords: HbA_1c, diabetes, Cobas b101, D-100
Background: The hemoglobin A_1c (HbA_1c) is employed in monitoring of patients with diabetes. Point of care (POC) instrument, characterized as fast, portable, and easy to use, have been shown to be suitable for providing rapid feedback of HbA1c levels. Ensuring the quality of the POC testing instrument used is crucial and should be embedded in a chain of quality control; otherwise the impact on patients will be immense.
Materials and Methods: We randomly selected samples received in our routine laboratory for HbA_1c determination. We use the D-100 (Bio Rad®) as a reference method which employs cationic exchange High Performance Liquid Chromatography (HPLC). The Cobas b101 (Roche Diagnostics®) is based in the principle of latex agglutination inhibition immunoassay methodology. For the comparison study, we selected 100 samples divided in 4 ranges and analyzed in duplicate. In the inter-assay variability test, a pool of different samples was divided in aliquots, frozen and measured in 20 consecutive days. In intra-assay study a low and a high level HbA_1c samples were chosen and measured 20 consecutive times. Passing-Bablok regression and Bland-Altman plot were used for statistical analysis of the results.
Results: Cobas b 101 showed Inter-assay CV: 1,87%. Intra-assay s CV were: 2,06% (low level), and1,92% (high).Passing- Bablok: slope: 0,911, confidence interval (0,889-0,933), intercept: 0,81, CI (0,65-0,99) $p<0,05$)The correlation coefficient was 0,992. The Bland-Altman plot: bias mean difference: 0,132, CI(0,0726- 0,191) $p<0,05$.
Conclusions: The Passing-Bablok linear regression shows a good correlation between Cobas b 101 and D100 (r: 0,992). The CI of the Bland-Altman plot does not includes the value 0, thus we find a systematic error of 1,69% between Cobas b101 and the reference method. The small systematic error calculated and the variability (both, inter and intra-assay) fulfill the quality specifications of total admissible error in the determination of HbA_1c (3%).

P18

STANDARDIZATION OF THE METHOD FOR CIGARETTE SMOKE EXTRACT PREPARATION AND ITS TOXICITY TOWARDS POLYMORPHONUCLEAR LEUKOCYTES

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Key Words: cigarette smoke extract, method standardization, poymorphonuclear leukocytes, toxicity
Background: Cigarette smoke is known as oxidative stress causing agent. For laboratory studies of its toxic effects on different cell models cigarette smoke extract (CSE) is used. The aim of this study was to evaluate the method for CSE preparation and to examine cytotoxic effect of CSE on polymorphonuclear leukocytes (PMN).
Materials and Methods: 100% CSE was freshly prepared by bubbling the smoke from two Kentucky 3R4F research-reference cigarettes through the 25 mL of cell culture medium with the use of vacuum pump. Standardization of CSE preparation was performed by measurement of the absorbance of 1.25-100% CSE at 280 and 320 nm using the UV/VIS spectrophotometer (Cecil Aquarius CE 7200). PMN were isolated from blood of healthy volunteer and exposed to 4% CSE or cell culture medium (control). After incubation for different time periods (1, 4, 20 or 22 h), cytotoxicity of CSE was examined by measurement of LDH activity (Herbos Dijagnostika reagent; Trace 30 analyzer) in PMN supernatants. Statistical analysis was performed using the SigmaStat program.
Results: Mean absorbance of 100% CSE at 320 nm was 1.717 ± 0.386 ($N = 6$; $CV = 22.5\%$), while it was too high for measurement at 280 nm. Variations in mean absorbances for diluted CSE solutions were slightly higher than for 100% CSE at 320 nm. The catalytic activities of LDH measured in PMN supernatants exposed to 4% CSE were not statistically different when compared to untreated PMN for all tested time periods ($P = 0.086$).
Conclusions: In this work the method for CSE preparation was evaluated and no cytotoxic effects of low dose CSE on PMN was found. Standardization of CSE preparation is the basis for further research of the role of cigarette smoke in pathogenesis of diseases caused by cigarette smoking.

P19

GESTATION SPECIFIC REFERENCE INTERVALS FOR THYROID FUNCTION TESTS IN PREGNANCY

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Key Words: thyroid function tests, pregnancy
Background: Maternal thyroid dysfunction has been associated with a variety of adverse pregnancy outcomes such as intrauterin growth retardation and preterm delivery. Thyroid function tests are frequently assessed during pregnancy to evaluate thyroid dysfunction or to monitor pre-existing thyroid disease. However, using non-pregnant reference intervals can lead to misclassification of normal pregnant women. International guidelines recommended that institutions should calculate their own pregnancy-specific reference intervals for free thyroxine (FT4), free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH). The objective of the study was to establish gestation-specific reference intervals (GRI) for thyroid function tests in pregnant Turkish women and to compare with the non-pregnant women.
Materials and Methods: Serum samples were collected from 2460 pregnant women and 220 non-pregnant women, aged between 18–45 years with 945 (39%) in the first trimester, 1120 (45%) in the second trimester, and 395 (16%) in the third trimester. TSH, FT4 and FT3 were measured using the Chemiluminescent Microparticle Immunoassay on the Abbott Architect i2000SR analyzer.
Results: In our study, GRIs of TSH, FT4 and FT3 for first trimester pregnancies were 0.49–2.33 mIU/L, 10.30–18.11 pmol/L and 3.80–5.81 pmol/L, respectively. GRIs for second trimester pregnancies were 0.51–3.44 mIU/L, 10.15–18.15 pmol/L and 3.69–5.90 pmol/L. GRIs for third trimester pregnancies were 0.58–4.31 mIU/L, 9.85–17.89 pmol/L and 3.67–5.81 pmol/L. GRIs for TSH and FT4 were lower, but FT3 was higher from age matched non-pregnant control group ($p<0.005$).
Conclusions: During pregnancy, TSH levels showed an increasing trend from the first trimester to the third trimester, but no significant difference was observed in FT4 and FT3 levels with progression of gestation. Instead of using universal cut-off concentrations, our GRIs should help in the diagnosis and appropriate management of thyroid dysfunction during pregnancy which will prevent both maternal and fetal complications.

P20

REVIEW AND OPTIMIZATION OF ANALYTICAL QUALITY SPECIFICATIONS IN OUR LABORATORY

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Key Words: quality control, laboratory, total error, biological variation, quality specifications
Background: The aim of this study is to evaluate our quality specifications for twelve biochemical magnitudes during 24 months and compare them to quality specifications based on biological variation (BV) defined by SEQC (Sociedad Española de Bioquímica Clínica y Patología Molecular) in 2014.
Materials and methods: We used total error (TE%) data from our control internal/external results and a computer program to manage quality control named Unity Real Time®, both from BioRad Laboratories, from May 2013 to April 2015. The biochemical analyzer was Dimension Vista® 1500 (Siemens Healthcare). Our previous quality specifications proposals were from CLIA (Clinical Laboratory Improvement Act, United States), Spanish Consensus and those proportionate by SEQC in 2011, which are less relevant today.
Results: Uric acid and Triglycerides met optimum performance based on SEQC 2014 BV, which was 6 and 13 respectively. Total Cholesterol, LDL Cholesterol, Glucose, AST (aspartate aminotransferase), Lipase, Phosphate and GGT (gamma glutamyl transferase) met desirable performance which was 8.5, 11.9, 6.96, 16.69, 37.88, 10.2 and 22.2 respectively, and Cholesterol HDL met minimum performance which was 16.6. Calcium and Magnesium did not meet minimum performance provided by SEQC 2014, apart from the Spanish Consensus and CLIA specifications respectively. Twelve parameters were evaluated: 58.3% met current quality specifications (SEQC), 28.57% of this reached optimal performance, 57.14% desirable performance and 14.28% minimum; 33.33% kept the old specifications, desirable and minimum performance (75% y 25% respectively). The magnitudes that not reached the minimum were those with a small biological variability, being 16.6% of the total. The degree of compliance did not suffer significant variations during the period.
Conclusions: As a result of the study we have improved the quality of our analytical results, reaching mostly the quality specifications currently proposed.

P21

PREANALYTICAL ERRORS IN ERYTHROPOIETIN ASSAY REQUEST

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Key Words: erythropoietin, samples carry on ice, hospital phlebotomy centres, extrahospital phlebotomy centres
Background: It has been reported that serum samples clotted at room temperature (22°C to 28°C) causes a decrease in erythropoietin value. This is why the sample should be transported on ice and promptly separated. The objectives of this research were to compare the preanalytical errors in erythropoietin request from hospital and extrahospital phlebotomy centres, and to develop a strategy to improve this kind of sample extraction.
Materials and Methods: The study was conducted from January 2014 to December 2014. A total of 139 erythropoietin requests were received from the decentralized and the hospital phlebotomy centres. When an unsuitable sample was received, specific coded results were registered as test results to inform the physician that an error had occurred and a new specimen extraction was recommended. The preanalytical errors were identified by looking for these coded results in the laboratory data base. Analysis of data was carried out using STATA 13.
Results: Only 58% of erythropoietin request were reported without preanalytical error. The highest number of incidences occurred in extrahospital centres (81%). Fewer errors were observed when the sample drawing was carried out by the hospital personnel (33%), showing distinct preanalytical quality specifications. With regard to the type of error, the largest proportion of errors was due to failures of transporting process (93%), followed by hemolyzed samples (4%). The proportion of preanalytical errors in the extrahospital centres was 2.47 times more than in the hospital (95% CI: 1.79 to 3.39). This risk could be reduced 48.45% (95%: CI: 28.10 to 61.53%) if the sample extraction was carried out in the hospital.
Conclusions: The high incidence of preanalytical errors and variability between centres suggests that there is a need to standardize the drawing practice. An extraction of blood at the hospital phlebotomy centres is proposed for this test.

P22

QUALITY SPECIFICATIONS IN HAEMATOLOGY. A RETROSPECTIVE STUDY

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Key Words: quality specifications, haematological quality control, total error
Background: The aim of the study is to change 2011 quality specifications based on biological variation to the latest ones from 2014. A retrospective study was carried out to verify the performance of the new specifications in our IQC data and to evaluate the change.
Materials and Methods: The new quality specifications for the tests have been defined using BV data provided by SEQC (Sociedad Española de Química Clínica) in 2014. The previous used specifications were from different organizations with less relevance (2011 Spanish Consensus). A year IQC (ABX Minotrol Retic HORIBA®), from May 2013 to April 2014, were evaluated. The haematology analyzer was ABX Pentra DX120. We compare the quality control result using a simple 2x2 analysis table. This table shows comparison of total error (TE) values obtained from IQC against BV data references. We chose to apply the TE limits as quality goals because a single measurement is always affected by both random and systematic error.
Results: Leukocyte count, erythrocyte count, haemoglobin and hematocrit, MCH TE met minimum performance based on 2011 BV, which was 18.5, 6.5, 6.2 and 6.1 respectively. Calculated parameters such as MCHC, CVM, MCH met optimum, which was 7.3, 5.8 and 6.5 respectively. Platelet count did not meet any criteria, apart from the BV 25 of CLIA specifications. Some parameters such as leukocyte count, erythrocyte count and hemoglobin met desirable performance based on 2014 BV, which was 15.5, 4.5 and respectively. Calculated parameters such as Hematocrit, MCHC, CVM, MCH did not meet minimum, which was 5.9, 3.8 and 3.6 and 1.9 respectively, so none changes could be made. Platelet counts did not meet any criteria, apart from the BV 25 of CLIA specifications.
Conclusions: The only parameters which meet the new specifications were leukocyte count, erythrocyte count and haemoglobin.

P23

PROTECTIVE EFFECT OF NANO-ALBUMIN BOUND COPPER COMPLEX AGAINST PHOTOOXIDATIVE STRESS INDUCED BY ULTRAVIOLET RADIATION

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Key Words: ROS, photooxidative stress, ultraviolet radiation, albumin
Background: Ultraviolet B (UV-B) irradiation is known to initiate a photo-oxidative reaction by increasing reactive oxygen species (ROS). In the living cells, there are different enzymes and small molecular antioxidants to inhibit oxidative stress.
Materials and Methods: Nano-albumin bound copper amino acid complex (NA-C) as a novel antioxidant was synthesized and characterized by circular dichroism and dynamic light scattering. Additionally, its protective effect on human white blood cell (WBC) against UV-B (50 mJ/cm2) was investigated by cell viability assay.
Results: The circular dichroism data showed that the secondary structure of albumine in nanoparticle and NA-C forms are almost the same. According to dynamic light scattering data the average diameter for diluted NA-C was 150 nm and the Zeta potential was -38 mV in pH 6.46. Additionally, WBC viability assay clarified that the cell viability was decreased by UV-B irradiation, but in the presence of NA-C the UV-B irradiation did not change the cell viability any more.
Conclusions: Taken together our results suggest that NA-C could be an efficient antioxidant to protect the WBC against UV-B induced ROS.

