Abstracts of Scientific Sessions

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The proteasome in collaboration with ubiquitin, whose covalent modification of target proteins marks them for degradation, rapidly and selectively degrades unnecessary proteins that must be eliminated from the cells, such as short-lived regulatory proteins as well as proteins with aberrant structures caused by various stresses. Over the past 30 years, our research work has focused on elucidating the basic mechanisms of eukaryotic proteasomes. The 26S holoproteasome is a 2.5-MDa multisubunit complex that contains a catalytic core particle (CP) and two regulatory particles (RP). The CP consists of four heptameric rings (two outer α-rings and two inner β-rings), which are made up of seven structurally related, but not identical, α and β subunits (i.e., α1, β1, β2, and β5 respectively, which emphasized their key role for the thymoproteasome in the development of the MHC class I-restricted CD8+ T cell repertoire during thymic selection. The discovery of these two types of vertebrate-specific immuno-type proteasomes highlights the significance and impact of our biological studies on the proteasome.

EXTRACELLULAR CONCENTRATIONS OF IRON ARE REGULATED BY THE PROTEASOME

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Extracellular concentrations of iron are regulated by the peptide hormone hepcidin. Hepcidin controls the transfer of iron from cells to plasma by regulating the cell membrane concentration of the sole known iron exporter, ferroportin. Hepcidin binding to ferroportin causes its endocytosis and proteolysis, trapping iron inside cells that normally transport iron to plasma: duodenal enterocytes, macrophages recycling erythrocyte iron, and hepatocytes that store iron. Iron consumption, mainly for hemoglobin synthesis, then depletes plasma of iron causing hypoferremia and limiting iron delivery to tissues. Hepcidin production is transcriptionally induced by iron-transferin through a complex pathway centered on the bone morphogenetic protein (BMP) receptor, interacting directly or indirectly with iron sensors transferrin receptors 1 and 2, and associated modulators HFE, hemojuvelin, TMPRSS6 / matriptase 2, BMP-6, and others. Another unknown pathway regulates hepcidin in response to intracellular iron concentrations in the liver, partly by modulating the secretion of BMP-6. As a mediator of innate immunity, hepcidin is greatly increased by inflammation, predominantly through IL-6. Finally, hepcidin is suppressed during stress erythropoiesis, induced by endogenous or exogenous erythropoietin. Hepcidin suppression with resulting iron overload is a pathological manifestation of ineffective erythropoiesis, e.g., in thalassemias. Hereditary hemochromatosis is an iron overload syndrome commonly caused by hepcidin deficiency leading to hyperabsorption of dietary iron. Hepcidin is deficient because of mutations in one of its regulators or rarely the hepcidin gene itself. Iron-loading anemias result from the suppression of hepcidin production by hyperactive but ineffective erythropoiesis. Erythrocyte transfusions partially relieve the erythropoietic suppression of hepcidin but cause severe iron loading because of their high content of iron. Iron-restrictive anemias result from excessive hepcidin production due to inflammation or mutations in matriptase 2/TMPRSS6, causing hypoferremia that limits iron delivery to hemoglobin synthesis. Understanding of these regulatory pathways is leading to improvements in diagnosis and treatment of disorders of iron homeostasis.

SICK MOLECULES AND AMYLOIDOSIS

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An increasing number of diseases are recognized to arise from the failure of proteins to adopt functional conformational states. These pathologic conditions are generally referred to as protein misfolding (or protein conformational) diseases. These proteins behave like "sick molecules", a term coined by Jan Waldenström, since they display a pathological conformation prone to aggregate and become toxic for cells and tissues, producing devastating damage. The largest group of misfolding diseases is associated with the conversion of peptides or proteins from their soluble functional states into highly organized fibrillar aggregates showing a cross-beta secondary structure termed "amyloid." It is becoming increasingly apparent that amyloid-forming proteins exist in a complex dynamic equilibrium between soluble monomeric or oligomeric states and various insoluble states of higher-order aggregation. The formation of these aggregates depends on the protein concentration, complex interactions with other molecules and the specific cellular environment. Several lines of evidence support a role for extracellular chaperones in the in vivo clearance of aggregation-prone proteins. To date, at least 28 different proteins have been identified as causative agents of amyloid diseases, ranging from localized cerebral amyloidosis in neurodegenerative conditions, to systemic amyloidoses such as immunoglobulin monoclonal light chain amyloidosis and transthyretin amyloidosis. The process of amyloid formation results in cellular injury, tissue damage, and organ dysfunction through mechanisms that are incompletely understood. The simple explanation of a physical, mechanical replacement of parenchymal tissue by amyloid deposits seems to be insufficient. A growing body of literature has implicated prefibrillar oligomers, rather than the fibrillar form, as the primary...
primary pathologic species. Direct cytotoxicity of amyloidogenic immunoglobulin light chains to cardiac cells has also been demonstrated. The clinical chemist plays a central role in the diagnosis and management of these complex diseases. Advances in biomarker studies have enabled detection of amyloid pathology in vivo in presymptomatic stage, before irreversible organ damage has occurred, providing the basis for early intervention trials. The accurate typing of the amyloid deposits is the prerequisite for designing the appropriate therapeutic strategy and involves the precise identification of the amyloid protein by mass spectrometry-based technologies. The assessment of the organ damage by novel biomarkers allows monitoring the efficacy of treatment. Advances in deciphering the molecular mechanisms underlying the amyloid process are leading to the development of novel therapeutic resources and strategies.

PL3 MOLECULAR BASIS AND CLONAL EVOLUTION OF MYELOPROLIFERATIVE NEOPLASMS

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Excessive production of terminally differentiated myeloid cells is the hallmark of myeloproliferative neoplasms (MPN). Current classification of MPN by the World Health Organization includes nine disease entities, however, only the classical BCR-ABL negative MPN are the subject of this overview. The classical MPN include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The discovery of a gain of function mutations of the JAK2 kinase facilitated the molecular diagnosis of MPN and also offered a target for therapeutic intervention. Although the majority of MPN patients are positive for the JAK2 exon 14 mutation (V617F), one third of patients with MPN are negative for JAK2 mutations. The MPN phenotype of JAK2-V617F negative patients has been in some cases associated with exon 12 JAK2 mutations or mutations in the thrombopoietin receptor gene MPL but they are present only in 1-5% of the cases. Therefore, considerable effort has been exerted to identify other disease associated mutations. This review will be providing an overview of these findings and discuss the role of other genes in the pathogenesis of MPN.