P24

USING S-MONOVETTE® LOWER THE RATE OF HEMOLYSED SPECIMEN FROM A BELGIAN ACADEMIC EMERGENCY DEPARTMENT

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Key Words: blood specimen collection, hemolysis, preanalytical phase, catheter
Background: Optimal preanalytical work is a basic prerequisite for precise and conclusive laboratory diagnostics. Assays can be affected by hemolysis wich is the main cause of nonquality samples in clinical laboratories. High prevalence of hemolysis is especially observed in specimens received from the emergency department (ED), collected from intravenous catheters which lead to more hemolysis than needles. Few assays can particularly be affected by light hemolysis like bilirubin, aspartate/alanine aminotransferase, potassium, creatine kinase and lactate deshydrogenase.
Materials and Methods: We compared hemolytic indexes (HI) caused by venipuncture with vacuum systems Terumo Venosafe (n=1986 plasmas) and with manual aspiration techniques S-Monovette (n=1555 plamas) on samples collected in an academic ED. The HI were measured on Roche Cobas c8000 by photometrical measurement.
Results: Among the 1555 samples collected without vacuum system, 84.4% were non hemolysed with HI < 25mg/dL cell-free hemoglobin concentration) whereas only 76.3% collected with vacuum system (p100) affected 4.1% of Terumo's samples while only 0.9% were with SMonovette (p25) drop from 23.7% to 15.63% using S-Monovette.
Conclusion: S-Monovette blood collection system with aspiraton technique is effective to lower the risk of hemolysis in venous blood samples in ED. Second venipuncture for obtaining a suitable serum sample could be avoided in half cases. This is associated with lower financial costs, human resources and diagnostic delay. In the end, general quality of care is improved.

P25

HOW TO ASSESS QUALITY OF SAMPLE – AUTOMATED SAMPLE INTERFERENCE TESTING

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Key Words: sample integrity, sample interferences indices
Background: The accuracy of laboratory tests is higly dependent on sample integrity. Quality of sample is often compromised by presence of intereferents such as hemolysis, lipemia and icterus. Traditionally, presence of interferents is visually estimated. It's not confident, is technician dependent and time consuming. Later years, laboratory analyzers offer automatic detecting of interferents present in sample, known as HIL testing. The aim of this paper is to show our experience with benefits of instrumentally testing serum interferences and also automatically ordering of that testing.
Materials and methods: Comparison of working process before and after automated HIL testing implementation.
Results: During october 2012. we applied sample interferences indices saline protocol on our clinical chemistry analyzer Architect c 16000. The protocol involves measurement of the sample at multiple wavelenghts, following dilution with isotonic saline. Pre-programmed formulae for each index value use these absorbance readings to calculate the index value in the appropriate units for that interferent. Furthermore, we defined in LIS which analyte requires testing for one or more of these interferents, so that operators don't have to make orders for HIL testing, LIS alone order it from Architect. Results from the analyzer are sent to LIS and are available to clinical chemistry specialist for evaluation and decision making. We've made some algorithms for further management of samples and results if HIL indices are elevated.
Conclusions: After nearly 3 years of systematic approach, there are at least 3 benefits our laboratory experienced with automatic HIL testing: more reliable discovering of interferents present, less time consumption, more data for decision making during validation results in LIS. Of course, there is need for harmonisation of HIL indices among different manufacturers and for proffesional guidelines and reccommendations for management of unreliable samples and results.

P26

VERIFICATION OF LC-MS/MS METHOD FOR IMMUNOSUPRESSANTS THERAPEUTIC DRUG MONITORING ON THE EXAMPLE OF CYCLOSPORIN A

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Key Words: immunosupressants, therapeutic drug monitoring, liquid chromatography-tandem mass spectrometry
Background: Patient care after solid organ transplantation involves the continuous immunosupressants therapeutic drug monitoring (TDM) to prevent graft rejection and adverse effects of drugs. Liquid chromatography-tandem mass spectrometry is a a gold standard in TDM. Before routine use, it is necessary to verify the analitycal performance characteristics of method, which was the aim of this study.
Materials and Methods: Verification of online SPE LC-MS/MS method for immunosupressants therapeutic drug monitoring (Recipe GmbH, Germany) on the analytical platform Shimadzu UPLC NEXERA X2-LCMS-8040, was performed according to CLSI Protocol EP15-A2 for precision using Recipe ClinChek controls in three concentration levels:55.79 µg/L, 113.38 µg/L and 227.64 µg/L.Verification of bias was performed by participating in external quality assurance programme (Referenzinstitut für Bioanalytik, Deuchland, Survey for immunosuppressives). Limits of quantitation (LoQ) was determined according to CLSI Protocol EP17-A.Expanded measurement uncertainty was estimated from the components of measurement uncertainty.
Results: Precision profile for three concentration levels included: repeatability CV% 2.56, 3.07 and 3.38, within-run precision CV% 5.18, 7.29 and 5.99, and within-laboratory precision CV% 5.58, 7.71 and 6.60, respectively.Analysis of four samples from external quality assurance programme,deviation from -3.9% to -11.9% from group median were obtained. Linearity of the method was confirmed by 6-point calibration curve (r=0,99).Determined LoQ was 1.0 µg/L. Expanded measurement uncertainty (U,k=2) for three concentration levels was ±11.4%, ±15.7% and ±13.4%, respectively.
Conclusions: The values obtained for precision are within goal for liquid chromatography-mass spectrometry methods according to CLSI C62-A (CV ≤ 15%). By participating in external quality assurance programme we have achived results that are within required criteria (deviation ≤ 30%). Values for expanded measurement uncertainty are within desirable specification for allowable total error based on biological variation (≤ 15%). Verification of the method has confirmed that all set criteria are achieved, therefore this method is suitability for routine use in clinical laboratory.

P27

ESTIMATION OF MEASUREMENT UNCERTAINTIES OF SERUM ELECTROLYTES BY TOP-DOWN METHOD

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Key Words: measurement uncertainty (MU), top-down approach, electrolytes, serum, direct ISE
Background: The measurement uncertainty (MU) is defined as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand. Estimating and reporting the UM is required for laboratories accredited to ISO/IEC 17025 and ISO/IEC 15189, but there are many papers about the method performance data in evaluation MU. The aim of this study was to demonstrate MU evaluation of three serum electrolytes, using the top-down approach with a main goal to improve the quality of the test.
Materials and methods: Top-down approach was used as a method for estimation of MU for serum sodium, potassium and chlorides measured by direct ISE, on electrolyte analyzer Humalyte Plus5. Reagents and control material were from Human Diagnostics. Data from internal quality control and EQUAS were used in: estimation of$\%u_{rw}$ from intralab imprecision (reproducibility and repeatability) as well as calculation of$\%u_{bias}$, combined uncertainty$\%u = (\%u^2_{rw} + \%u^2_{bias})^{1/2}$ and expanded UM (with a 95% confidence).
Results: Results were similar for both control levels, here higher values are presented. These are: for sodium$\%u_{rw}$ =3.1, %u=3.9 and UM (mmol/l)=9; for potassium$\%u_{rw}$ =4.4, %u=5.5 and UM (mmol/l)=0.4 and for chlorides$\%u_{rw}$ =5.0, %u=7.8 and UM (mmol/l)=14.
Conclusions: In cases where no demands have been published, a guiding principle that was accepted in this study could be that expanded MU should be approximately equal to, or less than 2 times the reproducibility. Our results showed just a little bit higher MU. So, the main goal that arose from the study is to reduce the uncertainty by using higher quality calibrators, checking the measurements by repeating them and better control of the analytical process.

P28

EFFECT OF MEASUREMENT UNCERTAINTY ON CLINICAL BIOCHEMISTRY CRITICAL TEST VALUES

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Key Words: measurement uncertainty, critical values, patient safety
Background: Measurement uncertainty characterises the dispersion of the results that could be reasonably attributed to the measure. Critical value reporting is crucial for patient safety and a requirement for effective patient treatment. Adding uncertainty to the measurement may cause it to become a critical value. It was aimed to investigate the effect of measurement uncertainty on clinical biochemistry critical test values.
Materials and Methods: Critical test results of patients (total critical test number/total test number =18,642/1,047,837) were evaluated for a period of 4 months. 16 tests of clinical chemistry, 4 tests of hematology and 3 tests of coagulation group were accepted as critical values. Expanded uncertainty values were determined for all critical tests. Critical values were evaluated before and after adding measurement uncertainty of tests. Intra-laboratory repeatability, external quality control values, uncertainty of the certificated reference materials and calibrators were used for calculating uncertainty.
Results: Critical values were evaluated upon whether uncertainty of the tests would change the results toward to critical value. 16% of all patient results (2972/18642) were changed to critical after adding uncertainty values. Among the other groups, clinical chemistry group had the highest ratio of this change as 2065/2972 (70%). Especially electrolites, magnesium, calcium levels were changed to critical values. Hematology group's ratio was 285/2972 (10%) and coagulation group's ratio was 622/2972 (20%). WBC and platelet parameters of hematology and prothrombin time of coagulation group were found as the most changing tests.
Conclusions: Uncertainty could be attributed to various sources in clinical biochemistry analysis from calibration to various analytical indicators. The extent of uncertainty of each test may affect the test results and change them to critical value. Reporting results with the measurement uncertainty will provide better care for patients and contribute to patient safety.

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ASSESSMENT OF ANALYTICAL RELIABILITIES AND CALCULATING THE SIGMA-METRICS OF HbA1c ANALYSİS

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Key Words: sigma-metric, HbA1c, analytical quality assessment
Background: Different evaluation procedures were available for assessment of analytical qualities of tests. Calculating Sigma- metrics is a reliable procedure in laboratory quality management and includes both imprecision and bias data. In this study, we aimed to assess the analytical performance characteristics and calculate sigma-metrics according to various analytical quality goals for the two HbA1c analyzers.
Materials and Methods: In this study, two level control materials (low and high) were used in Bio-Rad Variant IITurbo and Trinity BioTECK Premier H69210 HbA1c analyzers. Internal quality control results were evaluated for one month in the first analyzer and one year period in the second analyzer. Imprecision, bias, total error and sigma metrics were calculated. Quality requirement goals were achieved from Rilibak, CAP/NGSP PT 2014 and Ricos et al. biologic database.
Results: Sigma metrics were found to differ according to various quality requirement goals. Imprecision, bias, total error were found 2.5%, 7.7%,12.7%(level1); 2.88%, 0.08%,5.84%(level2) respectively and Sigma metrics according to CAP/NGSP PT goal were found 2.05 at the level of 9.7% on Bio-Rad Variant analyzer and 1.15%, 0.8%, 3.11% (level1);0.84%,1.23%,2.91% (level2) and Sigma metrics were found 4.9 at the level of 5.7%,6.4 at the level of 10.6% on Premier HbA1c analyzer.
Conclusions: It's necessary to reach a consensus both nationally and internationally, which the quality requirement and achievable goals should be regarded, for level of sigma metric should be aimed in quality journey for routine laboratories. Laboratory managers should choose the methods and analyzers that have better analytical performances and have above 2σ level to strengthen their laboratory control systems.

P30

WHICH METHOD ENHANCES ACCURACY OF QUICK PARATHYROID HORMONE IN PREDICTING POSTTHYROIDECTOMY HYPOCALCEMIA?

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Key Words: parathyroid hormone, total thyroidectomy, postoperative hypoparathyroidism, prophylactic calcium therapy
Background: Hypoparatiroidism leading to hypocalcemia is the main complication of total thyroidectomy. Many authors suggest to test postoperative quick parathyroid hormone (qPTH) in attempt to select patients needing prophylactic calcium therapy. A cornerstone of this strategy is to use a reliable method to measure qPTH. The aim of our study is to analyse the agreement of two different methods in selecting patients with low qPTH at risk to develop hypocalcemia.
Materials and Methods: From January to June 2015 we collected blood samples taken at the skin closure after total thyroidectomy and we checked qPTH by two different methods for each patient. qPTH was determined using: Abbott ® Architect Method CMIA, Intra-assay CV,% 4.1-9, Antibodies used polyclonal goat Healthy reference range 15-68 pg/mL; N-tact Liaison PTH Diasorin® Architect Method CLIA, Intra-assay CV,% 3.9-6.1, Antibodies used polyclonal goat, healthy reference range 14.5-87.1 pg/mL. Statistical analysis: data were described as number, percentage and 95% confidence interval.
Results: We analysed 25 patients, 7 (28%) male, median age 54 years (24-77). In 15 patients both the methods were under the normal range, without a clear dominance of one over the other. We had 3 patients (12%, 95% CI 3-31%) in normal range for Architect but considered as ipoPTH for Liaison. In the remaining 7 patients (28%) over the lower normal value, Architect always overestimates Liaison, being 40% (± 11) greater than Liaison.
Conclusions: These two methods analysing qPTH are not always in agreement. Therefore, before to transfer in clinical setting the indication to treat patients with low postthyroidectomy qPTH, it is compulsory to identify which method is most reliable in predicting postoperative hypocalcemia.

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WHEN LINEAR REGRESSION IS NOT ENOUGH: AMMONIA METHODS COMPARISON

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Key Words: method comparison, linear regression, ROC curve, plasma ammonia
Background: Ammonia is a waste product of protein catabolism and is synthesized within all organs. Under normal conditions, ammonia is metabolized to urea by liver enzymes. Several diseases, both inherited and acquired, cause elevated ammonia. Increased plasma ammonia may be indicative of hepatic encephalopathy, hepatic coma in terminal stages of liver cirrhosis, hepatic failure, acute and subacute liver necrosis, and Reyeís syndrome. Hyperammonemia may also be found with increasing dietary protein intake. Monitor of plasma ammonia levels in patient with liver diseases or renal failure is mandatory, because of it is potentially toxicity to the central nervous system.
Materials and Methods: Plasma ammonia is measured by either chemical or enzymatic methods. In our work we compared two methods for plasma ammonia determination: colorimetric (dry chemistry) method where ammonium ions react with bromphenol blue, and an enzymatic method that uses glutamate dehydrogenase and NADPH. We collected 99 plasma samples, after being routinely analysed for ammonia ion concentration with the routinely used dry chemistry method (Vitros350, Ortho Clinical Diagnostics), and immediately analysed on the wet chemistry analyser (Advia1800, Siemens). Results were statistically reviewed with linear regression analysis and ROC curve evaluation.
Results: Linear regression analysis showed acceptable correlation between methods (R=0,944, P<0.0001). Because of methods different analytical range (1.7-851.5 µmol/L for Vitros350 method, and 5.9-767.0 µmol/L for Advia1800 method) we presented the results as ROC curve, where we find a 97.7% sensitivity and 87.3% specificity. We checked external laboratory controls, where dry chemistry results were lower then other methods; our results were in agreement with this finding.
Conclusions: CLSI guidelines for laboratory method comparison suggest 0.975 correlation value to describe methods as comparable. In our study we gained a lower but acceptable correlation. For routine use of Advia1800 enzymatic method we should verify the method according to the national guidelines.