PL4 ACUTE CORONARY SYNDROMES – THE PRESENT AND FUTURE ROLE OF BIOMARKERS

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Over the past two decades there have been dramatic changes in the diagnosis, treatment and prognosis of acute coronary syndromes (ACS). Several new treatment modalities have been added. The prognosis after acute myocardial infarction (AMI) has improved, e.g. the 30 day mortality has been more than halved the last decade. Biomarkers have crucial roles in the management of ACS: for diagnosis as well as for prognosis and selection of treatment. At present, cardiac troponin (cTn) is the biomarker of choice for diagnosis of AMI. Currently, there are no other biomarkers, especially after the introduction of the high sensitivity assays, which can compete, neither regarding specificity nor regarding early sensitivity. However, there is still a clinical need of a biomarker able to reliably rule-in or rule-out AMI, immediately on admission. Secondly, a marker able of separating type I from type II infarctions would be clinically very useful. Thirdly, for the diagnosis of unstable angina a biomarker of cardiac ischemia is needed. MicroRNAs seem to be the most promising new candidates for diagnostic purposes. The optimal combination of biomarkers and new imaging techniques is another important area for research. The list of biomarkers associated with an adverse prognosis in ACS is long. However, for most of them it has been very difficult to prove an added clinical value. Only cTn, and to some degree also B-type natriuretic peptides, is widely used in clinical practice for risk assessment. Among new markers, growth differentiation factor 15 and the mid-regional part of the prohormone of adrenomedullin, have shown some promising results. The importance of moderate decrease in renal function for the prognosis of ACS has been much overlooked in the past. And since the renal function must be assessed anyway, i.e. for correct dosing of many drugs, it seems logical to utilize also the prognostic capacity of markers of the renal function. Cardiac troponin has been proven useful for selection of antithrombotic, antiplatelet and invasive treatment. Besides cTn, no other markers have consistently been shown to be useful for selection of specific treatments. However, in the predicted future era of “personalized medicine”, new biomarkers for selection of treatments are much needed.

SY01 BEYOND CREATININE STANDARDIZATION

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Background: Recent global restandardization has enabled clinicians to use eGFR values. National implementation programs have promoted the use of eGFR, which is an important tool to detect chronic kidney disease at population level. However, some problems remain after standardisation. Results: eGFR formulas (e.g. MDRD, CKD-EPI) show limitations (e.g. age, ethnicity). In clinical practice, laboratories and clinicians often do not take these limitations into account. Expanding the formula to normal range results in remarkable findings: e.g. increased hazard ratio of death among patients with an eGFR >90 mL/min per 1.73m2. This finding may be due to inadequacies of eGFR formulas at low serum creatinine levels. In children, where serum creatinine values are low, uncertainty remains a problem. Cystatin C determination might offer an interesting alternative for GFR estimation. The recent availability of the IFCC cystatin C standard will contribute to the popularization of cystatin C as a renal function marker. As the lion share of literature dealing with drug dosage in renal insufficiency is based upon older (often poorly characterized) creatinine methods, the effects of creatinine standardization on drug doses calculation are important. Pharmacokinetic studies based on nonstandardized methods obtained results that were method dependent. Recommended drug dosages were inconsistently translated into clinical practice due to methodological variability. It is impossible to re-express all current drug-dosing recommendations for use with standardized creatinine values. For the majority of patients and for most drugs, there is little difference in the calculated dose according to the equation used. However, using eGFR in very large or very small patients, reported GFR values should be multiplied by the body surface area to obtain eGFR. Assessing kidney function using alternative methods (e.g. measured CrCl or measured GFR using exogenous markers) should be considered for drugs with narrow therapeutic indices, or for individuals in whom eGFR and CrCl provide different estimates, or for patients in whom estimates based on creatinine are likely to be inaccurate.
Acute kidney injury (AKI) is a frequent complication in hospitalized patients and associated with substantially increased morbidity and mortality. There is a delay in the diagnosis of AKI, at least in part, resulting from currently used diagnosis parameters basing on renal function such as serum creatinine and urine output. Such delay contributes to the fact that to date, no effective intervention to prevent or successfully treat AKI has been found. At the time of diagnosis of established AKI, irreversible organ damage may already have occurred. Currently, several acute tubular damage markers have been introduced, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18, liver-type fatty acid-binding protein and kidney injury molecule 1. Exemplary for other acute tubular damage markers, in the following the presentation will focus on NGAL given the most comprehensive experimental and clinical data available in the literature. NGAL is a protease-resistant polypeptide with a molecular weight of 25 kDa, which is released from the distal nephron in response to ischemic, toxic, or inflammatory insult to the kidney or from other organs. NGAL is a transcellular transporter of iron in specific conformation. As a siderophore-iron complexing molecule, NGAL is involved in renal tubular cell differentiation and growth. Within a few hours after renal insult NGAL and other tubular damage markers are detected in urine and plasma which is 24 to 72 hours prior to renal function marker-based AKI diagnosis, which is the gold-standard in current clinical practice. Available data from experimental and clinical studies suggests NGAL to be a reliable 'real-time' biomarker for AKI of diverse etiology. NGAL may also be helpful in distinguishing volume depletion from oliguria in the setting of AKI. Novel renal biomarkers indicating cellular damage in real-time might soon guide patient-tailored earlier initiation of nephroprotection, improved fluid management or withdrawal of nephrotoxins directed at improvement of outcomes in patients developing AKI. Since, the recent, Acute Dialysis Quality Initiative' Consensus Conference has now recommended the use of tubular damage markers for AKI diagnosis complementary to renal function markers.
technique to classify renal biopsies, reading beyond the traditional histopathology. Differentially expressed proteins detected through MALDI-IMS represent powerful classifiers of nosologic groups and may help subclassify each disease according to glomerular cellular composition, interstitial damage or systemic co-factors. Moreover, this approach may detect early changes in the tubules or the surrounding tissue, possibly of prognostic value for the outcome of the pathologic process.

OC02

CYSS27CYS POLYMORPHISMS OF THE PAPP-A GENE (PREGNANCY ASSOCIATED PLASMA PROTEIN A) IS RELATED TO MORTALITY OF LONG TERM HEMODIALYSIS PATIENTS

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Background: PAPP-A is an independent mortality predictor of long term hemodialysis patients and a prognostic marker of acute coronary syndrome in general population. Cys327Cys PAPP-A polymorphism(SNP) (rs12375498, exon 2) was found to be of significance in preeclampsia and the C allele of the PAPP-A C/G SNP (rs13290387, intron 6) was defined as an independent risk factor for acute myocardial infarction. The aim of the study was to test the role of these two PAPP-A SNPs in long term hemodialysis patients.

Methods: The studied group consisted of 464 subjects – 319 long term hemodialysis patients (183 men and 136 women, mean age 62±14 years) and 145 controls (65 men and 80 women, mean age 49±14 years). A subgroup of 211 hemodialysis patients (118 men and 93 women, mean age 63±13 years) was prospectively followed up for 4.5 years. During the follow up, 111 patients died, 51 of them due to cardiovascular events. PAPP-A SNPs were analysed by DNA sequencing and serum PAPP-A was measured by TRACE technique to classify renal biopsies, reading beyond the traditional histopathology. Differentially expressed proteins detected through MALDI-IMS represent powerful classifiers of nosologic groups and may help subclassify each disease according to glomerular cellular composition, interstitial damage or systemic co-factors. Moreover, this approach may detect early changes in the tubules or the surrounding tissue, possibly of prognostic value for the outcome of the pathologic process.

Results: Both SNPs were in Hardy-Weinberg equilibrium. Allelic and genotype frequencies did not differ between patients and controls and were not related to serum PAPP-A concentrations. Cys327Cys SNP was significant for patients’ survival while C/G SNP was not. Presence of the mutated allele was significant for overall and cardiovascular mortality (HR(95%CI): 1.616 (1.110-2.353), P=0.012; 1.757 (1.018-3.030), P=0.043, respectively).