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ASSESSING QUALITY MEASUREMENT OF THYROGLOBULIN IN SERUM

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Key Words: thyroglobulin, recovery test, dilution, interferences
Background: The immunoassay measurement of serum thyroglobulin (TG) plays a role in the diagnosis and monitoring of thyroid disorders. Antibodies against thyroglobulin (aTG) can contribute to the false measurement of TG, but other interferences are possible. To verify a TG measurement a parallel TG-recovery test (TG-rec) is done. Its value outside the range of 70-130% indicates interferences in the sample. There are no guidelines how proceed the analysis of TG when TG-rec is outside the range. Therefore, our aim was to assess the protocol implying a dilution of serum sample to minimize interferences in order to report a valid TG result.
Materials and methods: In 8-months period 28 samples were collected, 27 samples with TG-recovery result <70% and one with >130%. For all sera, TG and TG-recovery measurements were performed in dilution 1:5, and, with respect to available sample volume, also in dilutions 1:10 (n=17) and 1:100 (n=15).TG measurement (ng/mL) and TG-rec (%) were performed using Kryptor CompactPlus (Brahms) analyzer, and aTG measurement (kE/L) using AdviaCentaur (Siemens) analyzer. Statistical analysis was performed using Wilcoxon test.
Results: Compared to undiluted sera, TG results of 1:5 diluted sera were not significantly different (median, 113.2 ng/mL and 121.8 ng/mL, respectively, P=0.122), whereas TG-rec significantly improved from 63 to 80.3% (P<0.001). Further improvement of TG-rec was observed in dilutions 1:10 and 1:100 (87%, P<0.001 and 92%, P=0.013, respectively), but TG results in dilution 1:10 decreased (P=0.008), and in 1:100 dilution increased (P=0.015). Results showed that aTG do not importantly contribute to interferences in determination of TG-recovery (63% samples had aTG<15kE/L, r=0.06).
Conclusions: Our results showed that serum dilution 1:5 efficiently lowers impact of interferences and a valid TG result in this serum dilution can readily be obtained. Consequently, for TG-rec outside the range 70–130%, a serum dilution 1:5 was introduced into practice.

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THE TRANSFERRIN ISOFORM ANALYSIS INTERFERENCE STUDY

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Key Words: CDGs, transferrin isoforms, capillary electrophoresis, HPLC
Background: The congenital disorders of glycosylation (CDG) were originally called carbohydrate-deficient glycoprotein syndromes, affecting primarily N-glycans. In CDG, serum glycoproteins have altered glycosylation. Transferrin is reliable, sensitive, and simplest indicator. Therefore the current diagnostic test for CDG is analysis of serum transferrin isoforms. Transferrin glycoforms can be identified by using isoelectric focusing electrophoresis (IEF), high performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE) and by mass spectrometric analysis. Our aim was to compare HPLC and two different CZE methods' performances in presence of hemolysis, bilirubinemia, and lipemia interferences according to determination of percentage of transferrin isoforms.
Materials and Methods: Interference samples were prepared for biliribunemia (total bilirubin concentrations: 11.628–377.91 µmol/L), lipemia (trigliseride concentrations: 1.08–33.4 mmol/L) and hemolysis (hemoglobine concentrations: 0.0 g/L to 5.0 g/L). HPLC analysis was performed on a gradient HPLC (Shimadzu Europe, Germany), using column and reagents provided in kit (EUREKA-CDT test in serum by UV/VIS-FAST) with spectrophotometric detection at 470 nm. CZE analyses carried out with The Helena Biosciences' V8® E-class analyser (Helena Biosciences Europe, UK) and with Sebia 2 Capillarys™ (Capillarys™, Sebia, France). Statistical calculations were performed using Microsoft Excel 2010.
Results: All isoforms percentage were compared with their zero concentrations in all samples. We observed that Helena CZE system were affected by samples with hemolysis. Analysis with HPLC system were affected by samples with lipemia (except tetrasialo transferrin). CZE systems have been influenced minimal by lipemia.We observed Helena CZE and HPLC systems had significant interferences with all concentrations of bilirubinemia (except tetrasialo transferrin).
Conclusions: We demonstrated some interferences which have not been reported previously about transferrin isoform analysis. The conscious implementation for interferences on these methods would provide more accurate results for CDG diagnosis.

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DOES RE-CENTRIFUGATION OF GEL-SEPARATOR TUBES AFFECT METABOLIC PANEL ANALYTES' DETERMINATION?

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Key Words: re-centrifugation, gel-separator tubes
Background: Clinical Laboratory Standards Institute (CLSI) guidelines and manufacturers do not recommend the re-centrifugation of gel tubes. Re-centrifugation can affect measurement of metabolic panel analytes, especially potassium, leading to potentially erroneous results. Our aim was to investigate the effect of short-term re-centrifugation of gel-separator tubes on metabolic panel analytes.
Materials and Methods: Samples from 40 participants were collected in gel-separator tubes, mixed immediately by inverting 5-10 times and stored at room temperature for 30 minutes allowing complete clot formation. Immediately after centrifugation at 2000xg/10 minutes/20°C baseline glucose, urea, creatinine, potassium, sodium, chloride and calcium concentrations were measured. Tubes were recapped and stored at room temperature for 4 hours. After storage, serum was aliquoted; original tubes and aliquots were re-centrifuged under the same conditions. Metabolic panel analytes concentrations measured in original tubes and aliquots after re-centrifugation were compared to respective baseline concentrations. The Kruskall-Wallis test was used for statistical comparisons. The level of statistical significance was set at $P<0.05$. Mean absolute bias from baseline concentrations was compared to desirable specifications for total error to define clinically relevant variations.
Results: Baseline concentrations of metabolic analytes were: glucose 6.0 (5.5–7.0) mmol/L, urea 6.4 (4.7-7.8) mmol/L, creatinine 67 (56-77) µmol/L, potassium 4.3 (4.0-4.5) mmol/L, sodium 138 (134-140) mmol/L, chloride 104 (100-105) mmol/L and calcium 2.21 (2.11-2.36) mmol/L. No significant differences were found comparing baseline concentrations of all analytes tested to concentrations measured in original tubes and aliquots after re-centrifugation ($P>0.05$). Mean absolute biases for all analytes tested in original tubes and aliquots after short term re-centrifugation did not exceed the set criteria for clinical significance.
Conclusions: No statistically or clinically significant differences were observed comparing baseline concentrations of metabolic panel analytes to concentrations measured in original gel-separator tubes and aliquots stored at room temperature and re-centrifuged 4 hours after collection.

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DOUBLE REFERENCE RANGES FOR CEA SMOKERS AND NON-SMOKERS; TRUE OR FALSE?

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Key Words: carcinoembryonic antigen, reference ranges, smokers, non-smokers
Background: Carcinoembryonic antigen (CEA) belongs to the group of carcinofetal antigens that are produced during embryonic and fetal period. CEA is found in the fetal gastrointestinal tract, in the fetal serum and in slight quantities in intestinal, pancreatic, and hepatic tissue of healthy adults (slight to moderate CEA elevations occur in benign diseases of mentioned tissues). Smokers have slightly increased CEA values but high concentrations are found in colorectal adenocarcinoma.
Materials and Methods: From all 50 healthy subjects (25 smokers and 25 non-smokers); median of age 38 with range 19–63 years; 33 women and 17 men, were taken venous (6 ml Vacuette; red cap; 21G needle) blood samples according to standards of good laboratory practice. Materials were from Greiner (Greiner Bio-One, Austria) and measurements were performed within 4 hours of blood sample collection. CEA was determinated on immunoassay analyzer Cobas e411(Roche Diagnostics, Germany) with electrochemiluminscence method, original reagent, calibrators and controls. Serum CEA level >5.0 ng/mL was defined as CEA-positive for non-smokers and >6.5 ng/mL for smokers according to the manufacturer's instructions with lower detection limit 0.20 ng/mL. Statistical analyses of descriptive analysis, correlation and Welch's corrected unpaired t-test were performed using statistical software MedCalc version 10.4.
Results: Data were normally distributed and Welch's t-test presented significant difference between smoker and non-smoker samples (P=0.021). CEA median for smokers was 2.43 (95% CI=1.72-3.14) and for non-smokers 1.38 (95% CI=0.83-1.94), with correlation coefficient r=0.10 (P=0.620).
Conclusions: There is relevant difference and no significant correlation between CEA in healthy smokers and non-smokers. Our data suggest a slight elevation of CEA in smokers according to non-smokers, but all tested samples are within declared manufacturer's expected values for non-smokers. Our study leaves question of necessity for double CEA expected values. Further investigation is needed to determine single or double reference range for CEA.

P36

THE VALIDATION OF INSTRUMENTATION LABORATORY ACL TOP 300 AND ACL TOP 500 COAGULATION ANALYZERS

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Key Words: precision, trueness, Passing-Bablock estimation, instrument validation
Introduction: Novel Instrumentation Laboratory (Bedford, USA) ACL-TOP 300 and 500 coagulation analyzers were simultaneously validated in order to assess their routine work applicability.
Materials and Methods: Original HemosIL reagents: PT (RecombiPlasTin 2G), APTT (SynthASil), fibrinogen (Fibrinogen-C), AT (Liquid Antithrombin) and D-dimer (D-Dimer HS 500) together with Normal and Low Control Assayed (PT, APTT, fibrinogen, AT) and Low and High Control (D-dimer) were used. Precision study results (within-run: 10 measurements; between-run: 10 days, 2 measurements) and trueness were estimated according to Westgard criteria. Method comparison (Passing-Bablock analysis, n=30 for each analyte, wide range of concentrations) with referent methods was performed using Innovin (PT), Actin FS (APTT), Berichrom Antithrombin III (A) (AT), Multifibren U (fibrinogen) on BCS XP (Siemens Healthcare Diagnostics, Germany) and Vidas D-Dimer Exclusion II (miniVIDAS, bioMérieux, France).
Results: Higher within-run precision coefficients of variation (CVs) were found for low fibrinogen (6.6%) and AT (6.1%) concentrations on ACL-TOP 300, and for AT (6.8%) on ACL-TOP 500. Between-run precision study on ACL-TOP 300 revealed slightly higher CVs for low PT (2.9%) and normal AT concentration (3.4%), and especially for low AT concentration (7.8%). Elevated biases were found for normal fibrinogen concentrations on both analyzers (8.2 and 7.6, respectively) and for low AT concentration on ACL-TOP 300 (13.1%). Method comparison criteria were fulfilled only for: APTT on ACL-TOP 300 (intercept -9.52, 95% CI -24.35, 0.44; slope 1.22, 95% CI 0.9, 1.68; r=0.775) and D-dimer on ACL-TOP 300 (intercept 0.05, 95% CI -0.04, 0.08; slope 0.87, 95% CI 0.78, 1.05; r=0.956). Comparison of results obtained on ACL-TOP analyzers was excellent for all analysis (r>0.970, P<0.0001).
Conclusion: ACL-TOP 500 was found to be superior to ACL-TOP 300 for all analysis. As precision and trueness results indicate important disagreements for low AT concentration on ACL-TOP 300, we find it less applicable for routine work than ACL-TOP 500.

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VALIDATION OF URINARY TOTAL GLYCOSAMINOGLYCANS FOR SCREENING OF MUCOPOLYSACCHARIDOSES IN TURKISH POPULATION

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Key Words: validation, reference intervals, glycosaminoglycans, DMB, Turkish population
Background: Detection of excessive glycosaminoglycans (GAG) excretion in the urine with 1,9-dimethyleneblue test (DMB) is used as a screening/monitoring test for mucopolysaccharidoses (MPSs). Validation of DMB and reference intervals of GAG in Turkish population were presented.
Materials and Methods: Validation was performed according to CLSI guideline. Precision (EP05), linearity and recovery (EP06), trueness (EP09), limit of detection (EP17), stability (EP25), ROC analysis (GP10) and reference intervals (C28) were determined. Statistical analyses were performed with SPSS software v.21.0. Reference intervals depending on ages were were determined in 817 urine samples of healthy volunteers. Distribution normality and extreme outliners were tested by D'Agostino Pearson's and Tukey method, respectively.
Results: Calibration plot is linear up to 24 µg standard of chondrotin 6-sulphate (R^2 = 97.5%). Within-day precision was 99% (ICC) and 5.58% (CV); inter-day precision was 99.5% (ICC) and 10.95% (CV). Mean recovery was 100% ±1.05 (SD). The limit of detection was 1.99 µg (CV 0.86%). The ICC of stability analyses for 15 days were as follows: room temperature: 99.6%, +4°C: 99.1%, -20°C: 97.5%. Using a cut-off level 92.51 mg/g creatinine, sensitivity and specifity were 93.1% and 55.3%, respectively. The AUC was 74.9% (p<0.001) and PPV, NPV, positive LR and negative LR were 6.4%, 99.6%, 2.1 and 0.1, respectively. Reference intervals in females following males of different age groups were; 0-3 month (< 289; < 282), 4-6 month (<260; <282), 7-12 month (<278; <234), 1-2 age (<171; <249), 2-13 age (<109; <120), >13 age (<102; <101) (mg/g creatinine). Our results agree with the ERNDIM quality scheme.
Conclusions: The diagnostic validity and age-related highest reference values for GAGs with DMB were determined in Turkish population. This first-line screening test could be used for early diagnosis and monitoring of MPSs in Turkish population that has a high incidence.