Conclusions: Our study shows for the first time the significance of Cys327Cys PAPP-A SNP (rs12375498) for overall and cardiovascular mortality of long term hemodialysis patients.

SY04

ENSURING POCT QUALITY IN THE COMMUNITY

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POC instruments are increasingly used throughout the community. Instruments are used at GPs offices, pharmacies, nursing homes, departments in the hospitals and in the hands of patients. How can the quality of these instruments be ensured? There are relatively more errors of POC instruments in the analytical phase compared to central laboratory instruments. But, of course, errors in the pre-analytical and post-analytical phases are not less important. First of all, politically it has to be decided that quality of POC instruments is the responsibility of the laboratory profession. It should be a goal for laboratory medicine to ensure the quality of the equipment of POC instruments wherever they are situated. Most clinicians and patients do not know that POC results can be misleading and prone to errors. Therefore an important aspect will be education of the users of the instruments, both in pre-analytical errors, in how to choose the right instrument for their use and how to run it and of course the post-analytical aspects e.g. how to report the results. As a laboratory profession we have critically to judge if – and how to - use traditional internal quality control. External quality control for POC instruments have to be improved securing that we can get information on both participant performance as well as method performance. To be able to do this, improved quality control material has to be used and/or methods to improve the “commutability of the EQA schemes” have to be developed.

SY05

ENSURING POCT QUALITY IN THE HOSPITAL ENVIRONMENT

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The quality of point-of-care tests (POCT) within different segments and between different parts of the health-care chain is currently insufficiently harmonised. Status of guidelines regarding the different segments of POC glucose measurements as one of the most profound used POC test will be presented. Connecting all POCT instruments in and outside a hospital to central databases is an essential step in improving the continuity of data in the health-care chain. This lecture will address the involved necessary and standardised requirements from a laboratory point-of-view. Patients privacy is at stake with increasing digital communication. The White House in the US has launched an initiative in 2010 to improve the privacy of patients and others in this market. The increasing use of wifi and other digital applications in and outside hospitals makes this initiative even more relevant. The clinical chemistry society needs to be more involved in solutions in this area invented for telecom and finance market because these solutions will in the end or do already enter the medical POC-market. Currently, telemedicine based on remote POCT is increasing rapidly. However, tele-medicine can be a dangerous exercise if it is based on patient identification only. Patient authentication is an essential improvement for the near future. An example how to perform safe digital authentication with minimal patient credentials involved will be presented. This lecture will show some validation topics to ensure the quality of POCT in the hospital which may be useable in the whole health care chain. The challenges in the critical care setting will be described regarding e.g. continuous glucose monitoring vs. point-to-point glucose comparison studies, statistic parameters involved and power of these statistic parameters. Differences between glucose measurements in a hospital vs/home-use setting and consequences for the quality of the glucose measurement will be presented.
Patients' views of laboratory testing are they will "have some bloods done" determining either their diagnosis, treatment changes or that monitoring is effective. The lack of understanding of how tests are done does not mean that patients do not understand the need for the quality. Patients are also familiar with testing for themselves e.g. pregnancy tests or blood glucose checking for diabetics; either through OTC devices, Point of Care Testing (POCT) in pharmacies or through the internet; for a POCT service patients make the assumption it is as good as the bloods that are sent for "testing". As a patient I expect my results to be guaranteed as being from me, accurate and done immediately; I want the result without an anxious wait; I may not understand the concept of error, to me a mistake; nor do I understand probability, I assume the result is absolute! This is a rather simplistic view: there is a spectrum, some patients with knowledge and experience in other fields understand that there may be variation, that there may be mistakes (errors); however a value is assumed to be correct: many have no such insight. We know about the many errors in the sample-result pathway for central laboratories, surprisingly such work is unavailable for POCT, excepting the proximity of analysis and return of result element all the others are the same as the central laboratory pathway: there are the same requirements for quality. Patients are aware of quality, they may see company vehicles with an ISO 9001 a demonstrable quality standard, so there will be a standard for my test and it will be done to the required standard: this implies professionalism. Hence, I want my POCT test to be convenient, something I can trust, supervised by professionals so that I can have confidence in getting the quality investigations I need to ensure quality outcomes for my disease processes: it is up to you, as the healthcare professionals who understand these things, to make sure my expectations are met!

**OC03**  
**A SURVEY OF LABORATORY ANALYSES PERFORMED IN PRIMARY HEALTH CARE IN SWEDEN**


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In Sweden, in primary health care, there is a lack of basic information on the different types of laboratory test performed and at what quality. There is not even accurate statistics on the number of primary health care centers within the country. EQUALIS (the provider of external quality assessment for clinical laboratory investigation in Sweden) initiated a study to gather this valuable data by creating a survey. The purpose of the survey was to understand the analyses done in Sweden by primary health care providers and occupational health service, how they are done, and at what level of quality. This has included not only the tests performed, but also the types of personnel involved in proving the testing (doctors, nurses, or laboratory technicians). The main objectives were to learn whether the number of doctors (a necessity for providing certain tests) and distance to the closest hospitals from the primary health care center. Can the number of doctors and the distance to hospitals explain the number of analyses performed at the primary health care centers? Furthermore, since this study includes the entire nation of Sweden, one important research objective is whether there were regional differences present within Sweden? Since no actual estimate of the number of primary health care centers is available in Sweden, study purchased 700 addresses to complement those that were already members of EQUALIS. The survey results covered roughly 22 percent of the Swedish population. Statistical analysis, including generalized linear models (Poisson GLM), geographic information science (GIScience), and cluster analyses were performed. Results of the study show that distance to hospitals has no bearing on the number of tests performed or on whether a laboratory was quality assured. Furthermore, doctors showed a negative association with quality assurance. Statistics on individual tests were also gathered, including frequency of a test and at what quality it was done. Finally, there were strong regional differences discovered in the frequency of tests analyzed in various regions of Sweden.

**OC04**  
**ERRORS IN BLOOD GAS POCT: A METHOD TO QUALIFY NONCONFORMITIES AND ADDRESS EFFECTIVE TRAINING FOR POCT OPERATORS**

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Background: 22 blood gas analyzers (Instrumentation Laboratory; n. 10 Gem 4000, 12 Gem 3500) have been placed and used since 2010, as Point-of-Care testing (POCT), at the University Hospital of Bari. Laboratory's staff supervise all processes through Gem Web Plus system. Intelligent Quality Management (IQM) and External Quality Assessment (EQA) programs, ensure blood gas analysis quality assurance. GEM 4000 analyzers were interfaced with Laboratory Information System (LIS). All operators have been formally trained for the process management and the operating procedures. To tracking pre-analytical errors, major nonconformities (NC) we evaluated along 12 months.

Method and materials: Thanks to LIS and Gem Web Plus, we have identified two classes of NC: Management NC (MNC), strictly linked to the registration –phase (typing patient demographic into LIS and bar-code generation) and Process NC (PNC) strictly linked to the inadequate sample handling; corrective actions have been taken to specific operating unit, accordingly.

Results: Three MNC categories caused failed data -transmission to LIS: planned analysis but not run in LIS mode (MNC1); analysis with wrong sample ID (MNC2); analysis with re-used sample ID (MNC3). Three PNC categories caused the analysis (MNC1; analysis with wrong sample ID (MNC2); analysis with re-used sample ID (MNC3). Three PNC categories caused the analysis complaint: air bubble into the sample (PNC1), NC incidence was as follows: MNC1=67%, MNC2=7%, MNC3=26%, PNC=8% (n.2448/29298 test); PNC1=58%, PNC2=33%, PNC3=8%. NC showed a decrease across the year which was significant for MNC (6% vs 3%) but not for PNC (7% vs 6%). In the Emergency Room (E.R) was found the highest incidence in PNC (42%). Following a certified training session, where all
operators were undergone to an interactive and self-evaluation session thanks to the Gem Web Plus, after a three-months period, NCP in the E.R. were decreased to 23%.

Conclusions: A systematic method for identifying management and process Non conformities supports the lab to better manage blood gas POC testing. In a critical setting such as E.R., is essential to constantly monitor the operator’s skills and enforce corrective actions to reduce the clinical risk caused by errors in the “total POCT blood gas process”.

SY07
ANALYTICAL TOXICOLOGY OF EMERGING DRUGS OF ABUSE
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Background: In recent years an ever increasing number of novel psychoactive substances has emerged on the recreational drug market. They belong to various drug classes and may have psychostimulant, hallucinogenic or cannabis-like properties. At the time they first appear on the market, these emerging drugs are generally not scheduled as controlled substances and therefore sold as “legal highs” and in part labelled as e.g. incense, research chemicals, or bath salts.

Methods: To the analytical toxicologist the emerging drugs are an on-going challenge. At the time they first appear, often not even their chemical structures are known. Once these have been elucidated and reference substances have become (commercially) available, laboratories working in the field of clinical and forensic toxicology must either update existing methods or develop new ones to cover these novel psychoactive drugs. This presentation will provide an overview on the chemical structures of different classes of emerging drugs (e.g. cathinones, synthetic cannabinoids) and on analytical procedures for their analysis in biomatrices most relevant to clinical and forensic toxicology (blood, urine, oral fluid, hair). Different analytical strategies (targeted vs untargeted analysis) and techniques (immunoassays, gas chromatography-mass spectrometry [GC-MS], liquid chromatography-mass spectrometry [LC-MS/MS]) will be discussed. This will include aspects of sample workup, analyte separation, and detection modes.

Results: The majority of the emerging drugs are not sufficiently detectable by commercial immunoassays for conventional drugs of abuse testing, but amenable to analysis by hyphenated chromatographic-mass spectrometric techniques. While the respective equipment is available in most forensic and clinical toxicology laboratories, analysis of emerging drugs generally requires modification of established or development of new analytical methods.

Conclusion: The occurrence of ever new psychoactive compounds on the drug market calls for regular updates of analytical methods in clinical and forensic toxicology laboratories.

SY08
BIOMARKERS OF ETHANOL CONSUMPTION
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A clinician should evaluate not only a recent/acute alcohol abuse, but also a long-term ethanol consumption. The choice of a proper biological matrix as well as the right biomarker is crucial for this purpose. For example, the evaluation of “Driving under the influence of alcohol” is carried out through the determination of Breath Alcohol concentration (BrAC) or through the determination of Blood Alcohol Concentration (BAC). BAC is the analytical procedure that guarantee a result with the highest diagnostic sensitivity and specificity. The determination of alcohol in urine is performed whether a recent alcohol intake diagnosis is required. Also direct phase II metabolites of ethanol, ethyl glucuronide (EtG) and ethyl sulfate (EtS) are also frequently used as potential markers of alcohol intake. They can be detected in all the biological fluids for several hours (up to 80 h in urine). Another biomarker for recent alcohol abuse is 5-hydroxytryptophol (5-HTOL) that is a minor metabolite of serotonin, produced in higher amount when acetaddehyde (the main metabolite of ethanol) affect the transformation of serotonin. For several years liver enzymes such as serum gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) have been used as state biomarkers for chronic alcohol abuse. These enzyme reflect the activity of the liver; however they showed low sensitivity and specificity. So far, the most used biomarker for ethanol dependence is a version of the glycoprotein transferrin, the carbohydrate deficient transferrin (CDT). Despite a relative high rate of false negative CDT is a well-characterized biomarker for chronic alcohol abuse. Another promising biomarker is blood phosphatidylethanol (Peth). This is an abnormal phospholipid produced only in the presence of ethanol consumption by the phospholipase D. Recently EtG and fatty acid ethyl esters (FAEE) detected in hair showed a very high diagnostic sensitivity and specificity for chronic excessive alcohol consumption as well; the Society of Hair Testing (SoHT) edited an International Consensus on the use of these compounds for forensic purposes, indicating the cut-off and analytical procedures for their determination in the keratin matrices.

SY09
ORAL FLUID DRUG TESTING
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Background: Over the last decades, knowledge concerning oral fluid (OF) and its possibilities for monitoring the presence of drugs of abuse (DOA) for clinical and forensic purposes has increased enormously. Especially in the domain of testing drivers under influence of DOA (DUID), OF testing has gained popularity due to its non-invasive collected directly on-site without hampering privacy. Several on-site immunosorbent assays (OIA) were developed and evaluated over the years. For confirmatory toxicological analysis, the use of OF has been hampered by the influence of the salivary composition on the final drug concentrations. The OF collection protocol, the degree of stimulation of salivary flow, the physicochemical properties of DOA, and the type of drug administration are some of the parameters that influence the OF drug concentrations. In addition, OF influences the analytical procedure through matrix effects and recovery issues. For the final result, the analyst will also have to pay attention to the collected volume, the dilution factor of the collection device and to the stability of the sample. As a result, toxicological analysis of OF samples and the final interpretation is not always straightforward.

Material and methods: Laboratory studies and samples obtained from DUID will be used to evaluate OIA (Drugwipe and DrugTest) and quantitative methods using OF collection devices such as StatSure® (StatSure Diagnostic Systems Inc), Quantsis® (Immunalysis) and Certus® (Concateno) in
EMERGING TRENDS IN DRUG USE AMONG YOUTH

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Background: User surveys show that there have been significant changes over the last years in the recreational drugs being used by young people and available through conventional and new telematic sources. Whereas cannabis and cocaine remain the most used drugs of abuse new designer drugs, psychoactive medications, dietary supplements, alcohol mixed to energy drinks, anabolic steroids are emerging trends in youth consumption.

Methods: Forums, social media, online shops, websites and other internet sources have been extensively and regularly monitored in cooperation with the Italian anti-adulteration and safety bureau (NAS) for emerging trends of novel drugs among young people. Different products sold via internet or through illegal venue were seized, listed, photographed and analysed by systematic toxicological analysis using GC-MS and/or LC-MS/MS methodologies.