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COMPARISON OF DIFFERENT SPECIMENS FOR FT4 AND FT3 VERIFICATION: COMMERCIAL CONTROL VS. PATIENT SAMPLE

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Keywords: within-run imprecision, between-run imprecision, commercial controls, patient samples
Background: Precision is the most important component of the quality of laboratory results and precision check is one of the requirements for quality control and accreditation of laboratory. Guidelines for method verification recommend the use of control samples, calibrators or previously analyzed patient samples for precision check. The aim of this study was to compare parameters of precision for free thyroxine (FT4) and free triiodothyronine (FT3) obtained by analyzing internal quality control samples and pool of previously analyzed patient specimens on Unicel DxI600 (Beckman Coulter, Tokyo, Japan).
Materials and Methods: The methods verification was done using control samples BioRad Lypocheck Immunoassay Plus Control (Biorad Laboratories, Marnes-la-Coquette, France) and pool of previously analyzed patient serums, both with high and low levels of analyzed hormones. Samples were tested 10 times in the series (within-run imprecision) and every day in duplicate during 10 days (between-run imprecision). The results are expressed as coefficients of variations (CV) and compared with values provided by manufacturer and by Westgard desirable specifications for imprecision. CV was calculated using Microsoft office Excel 2007 software while the comparison of CV values between control samples and patient samples was performed using MedCalc 12.4.0.0. software (MedCalc, Mariakerke, Belgium). P<0.05 was set as level of significance.
Results: The results of within-run imprecision and between-run imprecision analyzed by commercial control samples and by patient samples for both tests were in accordance with specifications provided by manufacturer but not within desirable range according to Westgard. We did not found statistically significant difference in CV values between control samples and patient specimens (t-test: P=0.827).
Conclusions: Patient samples and commercial control samples are equally suitable for imprecision testing of FT3 and FT4. Although simpler and safer procedure is provided by using control materials, using of patient samples is a cheaper way for precision checking.

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DESIGNING AN ANALYTICAL QUALITY SYSTEM IN THE CLINICAL LABORATORY

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Key Words: quality control, six sigma, error
Background: The clinical laboratory objective is to provide useful information for screening, diagnosis and monitoring of disease. Within the analytical process, as well as pre-analytical and post-analytical phases, the laboratory must ensure the quality of it, based on some requirements of the same. In this way, the laboratory develops and implements a system of internal quality control which serves to detect errors displayed in the analytical laboratory process. In addition to comparing our control data with those of other laboratories, through an external quality control. So, we have a tool for the detection of compliance with the objectives set, which allows us to implement corrective actions, and ensure the reliability of the results, in the case of error detection during the process.
Materials and Methods: 28 magnitudes serum biochemistry, urine 12 and 15 immunochemical serum were evaluated using three different control materials. With the information obtained, an operating procedure for the development of an internal quality system was planned, following these steps: Select the control material, characteristics evaluate of a measurement procedure in a stability situation, performance calculation procedure measurement, definition of quality specifications, Sigma value calculation, Choice of operating control rules and Verification of compliance with specifications.
Conclusions: The internal quality control function is the acceptance or rejection analytical series. This should be designed to detect the presence of random or systematic errors that compromise the stability of the analytical system. In our case we observed a systematic error in alanine aminotransferase (ALT) to compare ourselves with our peer, group mean and standard deviation, detecting it as a problem arising from the reagent lot change. This systematic error was not detected with the quality objective charts where we meet predetermined specifications therefore system stability is maintained. However in the external quality control we checked the error.

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COMPARISON OF PT, APTT, FIBRINOGEN, ANTITHROMBIN AND D-DIMERS ON TWO AUTOMATED COAGULATION PLATFORMS (IL TOP500 AND SIEMENS BCS XP)

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Key Words: coagulation, method comparison, PT, APTT, fibrinogen, antithrombin, D-dimer
Background: Due to the lack of standardisation in coagulation testing, the comparability of results between diferent coagulation analysers/reagents is poor. The goal of this study is to compare the results of emergency coagulation tests obtained by IL TOP 500/HemosIL reagents and Siemens BCS XP/Siemens reagents in order to estimate their potential alternating use.
Materials and Methods: PT%, APTT, fibrinogen, antithrombin (AT) and D-dimers where determinated in 98,64,71,34,54 routine patient plasma samples. Analyses were performed on automated coagulometer Siemens BCS XP with reagents Innovin, Actin FS, Multifibren, Innovance AT, Innovance D-dimer and on automated coagulometer IL ACL TOP500 with reagents RecombiPlas Tin2G, SynthASil, Fibrinogen-C, D-Dimer HS500, Liquid Antithrombin. The results were statistically analyzed using MedCalc Statistical Software demo version 14.12.0 with Passing –Bablok regression and Bland-Altman plot.
Results: The regresion equation is established as follows: PT% equation is y=0,0745385+0,953846x with 95% confidence interval (95% CI) for intercept A 0,04000 to 0,09500 and 95% CI for slope B 0,9091 to 1,0000; APTT equation is y=0,176436+0,883037x with 95% CI for intercept A 0,06000 to 0,2925 and 95% CI for slope B 0,7500 to 1,0000; fibrinogen equation is y=0,809901+0,598020x with 95% CI for intercept A 0,6345 to 1,0329 and 95% CI for slope B 0,5429 to 0,6466; AT equation is y=-0,0662198+1,127016x with 95% CI for intercept A -0,4025 to 0,1646 and 95% CI for slope B 0,8800 to 1,5000; D-dimers equation is y=-37,533848+1,034584x with 95% CI for intercept A -94,7246 to 11,3844 and 95% CI for slope B 0,9621 to 1,1050.
Conclusions: There is a constant difference between PT and APTT, as well as constant and proportional difference between the fibrinogen results. There are neither constant nor proportional differences between the D-dimer and AT results. Only D-dimer and AT can be used interchangably within these two coagulation systems.

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VERIFICATION OF CHROMOGRANIN A TEST

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Keywords: chromogranin A, imprecision, bias, total error
Background: Chromogranin A is a biomarker for neuroendocrine tumors that correlates with tumor mass and has its use as a prognostic marker as well. Therefore, as an important marker, chromogranin A has to have excellent analytical performance. The aim of our study was to investigate if the ELISA test for chromogranin A (Demeditec Diagnostics GmbH, Germany) met the desirable criteria according to biological variation.
Materials and Methods: We evaluated our method according to CLSI EP15 A2: User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. For assessment of precision and trueness we tested two levels of commercial control material provided by the manufacturer: control1 100µg/L(±40%) and control2 300µg/L (±40%). We tested the controls in triplicate for five serial testings in a row from 13 th of June 2014- 15th of December 2014. We compared our results to Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biological variation for chromogranin A that are declared as: imprecision 6.4%, Bias 7.3%, TE 17.9%
Results: The verification procedure for our test gained an average CV of 6.23% (5.75% for control 1 and 6.83% for control 2), average Bias of 1.64% (-1.38% for control 1 and -1.90% for control 2), and total error of 13.97%.
Conclusions: Considering that we gained lower CV (6,23% vs. 6,4%), Bias (1,64% vs. 7,3%) and total error (13,97% vs. 17,9%) we have concluded that our verification results met the desirable specifications derived from biological variation for imprecision, bias and total error, so Demeditec ELISA test for chromogranin A could be used routinely in a laboratory work.

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IMPORTANCE OF OPTIMIZATION EXPERIMENTS IN ELECTROCHEMICAL BASED DNA/RNA BIOSENSORS

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Key Words: optimization, electrochemical biosensors, hybridization
Background: Advancements in the field of nano-biotechnology bring new aspects to the biosensor applications. As much as new sensing systems are being developed, a great deal of effort is spent for the integration of biosensor system within an efficient detection strategy to deal with for the real sample analysis. In order to achieve sensitive detection, experimental parameters such as probe, target and drug concentrations, hybridization, immobilization and interaction times, ionic strength and surface activation conditions should be optimized in detailed.In the presentation, the optimization methodologies for the sensitive detection of target DNA with electrochemical techniques will be discussed.
Materials and Methods: The electrochemical signals were measured with Differential Pulse Voltammetry. The signals obtained from different oligonucleotides were compared for the analysis of DNA with three electrode systems.
Results: In order to provide best hybridization, experimental parameters were optimized. Detection of target analyte was detected by the decrease of the probe signals after interaction with its complementary target due to all guanines in the probe were partly closed to oxidation after the hybridization. The detection limits (S/N = 3) were calculated as pmol range of target sequences in a µL reaction volume. The stability is characterized by a relative standard deviation (RSD) of the peak current signals obtained from 3 measurements where the RSD values are less than10% for different days.
Conclusions: Electrochemical biosensors meet the sensitivity requirements with its picomolar detection limit in real samples with respect to optimization studies. In the case of clinical specimens, the challenge of sample preparation and detection of biomarkers within these samples can be reduced by optimizating the each studied parameter.

P43

AN EXAMPLE OF ESTIMATING REFERENCE RANGE FROM HOSPITAL DATABASE: REFERENCE RANGE OF ALKALINE PHOSPHATASE (ALP) FOR PEDIATRIC POPULATION

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Background: The aim of this study is to establish indirect reference intervals for ALP from pediatric patient results obtained during routine laboratory work as an alternative to laborious and expensive producing of their own reference range values according to international instructions.
Materials and Methods: ALP levels were analyzed by Abbott ARCHITECT c16000 autoanalyzer. According to manufacturer, reference range of ALP was <500 for 1-12 years of age and <750 for 12-15 years of age. All ALP results of 0 to 18 years of age patients (n=11284) that were stored in our laboratory information system (LIS) between May 2013-May 2015 were included in this study. After logarithmic transformation of raw data, outliers were excluded (n=109). Non-parametric reference intervals were estimated statistically after visual observation of the distribution using stem-and-leaf plots and histograms. A standard normal deviation test was performed to test the significance of differences between sub-groups.
Results: Total number of included results were 11172. As 50.7% of the group was male, 49.3% was female. There was no significant difference in serum ALP concentrations between male and female participants ($p<0.01$). Indirect reference values were 118.3-347.5 U/L for total pediatric population, 113.8-344.2 U/L for females under the age of 18, 123.2-347.9 U/L for male population under the age of 18. Subgroups were also established according to age.
Conclusions: Establishing reference interval for ALP is very crucial as decreased levels of ALP is as important as increased levels of ALP during childhood. Using patient laboratory data values is a relatively easy and cheap method of establishing laboratory-specific reference values if skewness and kurtosis of the distribution are not too large.

P44

CALCULATION OF MEASUREMENT UNCERTAINTY OF ROUTINE BIOCHEMICAL PARAMETERS

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Key Words: measurement uncertainty, clinical chemistry, emergency laboratory, CLIA
Background: Measurement uncertainty is a functional parameter which shows the deviation of measurement results from real values and reflects the quality and performance of the measurement method. The aim of this study is to calculate measurement uncertainties of 20 parameters from two identical model autoanalyzers and compare with total allowable error values of CLIA and Fraser.
Materials and Methods: Measurement uncertainties of 20 routine biochemical parameters which are measured by two Abbott ARCHITECT c16000 autoanalyzers in Konya N.E.U. Meram Faculty of Medicine Hospital Biochemistry Laboratory were calculated by a "top-down" approach. Components of uncertainty were determined as uncertainty on the within-lab reproducibility (uRw) and measurement uncertainty on the bias (u_{bias}). u_{bias} was calculated with uncertainty related to the reference value of CRM (uC_{ref}) and uncertainty of external quality assessment results (u_{EQA}). Using internal and external quality control results of last 12 months together with calibrator information, expanded measurement uncertainties were calculated. For calculations and statistical studies, Microsoft Office Excel 2013 was utilized.
Results: Expanded measurement uncertainties (U) with 95% confidence interval were calculated. U values were compared with total allowable error (TEa%) values of CLIA and Fraser. TEa (%) values of all parameters from both devices were not found to be higher than TEa% values.
Conclusions: As all of the calculated measurement uncertainties were found to be within allowable limits, quality of the results given from our laboratory satisfies our expectations. This practical application should be repeated periodically in order to track quality of results.