Results: Products seized using “purchase by false identity” in websites or inn illegal venues by NAS contained e.g. psilocin found in “magic truffles”, 1,3-dimethylamylamine and sildenafil in dietary supplements, alcohol mixed to energy drinks, anabolic steroids are emerging trends in youth consumption.

Discussion: These emerging trends in drug use among young people are certainly a niche phenomenon in drugs market and present some peculiarities, which differentiate from “classical” drugs of abuse. These substances are available via the Internet with no contact with the dealer, no possibility to reach the ultimate user and marked “not for human use”, or “plant material for collection”, so the dealers are not legally responsible if the products are consumed in a different way from that indicated in the labels. Moreover, they are legal for the period of time when they are sold but when they are included in the specific banning laws, new products (structurally similar to the previous one) are sold in place of the previous ones.

Conclusions: Although the use of these new drugs is increasing, no toxicity studies in animal or human models and no analytical assays are available so that side effects, long-term adverse effects and eventual forms of intoxications are unknown, so is the way to treat them.

AN ELEVATED CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) LEVEL DUE TO PHOSPHOMANNOSE ISOMERASE DEFICIENCY IN AN ASYMPTOMATIC ADULT WOMAN WITH CONGENITAL DISORDER OF GLYCOSYLATION OF THE MPI-CDG SUBTYPE

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Background: In connection with a routine company health check-up, a 32-year old woman showed a markedly elevated level of carbohydrate-deficient transferrin (CDT) in serum, a biomarker for long-term heavy alcohol consumption. Because there were no other signs of excessive drinking, this study aimed to disclose the cause of her elevated CDT value.

Methods: Serum CDT (％diaryltransferrin) was determined by an HPLC candidate reference method and phosphatidylethanol (PEth) in whole blood, another long-term alcohol biomarker, by an LC-MS method. Analysis of enzymes causing defective transferrin glycosylation in congenital disorders of glycosylation (CDG), a family of rare complex metabolic disorders, was performed in cultured skin fibroblasts. The fibroblasts were also used for DNA sequencing analysis.

Results: The CDT level remained very high with 17％ diaryltransferrin (reference <2) and 3％ asialotransferrin (reference 0) on two separate occasions, despite a negative PEth test. This confirmed that the elevated CDT level was not related to heavy alcohol consumption. The HPLC analysis showed a “type I” transferrin pattern (i.e., elevated asialo- and diaryltransferrin) pointing to a defect in N-glycan assembly. The phosphomannomutase activity in fibroblasts was within normal limits but the phosphomannose isomerase (MPI) activity was very low (0.64 mU/mg protein; reference 2.3–6.9) indicating CDG of the MPI-CDG subtype (formerly called CDG-Ib). Mutation analysis of MPI revealed that the woman is homozygous for the c.656G>A mutation causing replacement of arginine by glutamine (p.R219Q). The women has not reported any of the clinical manifestations typically associated with MPI-CDG, and has thus never been treated by mannose.

Conclusions: CDT is considered a specific alcohol biomarker with little risk for generating false positive identifications of alcohol abuse. In this case report, a markedly elevated CDT level in an adult woman obtained in connection with a routine company health check-up was demonstrated not to be caused by heavy alcohol consumption but by MPI deficiency due to a homozygous mutation in MPI. The woman thus seems to be an asymptomatic MPI-CDG adult. Only one other similar case has been reported.

DETECTION, PROGNOSIS AND MANAGEMENT OF LARGE BULKS

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Background: Cellular DNA undergoes profound changes in methylation during cancer development, with hypermethylation occurring in specific gene promoters, amidst a backdrop of generalized hypomethylation. DNA methylation...
in cancer often causes the silencing of tumor suppressors and other genes important for cellular growth, regulation and differentiation. Thousands of publications describe the methylation status of hundreds of genes in cancer, however only a relatively small number of methylated genes are specific for an individual cancer type. Further, even fewer have been utilized as cancer biomarkers in the clinical laboratory.

Methods and results: The Septin9 gene was first described by Lofton-Day in 2008 to be differentially hypermethylated in colorectal cancer (CRC) tissue, and this hypermethylation was shown to be detected in the blood of CRC patients of all clinical stages using a sensitive molecular method. A more recently improved sensitivity method to detect methylated Septin9 has been demonstrated to detect 90% of CRCs with 88% specificity. Septin9 hypermethylation can be detected in the blood of patients with cancers arising from all regions of the large intestine, including the right side of the colon which is often difficult to reach in colonoscopy and which is challenging to detect using stool-based screening methods. The Septin9 test may have additional clinical applications as well as its established role in CRC screening and early detection. Individuals with inflammatory bowel disease, such as Crohn’s and Ulcerative Colitis, have an increased lifetime risk of developing CRC; studies are currently underway to determine if Septin9 testing may be useful in detecting cancer or even dysplasia in these patients who otherwise are subjected to frequent surveillance colonoscopies involving multiple biopsies at each procedure. Interestingly, Septin9, and like other genes differentially hypermethylated in cancer, has been shown to be highly methylated in the blood pregnant women. Conclusions: Septin 9 may represent the first example of a blood-based, DNA methylation test based a new class of oncofetal biomarker, akin to other well-known biomarkers such as CEA (carcinoembryonic antigen), AFP (alpha-fetoprotein) and CA-125, CA 19-9 and CA 15-3.

SY11

EPIGENETIC BIOMARKERS FOR EARLY DETECTION OF AERODIGESTIVE TRACT CANCERS IN BIOLOGICAL FLUIDS

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Cancers of the respiratory tract (lung and head and neck) contribute to more than 25% of human cancer-related mortality worldwide. Tumours along the respiratory tract share common aetiologies, risk factors and molecular characteristics. Major clinical challenges in reducing mortality from these cancers include the detection of early lesions, timely discovery of relapse and patient stratification into more efficient therapeutic regimens. Epigenetic reprogramming is one of the hallmarks of human cancer. DNA methylation is currently the best-studied epigenetic modification pointing to a large number of genes being silenced by hypermethylation. These genes are now looked as potential biomarkers for clinical management of cancer. DNA methylation possesses many characteristics, which make it advantageous in biomarker development. The biological function of DNA methylation, its covalent chemical nature, the stability during fixation and the durability of DNA is clinical specimens are some of such characteristics. The application of molecular biomarkers in biological fluids and specimens acquired in common clinical practice has been a long term demand. To date, there is significant literature on the applicability of DNA methylation biomarkers in a variety of specimens including bronchial washings, sputum, buccal swabs, saliva, plasma and serum. However, while the feasibility has been demonstrated, the diversity of methods and study designs makes comparison particularly complicated. In addition, lack of statistical power is a frequent problem. Last but not least, is the lack of a continuum in DNA methylation biomarker studies thus very few groups move into proper clinical validation. This underlines the need of large consortia contributing clinical samples and information as well as the use of a consensus on the use of robust, high precision assays. Clinical validation of DNA methylation biomarkers is very important, especially when running along computed tomography (CT) trials, where it may be able to assist in the management of indeterminate nodules.

SY12

DNA HYPMETHYLATION IN CANCER

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DNA methylation plays a central role in growth and development. Alteration of DNA methylation is a hallmark of cancer. Tumors present sites specific DNA hypermethylation and a global DNA hypomethylation. This overall loss of DNA methylation can be a relevant biomarker for cancer and other diseases. Thus, in recent years it has become apparent that there is a need to develop methods for the analysis of DNA methylation using different approaches: global, locus-specific, or genome-wide.

OC07

IDENTIFICATION AND ENUMERATION OF TUMOR SPHERES CULTURED FROM CIRCULATING EPITHELIAL TUMOR CELLS IN PATIENTS WITH SOLID CANCERS

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Background: The main cause of mortality of cancer patients is the formation of metastasis. A subpopulation of circulating tumor cells with stem-like properties is responsible for tumor initiation, invasive growth and metastasis formation. This population is termed cancer stem cells (CSCs). CSCs can be identified by different approaches one of them is assessment of their ability to grow as floating spheres. Methods: We enrolled 60 patients into the study suffering from breast (40), colorectal (10) and prostate (10) cancer in which circulating tumor cells (CETCs) were detected. CETCs were determined using the maintrac® method and viable CETCs were plated in suspension culture system allowed for tumor sphere formation. Cell viability and surface marker expression was evaluated by laser scanning cytometer. Results: Sphere formation was observed in 78% of patients with breast, colorectal and prostate cancer. The number of tumor spheres was dependent on the type of tumor and varied from 0 to 122 in breast cancer; from 0 to 30 in colorectal cancer and from 0 to 7 in prostate cancer per 1ml of blood. Furthermore, we found a highly significant correlation between the number of CETCs and the number of tumor spheres after
21 days of culture in breast cancer patients (r=0.867; P <0.005). The number of tumor spheres was always higher in patients with metastatic disease as compared to patients with non metastatic disease. There was no sphere formation in 10 healthy donors.

Conclusion: This study demonstrates that tumor stem cells are present in peripheral blood from metastatic and non-metastatic tumor patients. They represent a small subpopulation of circulating epithelial tumor cells with the ability to growth as tumor spheres.

OC08
MODULATION OF EXPRESSION OF CYTOKERATIN-19 FRAGMENTS (CYFRA 21-1) BY IRON AND RISK OF LUNG CANCER IN SOME WELDERS

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Background: The welding process is classified as probably carcinogenic and welders may be at risk of developing lung cancer. Limited evidence in humans and inadequate evidence in experimental animals of carcinogenicity of welding fumes and gases were therefore the underpinnings of this study.

Materials and Methods: Sixty (60) welders and fifty (50) healthy volunteers were recruited as controls. Interviewer styled questionnaire was administered to all participants for demographic, medical history and occupational features. Urine samples were obtained from all subjects for estimation of plasma iron, ferritin, transferrin saturation and Cytokeratin-19 fragments (Cyfra 21-1). Statistical analyses were performed using Student T-test, Mann – Whitney U, Kruskal Wallis and Pearson correlation.

Results Mean ages (yrs) of the welders and control were 42.97 ±8.75, 40.36±7.56 (P=0.100) respectively. There were statistically significant differences in the values of urinary iron, lead, zinc, nickel and cadmium using flame atomic absorption spectrophotometer. Blood obtained from venepuncture was used for the estimation of plasma iron, ferritin, transferrin, total iron binding capacity, transferrin saturation and Cytokeratin-19 fragments (Cyfra 21-1).

Conclusion: These findings suggest that the welders studied were at risk of developing lung cancer as evidenced by significant expression of Cytokeratin-19 fragments in their plasma which was most likely mediated by iron. Therefore, periodic biomonitoring of markers of exposure and effect would be beneficial to this group.

SY13
THE IMPORTANCE OF AUTOANTIBODY TESTING IN AUTOIMMUNE RHEUMATIC DISEASE

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Autoantibodies are diagnostic tools for most of the rheumatic diseases and in some cases they also are formal classification criteria. Moreover autoantibody profile is useful for identifying subgroups of patients in a given disease and in some cases are prognostic factors for co-morbidities or for peculiar clinical manifestations. In this regard autoantibody testing is not only useful but also essential for a correct management of autoimmune rheumatic diseases. There is evidence that an early diagnosis may allow a prompt treatment. Such a strategy has been found to be successful since an early and – in some cases – aggressive treatment may induce stable remission or allow the use of maintenance therapy at lower dosages with reduced side effects. Reliable results in autoantibody testing are mandatory for such treatment in the daily practice.

False negative results may delay the diagnosis with a consequent increased risk for complications or disease progression. At the same time, false positive results may require re-testing or un-necessary second step diagnostic assays. Altogether these processes may increase the direct and the indirect costs for the management of rheumatic autoimmune diseases. All these considerations explain the need for the best standardization of the available diagnostic test for the autoimmune rheumatic diseases.

SY14
ROLE OF ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES

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Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies mainly directed to proteinase 3 (PR3) and myeloperoxidase (MPO). By indirect immunofluorescence (IIF) on ethanol-fixed neutrophils (PMN) PR3-ANCA produce a cytoplasmic staining pattern (c-ANCA) and MPO-ANCA a perinuclear pattern (p-ANCA). The large majority of c-ANCA corresponds to PR3-ANCA but this is not the case for p-ANCA in relation to MPO-ANCA. So, a positive IIF-test should be followed by an antigen-specific test (mostly ELISA/ELIA). PR3-ANCA are very sensitive and specific for granulomatosis with polyangiitis (GPA, formerly Wegener’s) and MPO-ANCA for microscopic polyangiitis (MPA) and for Churg-Strauss syndrome with a vasculitic phenotype. Levels of ANCA follow disease activity to some extent, but a rise of ANCA titer is not highly sensitive nor specific for an ensuing relapse. A primary pathogenic role for ANCA in the associated small-vessel vasculitides is suggested by the observation that they appear before the clinical onset of GPA/MPA, the development of MPA in a newborn child from a mother with MPO-ANCA, and the associations of PR3-ANCA and MPO-ANCA with different MHC-class II antigens (being stronger than with the associated diseases themselves). Also in vitro and in vivo experimental data strongly suggest a pathogenic role for ANCA. In vitro, ANCA are able to activate primed PMN to full activation with the release of reactive oxygen species and lytic enzymes resulting in damage to endothelial cells, a process in which the alternative pathway of complement plays a reinforcing role. In vivo, induction of MPO-ANCA in mice and rats results in necrotizing glomerulonephritis and vasculitis also in the lungs. This is less clear for PR3-ANCA associated GPA in which granulomatous inflammation is present, particularly in the airways, in addition to vasculitis. Here, Th-17 mediated cellular immunity is involved as well.