P45

EVALUATION OF BIOLOGICAL SPECIMEN REJECTION RATE IN A UNIVERSITY HOSPITAL BIOCHEMISTRY LABORATORY

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Key Words: biological specimen rejection, biochemistry laboratory, quality assessment, specimen rejection rate
Background: Determining specimen rejection rate (SRR) is an important analysis of pre-analytical phase performance of the laboratory letting laboratory management to improve patient care and reduce unnecessary sample recollections, redraws and unwanted increases in turnaround time. The aim of this study was to determine SRR and analyze the details to reduce SRR in the long run.
Materials and Methods: A retrospective study was carried out by determining the specimen rejection rate (SRR) of routine biological samples received at Konya Necmettin Erbakan University Medical Faculty Hospital Biochemistry Laboratory in 2014 May-2015 May period. The rejected biological samples were categorized for the reason of rejection.
Results: A total number of 852,831 biological specimens were collected during May 2014-May 2015 period and SRR was calculated as 1,1% (n: 9381). Reasons of specimen rejection was analyzed and it was found that improper specimen (52.1%), inappropriate volume (22.9%), incorrect request (8.5%), improper specimen container (2.9%), inappropriate transport (2.8%) and barcode and labeling errors (0.8%) constitute main categories of specimen rejection. Improper specimen category mainly involves hemolysed (65%) and clotted samples (23%), incorrect specimen (5.5%), specimen contamination (5%), and lipemic specimen (0.5%). Inappropriate volume category involves mostly inadequate volume (95%) and excess volume (5%). Incorrect requests are mainly due to the mistakes during the usage of LIS, such as incomplete and repetitive requests. Inappropriate transport, barcode and labeling errors mostly arise from lack of attention of hospital staff.
Conclusions: As SRR has been defined as one of the quality indicators of analytical performance of our laboratory, it was a necessity to determine the exact SRR. Our results will lead us to solve each related issue ending up as specimen rejection by corrective actions and on-site training programs.

P46

NEW TUBES ON THE BLOCK: READY FOR A BETTER BLOOD COLLECTION TUBES FOR PLASMA GLUCOSE

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Key Words: blood glucose, glycolysis inhibitor, Glucomedics, Venosafe FC
Background: Accurate plasma glucose measurements are essential for correct diabetes diagnosis and management. The decrease of glucose concentration in blood samples before analysis is an important pre-analytical problem. Many studies have confirmed the superiority of citrate acidification of blood as a rapid and the most effective additive for glycolysis inhibition. Currently available citrate buffer blood collection tubes contain the additive in either lyophilized or liquid form. The aim of this study was to compare these two types of tubes.
Materials and Methods: The blood samples were collected from 20 non-fasting volunteers, in two tubes: Vacuette Glucomedics tube with liquid citrate buffer (Greiner Bio-One) and Venosafe Glycaemia tube with lyophilized FC mixture (Terumo). To minimize collection order bias, the order of draw was randomized. The tubes were centrifuged within 15 minutes from collection at 2000 x g for 15 minutes. Glucose measurements were performed in duplicate on Architect c8000 (Abbott Diagnostics) using hexokinase method (within-laboratory CV=1.49%). Glucose concentrations determined in Glucomedics tubes were multiplied by 1.16 as recommended by the manufacturer to compensate the dilution effect. Statistical analysis was performed with paired t-test, Bland-Altman and Deming regression analysis using MedCalc 12.5.0 statistical software. $P<0.05$ was considered as statistically significant. The biases between the tubes were compared to ADA recommended criteria based on biological variation (2.2%).
Results: Our results showed that glucose concentration was significantly higher in Vacuette Glucomedics (mean 5.8 ± 0.8 mmol/L) compared to Venosafe Glycaemia (5.6 ± 0.7 mmol/L) ($P<0.001$). The mean bias according to Bland-Altman was 3.2 ± 1.7%. Deming regression showed constant and proportional difference between the tubes: y(Terumo)=0.45 (95%CI 0.07-0.83)+0.89 (95%CI 0.83-0.96)x(Glucomedics).
Conclusion: Glucose concentration is statistically and clinically different between the tubes containing citrate buffer additive in liquid and lyophilized form and Vacuette Glucomedics and Venosafe Glycaemia tubes cannot therefore be used interchangeably.

P47

CORTISOL IN SALIVA: PREANALYTICAL AND ANALYTICAL CONSIDERATIONS IN A RAPID RESPONSE LABORATORY

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Key Words: cortisol, saliva, stability, preanalytical phase.
Background: The concentration of some steroid hormones in saliva is known to accurately reflect their endogenous status. For instance, extensive bibliography is available regarding cortisol measurements in saliva to detect conditions affecting the adrenal and pituitary glands. In this study, we first evaluated the analytical performance of our immunoassay for the measurement of cortisol in saliva samples, and then we used this assay for the assessment of the stability of saliva samples.
Materials and Methods: The electrochemiluminescence immunoassay for cortisol was purchased from Roche. Assessment of the analytical performance included the calculation of the interserial coefficients of variation (CV; by measuring three levels of an internal control in 12 non-consecutive days), and the correlation with an assay provided by a different manufacturer (Abbott Diagnostics). The non-parametric Passing-Bablok regression was used to compare assays, by using saliva samples from 21 individuals referred to our hospital. CVs were acceptable if <8%. Four healthy subjects were included in the stability study, and saliva samples were obtained in the morning, by means of a Salivette® (Sarstedt). Each sample was divided in 3 aliquots:(1) immediately measured,(2) kept at 4°C for 5 days,(3) kept at 4°C for 10 days. Samples were classified as stable if variation was <1.65·CV.
Results: Interserial CVs were 5.99%, 2.91% and 3.00% for the three levels measured. Although correlation was good (r=0.997), Passing-Bablok regression showed both constant (+62.1% for Roche; 95%CI:51.7-85.8%) and proportional biases between immunoassays. After 5 days, cortisol had increased in all samples (mean increase:14.6%,StdDev:5.5%), and also after 10 days (mean increase:19.7%,StdDev:4.0%).
Conclusions: Cortisol immunoassay from Roche meets the analytical requirements for the measurement of this glucocorticoid in saliva, although transferability with Abbott's immunoassay was poor. Since salivary cortisol concentrations were observed to increase over time in non-frozen samples, analysis is recommended as soon as possible after extraction, or freezing.

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RISK EVALUATION IN CENTRAL LABORATORY AND EMERGENCY LABORATORY

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Key Words: trainig of personnel, DPMO, sigma metrics
Background: Quality in preanalytical phase remains a continous concern for Laboratory due to the fact that the preanalytical phase brings most of the errors in the results despite great efforts wich are made.
Materials and Methods: We assesed in an earlier study the types of errors that occur in pre-analytaical phase in Central Laboratory of Emergency County Hospital Tg Mures.With this study we tried to asses if the types of errors that appear are the same in Central Laboratory and in Emergency Laboratory.We considered the following errors:haemolyzed specimens,icteric,lipaemia,misidentification of patients,insufficient sample volume,inappropriate collection tubes.We calculated DPMO and sigma metrics sepparately for the 2 laboratories.Further more we tried to asses if the training of the personnel in Emergency Unit decreases the number of errors that appear by comparing DPMO and sigma metrics before and after the training.
Results: We assesed in Central Laboratory over 19.000 samples in november-december 2014 and 17.146 samples in emergency unit in january-february 2015.The sigma metric was 3,4-4,0 in central laboratory and 3,2-3,8 in the emergency unit. We assesd the DPMO in Emergency Laboratory before the training in january-february 2015 and then again in april-may 2015.The calculated sigma had about the same value and the p was 1,1.After the training no significant improvement was observed.
Conclusions: The number of errors, as we expected is higher in Emergency Laboratory due to the fact that collection of samples is made without patient preparation and by a lot of unexperienced personnel. Altough we organized a course in sample collection and transport the assesment after training shows that no signifficant changes appear in DPMO in Emergency Laboratory.

P49

PORPHYRIN QUANTIFICATION IN URINE: CONTROL MATERIAL EVALUATION FOR AN IN-HOUSE METHOD

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Key Words: stability, coproporphyrin, uroporphyrin, control material
Background: Porphyries are a group of metabolic disorders caused by an enzyme deficiency at the heme group synthesis pathway. In our laboratory, total porphyrin quantification in light-protected cool-kept urine samples is performed if acute porphyria is suspected. The control material for the porphyrin quantification (Lyphochek®,BioRad) had been assayed only for methods including a column extraction step. The aim of this study was to assess the analytical performance of our column extraction-free in-house method, and to determine the stability of control materials after reconstitution.
Materials and Methods: Quantification was based on UV-visible spectrophotometry after addition of 500µL 2.7M HCl to 2.0mL of sample. Wavelength scanning was 380-430nm (maximum absorption at 407nm). Concentrations were calculated using molar absorptivity coefficient of coproporphyrin and uroporphyrin (ε=14300nmol/L). Four same-lot vials of control material were used (BioRad), and reconstituted according to manufacturer's instructions. The reported mean value was 1191nmol/L. Analytical coefficient of variation (CVa) was calculated from 20 same-vial consecutive analyses. Control stability threshold (CS_t) was calculated as CS_t=1.65·CVa. Relative difference to mean value (RD) was calculated for every analysis. Control was classified as stable if RD<CS. The other 3 control vials were assayed up to 42 times in a 60-day span to assess porphyrin concentration. Shapiro-Wilk test was used to assess sample distribution (α=0.05) and a two-tailed paired Student's t-test to compare experimental results with the concentration certified by manufacturer.
Results: CVa was 10.2%. For all three control materials, RDs were lower than CS_ts, so all three were classified as stable. Calculated mean value was 1289.2nmol/L (StdDev:233 nmol/L), while not different from the certified value ($p<0.01$).
Conclusion: Lyphochek® control material can be used as quality control for our in-house method for the assay of total porphyrins in urine. Control materials may be stored at 2-8°C for at least 60 days without any loss of stability.

P50

ASSESSMENT OF INTRALABORATORY ANALYSERS' COMPARISON USING NORMALIZED MEDx CHART

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Key Words: intralaboratory quality control, method comparison, normalized MEDx chart
Background: In CHC Rijeka there are three distant laboratory locations. Aim was to present a method for assessing the results of intralaboratory analysers' comparison (IAC) by using graphical tool, Normalized MEDx chart.
Materials and Methods: IAC is performed monthly in the CHC Rijeka. Blood sample from volunteer is distributed on three distant locations for the same analysis. For each parameter we assess Bias in comparison to reference analysers; Roche Cobas c501 (Manheim, Germany) for biochemistry, Siemens Advia 2120i (Erlangen, Germany) for hematology. Reference analysers are chosen on account of participation in EQA schemes. CV is calculated using monthly internal quality control results. Each method for each analyser is expressed as the operating point with coordinates (%CV/TEa,%Bias/TEa) on the same MEDx chart where TEa represents Total error available from Westgard's Desirable Biological Variation Database. Results are judged according to the sigma value for classification of method performance. Here we present results of IAC for glucose (Glu), urea, creatinine (Crea), sodium (Na), potassium (K), hemoglobin (Hb), erythrocyte (RBC), leucocyte (WBC) and platelet (PLT) count for November 2014 on 5 biochemistry (3 Roche Cobas c501 (c5011S, c5012S, c5011R), Beckman Coulter AU5800 and AU400) and 6 hematology analysers (2 Siemens Advia 2120i (Advia-S, Advia-R), 3 Abbott Cell-Dyn 1800 (CD-R, CD-S, CD-K) and Sysmex XT2000).
Results: According to the Normalized MEDx chart the comparability of methods are: world class: WBC_CD-S, PLT_Advia-S, WBC_Sysmex, PLT_Sysmex; excellent: Urea_c5011S, Glu_c5011R, WBC_CD-K; good: K_c5011S, RBC_CD-S, PLT_CD-S, Hb_Advia-S; marginal: WBC_CD-R, PLT_CD-K, WBC_Advia-S, Hb_Sysmex; poor: Glu_c5011S, Urea_c5011R, Crea_c5011R, K_c5011R, Urea_AU400, K_AU400, PLT_CD-R, Hb_CD-S; unacceptable: Glu_AU5800, Urea_AU5800, Crea_AU5800, Na_AU5800, K_AU5800, Crea_c5011S, Na_c5011S, Na_c5011R, Glu_AU400, Crea_AU400, Na_AU400, Hb_CD-R, RBC_CD-R, Hb_CD-K, RBC_CD-K, RBC_Advia-S, RBC_Sysmex.
Conclusion: IAC was unacceptable for many parameters. Corrective actions have been undertaken upon data analysis. Normalized MEDx chart is valuable for judging method comparability and point out critical analyses.

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ASSESSMENT OF QUALITY CONTROL IN AN EMERGENCY BIOCHEMISTRY LABORATORY

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Key Words: sigma metrics, quality control, emergency laboratory
Background: To ensure the quality of analytical results is widespread the use of Westgard Quality Control (QC) rules for many biochemical magnitudes. This rules generates false rejections and an unnecessary increase in controls and calibrators. In our laboratory we used "1-2s" rule. Sigma Metrics is a tool used for reduce the process variability, in which a sigma value of 3 represents the minimum required quality.
Materials and Methods: we selected a total of 15 magnitudes measured in the emergency laboratory for a period of four months. As a control material we used PreciControl ClinChem Multi 1 and 2 (Roche Diagnostics). We calculated Sigma values using allowable total error (TEa) based on biological variability (BV) (minimum and desirable) and on CLIA (Clinical Laboratories Improvement Amendments) guidelines. Mean, bias and CV (coefficient of variation) was calculated for each magnitude. The aim of the study was to assess whether our laboratory complies with Sigma Metrics, comparing different quality specifications (CLIA, BV) in order to implement improvements.
Results: Sigma value according CLIA specifications resulting >3 for both levels in alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, creatine kinase (CK), lithium (Li) and uric acid (UA). For creatinine, glucose, lactate dehydrogenase (LDH), magnesium (Mg), sodium (Na), total protein (TP) and chlorine (Cl) only one of the controls presented sigma above 3. According minimum BV: ALT, amylase and potassium (K) meet sigma >3 for both controls, and Cl, creatinine and glucose reach minimum sigma only for one control. According desirable BV: ALT and CK meet sigma >3 for both controls, and AST, amylase, Cl, creatinine, K and urea reach minimum sigma only for one control.
Conclusion: We complie with Sigma Metrics for most of the parameters studied. BV specifications resulted more stringent than CLIA ones. Sigma metrics can be an efficient way to control analytical quality.