In conclusion, ANCA are important diagnostic markers for small-vessel vasculitis with, supposedly, pathogenic significance.
However, to understand the causes and to address them, contributing to the variability of autoantibody measurements. There are many factors introducing more automation. There are many factors outside our direct control but as a scientific community, we are able to produce materials that can be evaluated and if appropriate, introduced as common calibration solutions for widespread use in assays. The IFCC is supporting the Harmonisation of Autoantibody Testing Working Group which has been working with the IRMM on evaluating and producing candidate materials for selected autoantibody tests. Our aims were to produce materials for the harmonisation of 5 clinically significant autoantibodies. An important component of the production of these materials was to generate the first robust definition of the process for making calibration materials for autoimmune serology. This is to ensure the long term availability of reference material but also to define the process sufficiently well to allow more analytes to added to the repertoire as necessary. The preliminary investigations of one material are complete and the development of the second material is progressing well. A plan to produce materials for further analytes will be developed. Reagent manufacturers in the field of autoimmune serology have been very supportive of this initiative but will want to see tangible benefits of using new, common materials. We are planning a project to investigate changes in between assay (method) variability that result from the introduction of these materials.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by autoantibodies against citrullinated antigens. The importance of citrulline for the epitopes bound by these autoantibodies, referred to as ACPA (anti-citrullinated peptide/protein antibodies), was first described in 1998. In addition to citrullinated proteins, cyclic citrullinated peptides (CCP) can be used as test substrates for detecting ACPA, which are only rarely found in diseases other than RA. The standard test for these antibodies is the second-generation CCP (CCP2) test, which is one of the best in terms of sensitivity and specificity. The generation of ACPA is an early event in the disease course, and is dependent on the presence of certain MHC class II alleles. ACPA in the inflamed synovium have been shown to associate with citrullinated antigens to form immune complexes, resulting in progression of the inflammatory process. The involvement of ACPA in the chronicity of RA is probably the reason why ACPA-positive patients have a more erosive disease course than ACPA-negative patients. The presence of ACPA has been included in the 2010 RA classification criteria. Thus, it is important to further standardize ACPA testing, for example by including an internal serum standard, which may lead to a better distinction between low and high ACPA levels. Recent data tend to indicate that ACPA-positive RA patients develop erosions earlier and the erosions become more widespread than in patients that are consistently ACPA-negative. The therapeutic window of opportunity to prevent the progression from early inflammatory synovitis to the chronic joint destructive phase of RA is narrow, and hence, early diagnosis and early therapy with disease-modifying agents under tight disease control is mandatory. Although citrulline is a common and essential feature of ACPA epitopes, ACPA represent a diverse set of antibodies that target distinct citrullinated epitopes. Multiplex ACPA assays are currently being developed to measure ACPA profiles and to investigate whether these have additional value for the early diagnosis and prognosis of RA patients.

The detection and quantification of IgG autoantibodies against normal components of tissue remain important tests in the diagnosis and management of patients with autoimmune diseases. The nature of the analysis and the lack of any robust reference materials have generated a group of tests that show astonishing variability. A sample known to be from a “normal” donor can show numerical values that vary 10 fold across the various methods, even though they are reporting results in the same units! This represents a very dangerous situation for patients that will be accentuated as labs consolidate and introduce more automation. There are many factors contributing to the variability of autoantibody measurements. However, to understand the causes and to address them, we need to begin the process of harmonisation. Many of the factors are outside our direct control but as a scientific community, we are able to produce materials that can be evaluated and if appropriate, introduced as common calibration solutions for widespread use in assays. The IFCC is supporting the Harmonisation of Autoantibody Testing Working Group which has been working with the IRMM on evaluating and producing candidate materials for selected autoantibody tests. Our aims were to produce materials for the harmonisation of 5 clinically significant autoantibodies. An important component of the production of these materials was to generate the first robust definition of the process for making calibration materials for autoimmune serology. This is to ensure the long term availability of reference material but also to define the process sufficiently well to allow more analytes to added to the repertoire as necessary. The preliminary investigations of one material are complete and the development of the second material is progressing well. A plan to produce materials for further analytes will be developed. Reagent manufacturers in the field of autoimmune serology have been very supportive of this initiative but will want to see tangible benefits of using new, common materials. We are planning a project to investigate changes in between assay (method) variability that result from the introduction of these materials.
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understanding where we are now, after active participation and strong involvement in the numerous meetings and questionnaires, propositions of amendments to the final draft proposed by the European Commission to the European Parliament. We will present and explain the main points of the revised directive, and stress the importance for the future of our profession: Professional cards, «Common training frameworks» replacing the common-platforms, back to harmonization instead of identifying differences for compensation measures, CPD, Partial access, rules on language skills. High quality practice working to an equivalence of standards across the European Union is the key guarantor for patient safety. In this regard, the European professional associations are taking the lead in devising “common training frameworks” for harmonisation and the EC4 Register of Specialists in Laboratory Medicine identifies individuals able to reach such equivalence at high level of education.

Results and conclusion : 9 EU countries where professionals and the Member States agree on the program of harmonisation according to the “common training frameworks”, are needed to make propositions the European Commission. If accepted the frameworks will be transposed into national legislation. We will report at what point we will be in May 2013.

SY19
THE COMMON TRAINING PROGRAMME FOR SPECIALISTS IN LABORATORY MEDICINE
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The Directive on Recognition of Professional Qualifications is there to facilitate professionals of professional in moving from one EU member state to another, and to practice in the host member state in the same way as in the member state of origin. In the directive seven professions are regulated specifically among which medical doctors. The rest of the professions fall under the so called General System. In Annex VII of the directive the medical specialties are named. Although the list suggests equivalence of names and equivalence of standards in education and training in practice this is not the case in Europe. A cardiologist in one member state may well have enjoyed a different training than a cardiologist in another state. In the same way an urologist is not an urologist. Harmonization of training is of the utmost importance for patient safety. Patients must know whether a specialist coming from another country is equivalent, and also whether specialists in his or hers country are equivalent to those in other member states. For specialists in laboratory medicine even two specialties are mentioned, clinical biology and biological chemistry. In the revision of the directive the system of Common Training is introduced. The EFLM Profession Commission focuses on the Common Training System to get our profession recognized for specialists in laboratory medicine for all educational groups, medical, pharmaceutical and scientific. The European Register for Specialists in Laboratory Medicine should serve as a basis. The criteria to be registered in this register are discussed.

SY20
THE EC4 REGISTER OF SPECIALISTS IN LABORATORY MEDICINE
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To be able to obtain an adequate level of quality of the profession and practice in healthcare within the European Union, it is vital to have an up-to-date, efficient and operational level of skills required to be a Specialist in Laboratory Medicine (SLM). This is based on two pillars: common training and code of conduct. The EC4 committee plays a central role in the coordination of the recognition of equivalence of standards for application to the EC4 Register. Today, more than 3200 Specialists in Laboratory Medicine are members of the register. The Netherlands and the UK have successfully implemented an automatic registration procedure. In France this procedure is ongoing and it will be the next country. Now it is time to modernize the website and make it easier to use, this will make it possible to increase the number of registrations and re-registrations in the future.