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BLOOD GAS ANALYZER INTERCHANGEABILITY

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Key Words: interchangeability, gasometer, patient safety, CLSI EP-31-A-IR
Background: According to ISO 15189:2003, clinical laboratories must periodically verify the interchangeability of test results among autoanalyzers at clinical decision levels, aiming at an improvement in patient safety. In this study, we applied the CLSI EP-31-A-IR protocol to check the interchangeability of blood gas analyses, and thus establish appropriate corrective actions.
Materials and Methods: In our laboratory, we use 2 gasometers GEM® Premier™4000 (Werfen Co). The analyzers have an integrated quality control material (IQM,Intelligent-Quality-Management), based on an analysis of four solutions with known amounts of analytes, which are automatically measured. Studied parameters were pH, pCO_2, pO_2, potassium ion and ionized calcium, in accordance with Emergency Department needs. Approximate analyte concentration was estimated on the basis of clinical decision levels and known imprecision; the latter obtained from the intraserial standard deviation (RS, n=20) and the total standard deviation (TS,n=number of IQM controls assayed in a 6-month period). We established the maximal allowed difference (MAD,%) between the results of both analyzers, according to quality specifications in our laboratory. From these, we calculated the critical difference (CD=MAD·analyte concentration), the ratios CD/TS and RS/ST to determine the number of sample series needed and the replicate number in every series, the mean of the results in either gasometer and the difference between means (DbM). In case of methodological difficulties when performing same sample replicates, quality specifications were lowered. Limit of acceptability was defined as LoA=CD·L, where L is the rejection range. Magnitudes were defined as interchangeable if DbM<LoA.
Results: DbMs were lower than LoAs in every case: pH (0.00<0.03), pCO_2 (1.25<1.53), pO_2 (12.00<13.17), potassium ion (0.00<0.13), ionized calcium (0.090<0.091). Therefore, all magnitudes were shown to be interchangeable.
Conclusion: The clinical laboratory must periodically verify the interchangeability of the results from its gasometers and establish pertinent corrective measures to improve result quality to ensure patient safety.

P53

UX-2000 AN AUTOMATED URINE SEDIMENT ANALYZER-NEED FOR SPECIFYING ADDITIONAL CRITERIA AND THRESHOLDS FOR MANUAL MICROSCOPY REVIEW

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Key Words: automated urine analyzer, flow cytometry, urine microscopy, urine sediment
Background: The Sysmex UX-2000 uses fluorescent flow cytometry to classify and quantify urine sediment particles, saving a considerable amount of time and labour, and offers an improvement in standardization over manual microscopy. Since this technology has limited ability to differentiating some elements a precise cut-offs need to be established by the lab for their patient population. Aims of this study were to compare the performance of the UX-2000 against manual microscopy urine sediment examination and specify criteria and thresholds for results to be flagged for manual microscopy.
Materials and Methods: A total of 280 specimens were collected and analyzed within 2 hours of receipt for urine components by automated analyzer and microscopy. The correlation between two methods was determined using *gamma statistics,* while agreement was established by *weighted kappa statistics.*
Results: Good correlation were shown for white and red blood cells (*gamma* 0.851 and 0.872 with P<0.001, respectively), epithelial cells, bacteria, mucus and yeast (*gamma* 0.764, 0.868, 0.874 and 0.754 with P<0.001, respectively). Good agreement was shown for crystals (*weighted kappa* 0.595, P<0.001), while for hyaline and pathological cast as for small round cells *weighted kappa* showed poor agreement (0.303, 0.137 and 0.359 with P<0.001, respectively). After specifying criteria and thresholds we set 8 rules for parameters flagged for manual microscopy.
Conclusion: UX-2000 an automated urine sediment analyzer is reliable in measurement of white and red blood cells, epithelial cells, bacteria, mucus, yeast and crystals, but for confirming of hyaline and pathological cast as for small round cells additional manual microscopy examination is necessary.

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IMPLEMENTATION OF ROUTINE RETICULOCYTE COUNTING ON SYSMEX XN-2000

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Key Words: reticulocyte, automated analysers, flow cytometry
Background: Various methods of automated reticulocyte counting are used in clinical laboratories. Here we performed a side-by-side comparison of two routine haematology analysers in order to implement new method of automated reticulocyte counting in clinical practice.
Materials and Methods: Residual K_2EDTA blood samples were taken from routine haematology workload. A total of 68 samples were analysed for reticulocyte parameters by both methods within 4 hr of specimen collection in random order. Two haematology analysers Advia 120 (Siemens) and XN-2000 (Sysmex) with the option of reticulocyte count in complete automation were evaluated. Recommended quality control was run on both instruments on a daily basis. The investigated haematology analysers utilise different technologies for determination of reticulocytes. Analysis of correlation and regression analysis (Deming) was done for measurements of absolute and relative reticulocyte count and reticulocyte haemoglobin content from each analyser. Within-run imprecision for XN-2000 was estimated by performing 10 consecutive measurements on one sample, after which coefficient of variation (CV%) for each parameter was calculated.
Results: Inter-instrument comparison of directly analysed reticulocyte parameters revealed correlation coefficients $r^2 \geq 0.85$ ($p<0.0001$) and $r^2 \geq 0.90$ ($p<0.0001$) for reticulocyte absolute counts and relative counts, respectively. Systematic differences between analysers were seen in both parameters showing lower values with XN-2000 (slope 0.89 for absolute counts and slope 0.85 for relative count). There was good correlation of reticulocyte haemoglobin content with coefficient $r^2 \geq 0.94$ ($p<0.0001$). The results of the within-run precision analyses revealed CV% being ≤2.07 for reticulocyte absolute counts (mean 86.88 $x10^9$/L), ≤2.35 for relative counts (mean 3.0%) and ≤0.77 for reticulocyte haemoglobin content (mean 30.6 pg).
Conclusions: Our parallel testing revealed a good concordance for reticulocyte haemoglobin content while a correlation between instruments for reticulocyte count was satisfactory to a lesser extent. Manufacturer's precision claim was assessed and confirmed.

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COMPARISON OF THE GLUCOSE RESULTS BETWEEN TWO METHODS IN INTENSIVE CARE UNIT

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Key Words: glucose, blood gas analyser, glucometer
Background: POCT analyses of glucose in the Intensive Care Unit in our hospital were performed interchangeably from arterial blood on blood gas analyser and on glucometer. Because of the introduction of new glucometers in our hospital a comparison in glucose results was made.
Materials and Methods: We included 25 samples of heparinised arterial blood collected in syringe from patients in Intensive Care Unit in our hospital. The syringe was first used for the analyses on Blood Gas Analyser (Rapid System 1265, Siemens Diagnostics, Germany) and then a drop of blood from the same syringe was placed on test strip on glucometer (Accu-Chek Inform II, Roche Diagnostic, Germany). Obtained glucose results were statistically evaluated.
Results: Measurement range on Rapidlab 1265 for glucose was from 4,9 to 14,6 mmol/L with average value 8,32 mmol/L and on Accu-Chek Inform II was from 5,5 to 14,8 mmol/L with average 8,72 mmol/L. With the nonparametric Wilcoxon test we demonstrated a statistically significant difference between two methods ($p < 0,01$). Glucose results were higher on glucometer Accu-Chek Inform II and the average difference was 0,40 mmol/L or 4,8%. According to regression by Deming the following results were obtained: Slope: 1,02, Intercept: 0,26 and correlation coefficient (r^2): 0,975.
Conclusion: There was small but statistically significant difference between glucose results on Accu-Chek Inform II test strips, which are plasma calibrated, and glucose results from blood gas analyser with direct reading glucose biosensor. For blood glucose monitoring in our Intensive Care Unit it is more important that the same type of blood sample is used than the instrument where analyse is performed.

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COMPARISON OF HEMOLYSIS RATE BETWEEN BECTON DICKINSON AND S-MONOVETTE-SARSTEDT SERUM SEPARATOR TUBES

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Key Words: blood sampling, serum separator tubes, hemolysis
Background: In vitro hemolysis is one of the main causes of preanalytical errors that lead to inaccurate test results, increased costs and time consumption. Although, use of standardized needles and vacuum blood collection systems reduced the rate hemolysis, it still remains being a common problem in daily practice. Therefore, two different manufacturers' serum separator tubes were compared for hemolysis by the means blood sampling units, time of collection and usage of either needle or intravenous catheters.
Materials and Methods: A total of 200 patients' blood samples were collected to BD and S-Monovette SSTs consecutively in emergency and coronary intensive care and clinical laboratory blood collection units between February to March 2015. Hemolysis index of samples were measured by Roche-Cobas C501 using Gen.2 kits. The data was evaluated by Chi-Square Tests and Wilcoxon Signed Ranks Tests. A logistic regression model was performed to predict the determinant(s) of hemolysis by using all evaluated parameters and SSTs as covariates.
Results: Evaluation of all results showed the frequency and the level of hemolysis were significantly high in BD SST than S-Monovette SST ($p<0.001$ for both). Comparison of results with regard to blood collection units also revealed that hemolysis index was high with BD SST in emergency and intensive care units. Similar results were obtained for blood collection by intravenous catheters ($p<0.001$). The logistic regression model revealed that the determinant(s) of hemolysis were the type of the SST used and blood collection unit ($p=0.000$ and $p=0.005$ respectively).
Conclusion: In conclusion blood collection by S-Monovette SST seems more favorable than BD SST in the prevention of hemolysis. S-Monovette SSTs may be the choice of blood collection tube in emergency and intensive care units for blood drawing from intravenous catheters.

P57

CRITICAL POINTS OF ACID-BASE BALANCE AND ELECTROLYTES TESTING IN EXTERNAL QUALITY CONTROL ASSESSMENT IN CROATIA

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Key Words: external quality control, acid base balance, electrolytes, inter-laboratory comparability
Background: External quality assessment (EQA) for analysis of pH, blood gases and electrolytes in Croatia is conducted through two modules with two different sample types. The aim of this study was to present inter-laboratory comparability of these analyses and to identify critical points in sample types used in EQA and/or laboratory work.
Materials and Methods: Control samples used during three cycles in 2014 and one in 2015 were commercial, but different origin (sample A, human origin and sample B, aqueous solution) in three different concentration ranges (normal, pathological low and high).They were used in 'one cycle-one range' manner. According to Tukey's model outliers were excluded and mean, standard deviation (SD) and coefficient of variation (CV) were calculated. According to quality specifications established in Croatian EQA, the allowable CVs were as follows: CV_{pH}=±1%, CV_{pO2}=±12%, CV_{pCO2}=±12%, CV_{Na}+=±3%, CV_{K}+=±5% and CV_{Ca2}+=±5%. Criteria used in assessing preferable sample type and laboratory work were CVs and success rate (SR) of all participating laboratories, regardless of method and instrument.
Results: Both samples indicated equal presence of unacceptable CVs according to established criteria (5/24; 21%) for following parameters: pO_2 (low range-2A,B), ionized potassium (low range-A,B; high range-B), ionized calcium (low range–B; high range–A,B). For all these parameters, SR was unaceptable (<67%, depending of analysis). Normal concentration range regardless of the sample type for all analysis indicates acceptable CVs and good inter-laboratory comparability. pH, pCO_2 and ionized sodium in all concentration ranges and for both sample types had acceptable CVs and high SR.
Conclusion: According to our results, sample type does not represent a critical point in the implementation of EQA for examined analytes. Our observational study indicates that a critical point is pathological range of both commercial controls. On the other hand, preanalytical and analytical factors in laboratory work should also be concerned.

P58

COMPARISON OF SIX SIGMA VALUES USING TWO DIFFERENT ALLOWABLE TOTAL ERROR CRITERIA

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Key Words: D-dimer, quality control, six sigma
Background: Every laboratory is responsible for maintaining analytical quality through adequate quality control (QC) schemes. Six sigma metrics provides an objective tool to measure quality. It is calculated using accessible laboratory parameters: allowable total error (TEa), bias and imprecision. Despite the simple equation, selecting right TEa criterion can be a challenge. The aim of this study was to compare the analytical quality of a D-dimer (DD) method using six sigma values calculated with two different TEa criteria.
Materials and methods: The six sigma was calculated using the equation: Sigma=(TEa-bias)/CVa, where CVa represents the calculated analytical coefficient of variation. The first TEa criterion was selected from the desirable biological variability database specifications (desirable TEa). The second criterion was the reference change value (RCV) calculated using the equation: $RCV = Z \times \sqrt{2} \times \sqrt{CVa^2 + CVi^2}$, where Z=1.65 and CVi denotes the within-subject biological variation. Bias was assessed from the Croatian Center for External Quality Control Assessment (CROQALM) and CVa was calculated from internal quality control data. Six sigma values for both criteria were compared.
Results: The calculated CVa for level 1 and 2 was 8.9%. The bias for D-dimer was 5.5%. RCV was 58.2%. TEa according to the biological variability database was 28%. Calculated six sigma values using the RCV and TEa criteria were 5.9 and 2.5, respectively.

Conclusion: The quality of our DD method would be classified as poor if the TEa criterion was used. Conversely, the quality would be excellent if RCV was used as the TEa criterion. Six sigma values for D-dimer strongly depend on the selected TEa criteria. The lack of studies meeting criteria suitable for defining TEa according to proposed quality hierarchy in Milan 2015, represents a challenge for implementation of six sigma metrics for DD.

P59

LABORATORY PERFORMANCES WHEN USING REFERENCE VALUE AS A TARGET VALUE IN EXTERNAL QUALITY ASSESSMENT SCHEME IN BIOCHEMISTRY

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Key Words: external quality assessment, reference value, consensus overall mean, allowable limit of performance
Background: External quality assessment provides an essential tool for medical laboratories which enables participants to evaluate both assay performances and established quality standards. Croatian Centre for Quality Assessment in Croatia provides quality assessment for biochemistry assays through corresponding module in every round of the scheme. The performance of each laboratory is evaluated using previously established allowable limits of deviation from consensus mean of comparison. In the last three rounds of the quality assessment scheme certified referent values based on reference methods has been assigned for 17 analytes. Reference values give the opportunity to access trueness of laboratory measurements procedures, especially when multiple instruments, reagents and calibrators are used. We compared the deviation of consensus overall mean from reference value for each of those 17 analytes and calculated the percentage of laboratories that met the criteria of allowable limits when both reference value and consensus overall mean were set as a target value.
Materials and methods: The data reported during three biochemistry rounds have been reviewed. The biases of consensus means were calculated using formula: ((consensus mean – target value)/target value)x100. The number of laboratories that met the allowable limits of performance criteria was calculated first by defining minimum and maximum values allowed (min/max= target value –/+ (allowable limit of performance% x target value)/100,) and then counting the number of laboratories that fulfilled the criteria.
Results: Average biases of consensus means ranged from 0,69% (Na) to 9,15% (CK). The percentage of laboratories that met the allowable limits of performance ranged from 66,3 – 100% when consensus overall mean was set as a target value, whereas the range is 51,1-100% when using reference value as target value.
Conclusions: Deviations from target values were higher when reference values were set as target value and the extent of deviation depends on the analytes and the value.

P60

VERIFICATION OF QUANTITATIVE REAL-TIME PCR (qPCR) microRNA PROFILING

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Key Words: microRNA, serum, plasma, MIQE, qPCR
Background: MicroRNAs (miRNAs) derived from various tissues or organs have critical functions in many biological processes. miRNA profiling either in human serum or plasma could be clinically usefull in noninvasive molecular diagnostic testing, but its clinical effectiveness is likely to be affected by various methodological issues like the efficiency of RNA recovery and qPCR platform used. The aim of this study was verification of miRNAs profiling performance on TaqMan qPCR platform.

Materials and Methods: Purification of miRNAs from human serum or plasma (200 µl) was performed using miRNeasy Serum/Plasma Kit (Qiagen). miRNeasy Serum/Plasma Spike-In Control (Qiagen) has been used to monitor sample preparation. miRNA reverse transcription (RT) was performed using miScript II RT Kit (Qiagen) according to the manufacturer's instructions. miScript SYBR Green PCR Kit and Custom miScript miRNA PCR Arrays (Qiagen) were used for qPCR miRNA profiling on ABI 7300 (Applied Biosystems) with multiple controls included on each array for miRNA quality, contamination and general qPCR performance.
Results: Each miScript miRNA PCR Array has been verified by the producer to ensure dynamic range/sensitivity. The calculated R^2 was 0,913 and slope -2,998. qPCR efficiency calculated from slope was 115,6%. Amplification plot of cell-miR-39 standard curve showed four 10-fold dilutions and sensitivity was estimated at 1000 copies. Specificity was assessed by the default software melting program immediately after the cycling program completion. Dissociation curves with a single peaks indicated presence of specific amplification products without primer dimers. The baseline settings were the same (0.2) across all qPCR runs in the same analysis to allow comparison of results. The data collected from two independent runs were normalized by the cell-miR-39 as a reference gene.
Conclusion: Verification of qPCR miRNA profiling on TaqMan qPCR platform according to MIQE guidelines and manufacturers recommendations demonstrated good reproducibility which is a prerequisite for its clinical application.

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VERIFICATION OF METHOD FOR TACROLIMUS IN WHOLE BLOOD ON AUTOMATIC ANALYSER ABBOTT ARCHITECT i1000sr

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Key Words: verification, tacrolimus, precision, trueness
Background: Tacrolimus is an immunosuppressive drug shown to be effective for treatement of organ rejection following transplantation. Current studies recomend whole blood rather than plasma as more appropriate sample to describe the pharmacokinetic characteristics of tacrolimus. Because of its nephrotoxicity, tacrolimus concentration should be determined to prevent severe side effects. The aim of this study was to verify method (CMIA – carbonylmetalloimmunoassay) for tacrolimus determination in whole blood on automatic analyser Abbott Architect i1000sr.
Materials and Methods: Verification was preformed according to CLSI protocol EP 15-A2. Using two levels of comercial control samples (Architect TP multichem WBT), tacrolimus concentration was measured three times a day during five days (N=15 for each level). Following parameters have been determined: precision (CV_p) and trueness (BIAS). Precision was interpreted according to external QC provider's permited values. For determining trueness, declared manufacurer values for control samples were interpreted.
Results: Obtained values of CV_p for precision were 3,73% (level 1; target value 6.41 µg/L) and 2,55% (level 3; target value 24.5 µg/L), and mean CV is 3,14%. Values for trueness (BIAS) were 18,25 (level 1) and 18,06 (level 3) with mean value of 18,16.
Conclusion: The coefficient of variation for the precision obtained in the verification of method for the tacrolimus meets the criteria of the providers of external quality control (for toxicology- drug monitoring 10-20%). This means that the measurement reproducibility is satisfactory, and thus the method is acceptable for the longitudinal monitoring of patients. The results for trueness show difference between target values of the commercial control, but are not clinically significant because it proves that the immunochemical methods on different devices can not be compared. Method for determination of tacrolimus in whole blood on analyzer Abbott Architect i1000sr should be implemented in routine work.

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ASSESSMENT OF UTILITY TWO IMMUNOASSAYS (ELISA AND CLIA) IN LYME DISEASE DIAGNOSTICS

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Key Words: borrelia, CLIA, comparison, utility
Background: Two different, routine methods are performed as a screening test in Lyme Disease (LD) diagnostic in Poland. The aim of the study was to compare utility of CLIA (chemiluminescenceimmunoassay) and ELISA method, use in Borrelia IgM antibodies identification on a group of high risk of LD.

Material and Methods: Tests were performed on a group of 27 patients (total group TG)(men and female), collected and stored at -20 °C without interruption, in 2015 at Diagnostyka Company, Cracow, Poland. All sera were tested with ELISA [Euroimmun – antiBorreliaIgM – extract from different strains of Borrelia] and with the CLIA [Diasorin – BorreliaIgM Quant – recombinant antigens OspC and VIsE]. Samples from 16 patients (high-risk group – HRG) with positive results one of the methods were tested with Western Blot [Mikrogen – recomLineBorreliaIgM].
Results: Median for Borrelia IgM CLIA method was 16,90 [quartile 15,80-19,30] (AU/ml) for TG and 17,65 [quartile 16,45-36,40](AU/ml) for HRG. Median for ELISA were 20,13 [7,14-23,06] for TG and 50,29 [quartile 23,48-83,20] (RU/ml) for HRG. Significant **p**-value was assayed in TG between methods (r= 0,411; p=0,033). No correlation between both methods was found (R=0,1941, p=0,471) in HRG. Using Borrelia IgM CLIA method 6 false positive, and 3 false negative results were assayed. Using ELISA 8 false positive and no false negative results were observed. Sensitivity, specificity, PPV and NPV for: CLIA were 40%; 45%; 0,250; 0,625 respectively and for ELISA: 100%; 27%; 0,384; 1,000 respectively.
Conclusion: ELISA method is characterized by significant higher sensitivity than CLIA method. Choice of antigens implicate more false positive results in ELISA method. Although a hypersensitive reaction, ELISA seems to be method of choice as a screening in two-step approach in LD diagnostics.

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PERFORMANCE VERIFICATION OF CLINREP HPLC METHOD FOR MYCOPHENOLIC ACID IN PLASMA

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Key Words: verification, mycophenolic acid, HPLC
Background: Mycophenolic acid has an important role in preventing acute organ rejection, but with side effects awareness, it is very important to determine the drug concentration precisely and accurately. The aim of this study was to evaluate the performance of ClinRep HPLC method for Mycophenolic acid in plasma on Shimadzu LC10 AD HPLC system.
Materials and Methods: Precision was evaluated according to CLSI EP15 A2 guideline. For precision assessment we tested two concentration levels of commercial control material (ClinChek plasma control for mycophenolic acid) in triplicate for five days in a row: 1.42µmol/L for control1 and 15.0µmol/L for control3. HPLC standard was traceable till mass spectrometry. For trueness assessment we used 6 previously reported samples of UK NEQAS Mycophenolate International Proficiency Testing Scheme, which were reported as target (4/6) and as pool concentration (2/6).
Results: The verification procedure gained precision profile including repeatability CV% 0.98 and 0.35, intermediate precision CV% 7.44 and 6.48, and within-laboratory precision CV% 7.48 and 6.48, for levels 1 and 2, respectively. Average deviation from target value for 6 retrospectively analysed UK NEQAS samples was -3.14%(-12.09%-3.13%). Expended measurement uncertainty (k=2), derived from within-laboratory precision, uncertainty of calibrator and pipettes, was 15.0% for control1 and 13.0% for control2.
Conclusion: Our results showed that precision was acceptable according to the desirable biological specifications based on the drug half life (10.9% -9h;6.0% -17h) with minor discrepancy for 17h. International proficiency testing results were within the obtained values for precision and are in compliance with the organizer's requirements (deviation <30%). Calculated deviations were much lower than allowed regardless of the fact that values were reported both as target and as pool sample concentrations. Therefore, ClinRep HPLC method for Mycophenolic acid, as a method of choice, ensures reliable determination of active substance concentrations providing clinicians undoubtable information for optimal patient treatment.

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VERIFICATION OF ABBOTT STAT HIGH SENSITIVE TROPONIN-I ASSAY

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Keywords: high sensitive troponin I, method verification, precision, carry-over
Background: Our aim was to verify the Architect STAT high sensitive troponin-I assay (hsTnI) prior to its introduction into our daily routine. The protocol included the verification of: (i) the 99th percentile of the reference population for both men and women declared by the manufacturer (34.2 and 15.6 ng/L, respectively); (ii) the declared imprecision at the 99th percentiles (3.5% at 34.2 ng/L; 5.3% at 15.6 ng/L); and (iii) the carry-over.

Materials and Methods: The 99th percentiles were verified according to the CLSI C28-A3c guideline on 40 male and 40 female healthy participants, without coronary heart disease. Method imprecision (repeatability and within-laboratory precision) was verified according to CLSI EP15-A2 protocol, by analyzing two serum pools with concentration near the 99th percentiles, for five consecutive days in triplicate. Carry-over was tested according to IUPAC protocol by analyzing one sample with hsTnI >50000.0 ng/L in duplicate followed by a triplicate measurement of a negative sample (hsTnI <4.7 ng/L). All measurements were performed on Abbott Architect i2000 analyzer.
Results: Median age was 55 years (range: 39-82) for women and 57 years (range: 31-83) for men. Values of hsTnI have exceeded the 99th percentile for 3/40 women (hsTnI values: 26.4, 41.9 and 44.2 ng/L) and for 2/40 men (hsTnI values: 41.8 and 71.0 ng/L). The hsTnI concentrations were above limit of quantitation (4.7 ng/L) for only 7/40 women and 6/40 men. The repeatability and within-laboratory precision at 18.4 ng/L were 6.10% and 6.69% and at 25.5 ng/L 5.83% and 9.53%, respectively. The carry-over of hsTnI was 0%.
Conclusion: Declared hsTnI 99th percentiles both for men and women can be used on our population. No carry-over was observed for the hsTnI assay and method imprecision is higher than declared by the manufacturer, but still below the recommended 10%.

P65

THE ROLE OF ALBUMIN IN CALCULATION OF FREE TESTOSTERONE

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Key Words: calculated free testosterone, albumin, Vermeulen equation
Background: Free testosterone concentration (cFT) can be calculated according to Vermeulen equation using total testosterone, albumin and sex hormone binding globulin (SHBG) concentrations. Our aim was to asses the differences between cFT using individual and fixed albumin concentrations.
Materials and Methods: A total of 815 patients (aged 13–75) were included into the study. Serum total testosterone, SHBG (Roche Cobas e601) and albumin (Abbott Architect c8000) were measured. cFT was calculated using: (i) individual albumin concentrations (cFT_i); (ii) fixed albumin concentration of 43 g/L (cFT_{43}, according to Vermeulen); (iii) fixed albumin concentration of 44 g/L (cFT_{44}, studied group median). Friedman test with post hoc analysis according to Conover, Passing-Bablok regression and Bland-Altman plot were used. Absolute mean biases were compared with current desirable specification for imprecision by Westgard (4.7%). Data were expressed as median and interquartile range.
Results: Studied group albumin was 44 g/L (42-46), and cFT_i, cFT_{43}, cFT_{44} were 1.40% (1.04-1.86), 1.41% (1.04-1.90) and 1.39% (1.03-1.88), respectively. Friedman test showed statistically significant difference (P<0.001) with post hoc significant differences between each pair of cFT calculation. Passing-Bablok analysis showed constant and proportional difference between cFT_i and cFT_{43}; while there was no difference between cFT_i and cFT_{44}. Mean difference from Bland-Altman plot between cFT_i and cFT_{43} and between cFT_i and cFT_{44} was -1.0% and -0.2%, respectively, with more than 5% of data exceeding limits of agreement. Absolute mean biases showed no clinically significant difference between cFT_i and cFT_{43} (2.0%) and between cFT_i and cFT_{44} (1.8%).
Conclusion: Clinically, it is unnecessary to measure individual albumin concentration and fixed concentration of 44 g/L can be used. Clinicians should be aware that cFT is not reliable in states with altered albumin concentration and measuring individual albumin concentration is recommended.

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VERIFICATION OF IMPRECISION FOR TSH, FT4 AND FT3 WITH 3 DIFFERENT METHODS

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Key Words: imprecision, thyroid-stimulating-hormone, thyroxin, triiodothyronine
Background: In laboratory routine, it is important to ensure that results of analysis are correct and precise. Verification of method is the way for users to check precision and accuracy of results, according to current standards. The aim of this study was to evaluate results for imprecision, obtained from 3 different analyzers, for thyroid-stimulating-hormone (TSH), free thyroxin (FT4) and free triiodothyronine (FT3), regarding to manufacturer's specifications and desirable specifications for imprecision derived from biological variation.
Materials and Methods: The verification of methods was performed on control samples BioRad Lypocheck Immunoassay Plus Control (Biorad Laboratories) (Level1 and Level3) for all 3 thyroid hormones, on Unicel Dxl600 (Beckman Coulter), Architect i1000 (Abbott) and

Dimension EXL (Siemens) analyzers. Control samples were tested 10 times in the series (repeatability) and every day in duplicate during 10 days (intermediate precision). Results are shown as coefficient of variation (CV%) and estimated through manufacturer's and biological variation's desirable CV% for total imprecision. Statistical analysis was performed using Microsoft Office Excel 2007 software.
Results: For all 3 analyzers, CV% for all parameters was within manufacturer's specifications. CV% (TSH) on all analyzers was within biological variation's desirable specifications (I=9.7%). The highest CV was obtained from Architect (I=6.36%). For FT4 (I=2.9%) and FT3 (I=4.0%) the measured values did not fulfilled biological variation's desirable specifications for neither analyzers. For both, FT4 and FT3, the highest CV was obtained from Unicel Dxl600 (I=8.36%, I=8.25%, respectively).
Conclusion: All 3 methods have satisfactory imprecision for all 3 hormones, according to manufacturer's specifications. Biological variation's specifications are too narrow and they can be accomplished only for TSH. Therefore, each analyzer can be used for routine analyzes of thyroid hormones separately. Future goal is to evaluate accuracy to determine referent analyzer and to compare all 3 methods for these parameters to enable backup for current method.

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COMPARISON OF CYCLOSPORINE A CONCENTRATIONS DETERMINED USING ECLIA AND FPIA METHODS

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Key Words: comparison, cyclosporine A, Passing and Bablok regression
Background: Cyclosporine A is a potent immunosuppressant which is used as a primary agent during immunosuppressive therapy for solid organ transplants. Monitoring patient's cyclosporine A blood concentrations is important for adjusting dosage to avoid overdose toxicity or underdose inefficiency. The aim of this study was to compare two methods for measuring cyclosporine A blood concentrations: ECLIA (electrochemiluminescence immunoassay) and FPIA (fluorescence polarization immunoassay).
Materials and Methods: The concentration of cyclosporine A was measured in 24 whole blood samples of patients on cyclosporine A therapy. After manual sample preparation, cyclosporine A was determined using ECLIA (on automatic analyser Abbott Architect i100SR) and FPIA (on automatic analyser Abbott AxSYM). We compared the two analytical methods using the Passing and Bablok regression analysis (Cusum test $P < 0.05$ indicates significant difference from linearity). The accepted mean bias value by providers for external quality control is ±10%.
Results: The regression equation according to Passing and Bablok was $y = 8{,}0793 + 1.0996x$ (95% CI for intercept was from -1.4238 to 14.6007 and 95% CI for slope was from 1.0239 to 1.1942). The mean bias value was -20.79%, and the calculated Cusum linearity test P value was 0.48.
Conclusion: Passing and Bablok intercept showed that there is no constant difference between the two methods. However, the slope showed that there is a proportional difference. The Cusum test indicated no significant deviation from linearity, and the mean bias value exceeded twofold the acceptable percentage. Thus, only one method should be used to monitor patient's cyclosporine A concentrations. Taken together, for obtaining reliable results, when monitoring patient's drug concentrations, it is recommended to use the same analyzer as well as the same immunoassay.

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VERIFYING THE COMPARABILITY OF RESULTS IN LARGE LABORATORIES – MISSION IMPOSSIBLE?

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Key Words: accreditation, quality control, comparability of results
Background: Laboratories accredited according to ISO 15189 standard which have two or more different analyzers or procedures for examinations should define a protocol for verifying comparability of results throughout clinically appropriate intervals. The aim of this study is to describe a procedure used in order to meet this requirement in large laboratory settings.
Materials and Methods: Department of Laboratory Diagnostics, the largest laboratory in Croatia, comprises four different locations. The procedure used to verify comparability of results had to include all locations, 73 analytes and 30 analyzers using venous blood samples (serum, plasma, whole blood) and urine samples of patients. Before implementing this procedure initial comparability for each analyte on 20 samples was assessed. Maximum allowable bias for each analyte was defined and >90% samples for each analyte had to meet this criteria. Results

obtained from initial comparability survey helped to define the neccessary frequency of performing the comparability survey for selected 73 analytes.
Results: Results of initial comparability showed that the majority of the included analytes met predefined criteria (89.04%). 8 of 73 analytes didn't meet the predefined criteria (10.96%) but absolute values of the differences were not clinically significant. In a one year period 12 surveys of comparability were performed. Corrective actions (N=67) were undertaken for those unacceptable results that had been recognized as clinically relevant (795 of total 6733 results;11.81%).
Conclusion: Before implementing the procedure for verifying comparability of results, many parameters must be taken in consideration (sample stability, number of analyzers and analytes, size of laboratory etc). According to results of initial comparability, monthly comparability surveys are performed. Daily comparability is performed for all accredited locations and analytes including those analytes that have not met the predefined criteria. Thus the established system of quality control allows us to have a better control over the working process.

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ASSESSMENT OF ACTH ASSAY VARIABILITY IN CROATIA

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Key Words: ACTH, EQA, immunoassay
Background: Determination of plasma ACTH plays an important role in differential diagnosis and treatment of disorders in hypothalamic-pituitary-adrenal system. Currently used immunoassays have high analytical specificity, require minimal plasma volume, and are performed in routine. The aim of this study was to determine which ACTH assays are used in Croatia and to assess the performance of those assays.
Materials and Methods: Results were obtained from laboratories that participated in all 4 cycles of ACTH EQA schemes (CROQALM) in 2013, 2014 (1/2014 and 3/2014) and 2015. The control samples were commercial, lyophilized human sera specimens with median concentrations of ACTH:33,15 pmol/L (2013); 2,9 pmol/L (1/2014); 10,6 pmol/L (3/2014) and 3,55 pmol/L (2015). In each cycle mean value (X), standard deviation (SD) and coefficient of variation (CV) were calculated.
Results: From 2013 until 2015 number of laboratories which measure ACTH has increased from 8 to 14. All the participant's results were divided into 3 groups according to the method/analyzer combination (Beckman Coulter CLIA,Roche Elecsys/Modular/Cobas ECLIA,Siemens Immulite CLIA). For all methods calculated CVs were: 31,5% (2013), 30% (1/2014), 22,9% (3/2014) and 46,7% (2015). For Roche Elecsys/Modular/Cobas ECLIA group calculated CVs were: 29,2% (2013), 27,3% (1/2014), 34,6% (3/2014) and 39,8% (2015). In other two groups the number of results was too small for statistical analysis.
Conclusion: Our results show a significant variability in interassay and interlaboratory performance for ACTH. The highest CV is obtained in control samples with low ACTH concentrations. However, this variability is also very important to keep in mind when cut-off ACTH values are interpreted. In our EQA scheme with upper cut-off ACTH concentrations two laboratories (14%) reported results which would be classified incorrectly. Every laboratory should evaluate the performance of their ACTH assays and ACTH results have to be interpreted with caution.

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PERFORMANCE EVALUATION OF THE NEW HEMATOLOGY ANALYZER SYSMEX XN-1000: A COMPARISON STUDY WITH SYSMEX XE-5000 AND XS-1000I

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Key Words: performance evaluation, comparison, hematology
Background: The new Sysmex XN-1000 hematology analyzer has been introduced to routine laboratory along with the XE-5000 and XS-1000i, so the aim of this study was performance evaluation as well as interchangeability of the obtained results for the clinical practice.
Materials and Methods: Verification studies were performed according to CLSI H26A2. Obtained results from XE-5000 and XS-1000i using analytical methods accredited according to ISO 15189 were within the target values of internal and external quality assessment-EQA (WHO

EQA Scheme for hematology, UKNEQAS and Labquality). The XN-series offers two new channels; WNR (separates NRBC, basophiles and other leukocytes for every sample) and WDF (provides improved differentiation between lymphocytes and monocytes using flow cytometry with semiconductor laser). Comparison studies were performed on 50 routine samples (without flags) on all three analyzers. Passing and Bablok regression analysis and Bland-Altmans plots were calculated using MedCalc software.
Results: Verification studies of the new Sysmex XN-1000 fulfil analytical quality specifications. Expanded measurement uncertainty was calculated for all measured parameters, including the declared uncertainty of Sysmex calibrator traceable to Internationally recognised reference methods for erythrocytes, leukocytes, platelets, hemoglobin and hematocrite. The conditions for Passing Bablok analysis were achieved for all analyzed parameters, except for platelets, showing a constant error. Specifically, intercept value was 7.36(95%CI:2.76-12.02), slope 1.01(95%CI:0.99-1.04), and the mean bias value calculated by Bland-Altman plot 7.0%, with the agreement range (±1,96SD) -23.6%-37.6% when comparing XN-1000 to XE-5000. When comparing XN-1000 and XS-1000i, intercept was 4.01(95%CI:0.34-7.42), slope 1.05(95%CI:1.03-1.07), and the mean bias value 9.2% with the agreement range -12.9%-31.4%.
Conclusion: The new Sysmex XN-1000 has fulfilled analytical quality specifications. Comparison studies showed possible interchangeability of the obtained results for the clinical practice. The more extensive evaluation study including samples with flags along with manual blood-film counts are planned in the second phase of analytical evaluation.

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INDICATORS OF QUALITY IN THE PREANALYTICAL PHASE FOR DETERMINING CARDIAC MARKERS IN ACUTE MYOCARDIAL INFARCTION-CK, CKMB AND TNT

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Key Words: miocardial infarction, quality indicators, haemolysis, immunological status, Troponin T, CKMB
Background: For the early diagnosis of acute myocardial infarction and increasing patient safety is important the standardization indicators of quality (QIs).
Matherials and methods: This study seeks influence of two types of factors that interfere in preanalytical phase: the primary sample aspect (hemolysis) and the immunological status of the patient. The prospective study, carried out for 6 months on hospitalized patients aimed at assessing the effect of these factors on the values of CKMB (immunoturbidimetric method) associated with total CK, and troponin T-TnT (hsst ECLIA). We assess the degree of non-compliance through visual inspection of samples after primary processing.
Results: We compared the results of the measurements performed on non-compliant samples with those obtained for samples receveid after repeated request. The analysis of the results showed the occurrence of falsely elevated CKMB values – even 200% higher for hemolysed samples. Very high TnT hsst values-more then 3000 ng/mL, was obtained in patients with altered immune status.
Conclusion: Study results confirmed the importance of QIs established as samples rejecting criteria in preanalytical phase and the need for effective communication with clinicians.

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COMPARISON OF TWO DIFFERENT CHEMILUMINESCENT IMMUNOASSAY ANALYZERS

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Key Words: immunoassay, thyroid hormones, fertility hormones, cardiac markers
Background: The aim of this study is to compare analytical performances of two chemiluminescent immunoassay analyzers (Beckman UniCel® DxI 800 and Siemens ADVIA Centaur).
Materials and Methods: Analytical performance was evaluated by measuring levels of thyroid hormones (fT_4, fT_3, TSH), fertility panel (β-HCG, E_2, progesterone, PRL, FSH, LH) and cardiac markers (CK-MB) by both instruments. Method comparison study was planned as linearity, within-run and between-day precision studies and performed for all parameters. Within-run precision study was performed by measuring control samples consecutively for 20 times for each parameter. Between-day precision study was performed by measuring high and low control samples for 10 days repeating twice a day. Measurement results were evaluated by linear regression analysis using Microsoft Office 2013 Excel.

Results: Correlation coefficients were between 0.89-0.94 for thyroid hormones, 0.96-0.99 for fertility panel and 0.93-0.99 for cardiac markers. Coefficient of variance (CV) values were found to be smaller than 6% for all parameters. CV values of between-day precision study were found to be below 10%.

Conclusion: The analytical performances of Beckman UniCel® DxI 800 and Siemens ADVIA Centaur are found to be similar for thyroid hormones, fertility hormones and cardiac markers.