SY21
MOLECULAR GENETIC APPROACHES TO THE DIAGNOSIS OF THYROID CANCER
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Thyroid cancer is the most common type of endocrine malignancy and its incidence has been steadily increasing in many regions of the world. Papillary and follicular thyroid carcinomas are the two most common types of thyroid cancer. Initiation and progression of thyroid cancer involves multiple genetic and epigenetic alterations, which mutations leading to the activation of the MAPK and PI3K/AKT signaling pathways are crucial. Non-overlapping genetic alterations, including BRAF and RAS point mutations and RET/PTC and PAX8/PPARγ rearrangements, are found in more than 70% of papillary and follicular thyroid cancers. They represent the most common genetic alterations in thyroid cancer, as well as molecular markers of diagnostic and prognostic significance. These mutational markers are being introduced into clinical practice, assisting the diagnosis of malignancy in fine-needle aspirates from thyroid nodules, and are particularly helpful for those nodules that have indeterminate cytologic diagnosis. Moreover, some of these markers, such as BRAF, provide additional prognostic information, which may facilitate more individualized operative and post-operative management of patients with thyroid cancer. New emerging laboratory technologies, such as next generation sequencing, will allow to significantly expand the extent and precision of molecular testing for thyroid cancer in the near future.

SY22
SUBCLINICAL THYROID DISEASE: IS IT CLINICALLY IMPORTANT?
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The introduction of ultrasensitive immunoassays for TSH and free thyroid hormones has changed the diagnosis of clinical thyroid disease and enables us to detect early mild failure or hyper function of the thyroid. The estimated prevalence of subclinical hyper- and hypothyroidism (defined as a TSH outside of reference range, with normal thyroid hormones) in the general population is higher than overt dysfunction and varies from 1.5% to 5.9%, and 2.9% to 16%, respectively. Clinicians should consider the limitations of diagnostic classification according to a single sample. Spontaneous individual variations of thyroid tests may occur over time and results may return to normal on follow-up. (Meyerovitch et al.,
Today it is much debated whether these abnormalities should be treated. The precise threshold at which excess or failure of the thyroid makes the clinical difference will ultimately be guided by the results of well-designed prospective cohort and intervention studies. There is general consensus on the need to treat subclinical hypothyroidism (SHypo) of any magnitude in pregnant women and women who are contemplating pregnancy, to decrease the risk of pregnancy complications. In non-pregnant adult patients SHypo with a TSH > 10 mIU/L, has been associated with a higher incidence of ischemic cardiovascular events and heart failure.(Rodondi et al., 2010; Gencer et al., 2012). Three recent meta-analyses provide evidence that subclinical hyperthyroidism (SHyper) may increase CV mortality in patients with undetectable serum TSH. The increased CV risk may be linked to the risk of atrial arrhythmias, especially atrial fibrillation, and heart failure. (Yang et al., 2012; Collet et al., 2012; Gencer et al., 2012). Although SHyper has been associated with lower bone density, data on fracture risk are limited and contradictory.

Most of the data linking adverse CV outcomes of subclinical thyroid dysfunction are derived from observational data. No appropriately powered randomized controlled trials have evaluated the effects of treatment to improve CV endpoints in SHypo and SHyper. Today, inference on the potential benefits from the treatment of the condition remains hazardous. Finally the risk benefit ratio of such an intervention needs to be addressed prospectively.

SY23
QUALITY AND STANDARDISATION ISSUES IN THYROID FUNCTION TESTING

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The diagnosis, treatment and monitoring of thyroid disease relies on accurate thyroid function testing. The volume of thyroid function testing in most laboratories is high and increasing, with reliance on a high capacity immunoassay analysers from the major diagnostics manufacturers. External quality assessment schemes reveal an unacceptable level of between method variability for the most commonly performed thyroid function tests – thyroid stimulating hormone (TSH); free thyroxine (FT4); and free triiodothyronine (FT3). This between method variability has significant implications for patients, especially for those being monitored during and after treatment. This variability limits the applicability and effectiveness of clinical practice guidelines and has caused a loss of confidence in thyroid function testing amongst patients with thyroid disease. In order to address the issue IFCC established a Working Group (now a Committee) for the Standardisation of Thyroid Function Tests (C-STFT) under the direction of Prof Linda Thienpont. C-STFT has established a reference measurement procedure (RMP) based on equilibrium dialysis isootope dilution mass spectrometry which has been validated for the measurement of FT4 and FT3. The RMP has been applied to the measurement of FT4 and FT3 in a panel of sera from normal subjects and from patients with thyroid disease. The same sera were used by the manufacturers to measure FT4 and FT3 using their commercial assay systems. TSH was measured in the same sera but in the absence of a RMP results were compared to the all method mean. The FT4 and FT3 results varied significantly across the methods tested. None of the commercial methods showed close agreement with the respective RMP. Careful analysis of the data demonstrated that recalibration of each method would dramatically reduce between method variability. Similar, though less dramatic results were found from analysis of the TSH data. These data have shown the potential for reducing between method variability for FT4, FT3 and TSH methods. The data is being assessed by manufacturers in terms of assay calibration and the impact on reference intervals. Coordinated implementation of recalibration would be of maximum benefit to patient safety.

OC09
DIAGNOSTIC VALUE OF THYROGLOBULIN MEASUREMENT IN FINE-NEEDLE ASPIRATE WASH-OUTS OF LYMPH NODES METASTASES OF PAPILLARY THYROID CARCINOMA.

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Background. Ultrasound-guided fine-needle aspiration biopsy cytology (FNAB-C) is the most common procedure for diagnosing lymph node metastases from papillary thyroid carcinoma (PTC). The thyroglobulin measurement in the wash-out of the fine-needle (FNAB-Tg) has been proposed to improve its accuracy. However, there is disagreement on the FNAB-Tg cut-off value. The aim of this study was to determine the FNAB-Tg cut-off on samples obtained from patients undergoing ultrasound-guided fine-needle aspiration of suspected lymph nodes metastases of PTC.

Methods. FNAB-C was performed on 73 lymph nodes in 60 patients with PTC before initial surgery or during post-thyroidectomy follow up. After obtaining a FNAB-C specimen, the needle was washed with 1 mL of saline solution. The FNAB-Tg was performed by chemiluminescence immunoassay method on the Beckman Coulter DXI800 (functional sensitivity: 0.1 ng/mL at 20 CV%). ROC curve was produced calculating the best FNAB-Tg cut-off to discriminate true-positive from true-negative subjects. Moreover ROC curve was created for FNAB-C; sensitivity and specificity were calculated according to the area under curve (AUC) for FNAB-Tg and FNAB-C. FNAB-Tg/serum-Tg ratio >1 in non-thyroidectomized patients were considered positive. Results. Overall, lymph node metastases were found at final histological examination in 47 cases (64.4%). ROC curve analysis for FNAB-Tg and FNAB-C showed an AUC=94% e 83% respectively (P <0.001); on the basis of this curve, the best FNAB-Tg cut-off was 1.2 ng/ml leading to 92% sensitivity and 81 % specificity. Overall accuracy, positive and negative predictive values were 92%, 100% and 81% respectively for FNAB-Tg and 82%, 100% and 67% respectively for FNAB-C. The integration of both methods resulted in 95% overall accuracy, 100% positive predictive value and 87% negative predictive value. Two out six patients with false negative FNAB-Tg result were correctly diagnosed by FNAB-C. Thirteen out of fourteen non-diagnostic FNAB-C were correctly classified by FNAB-Tg.

Conclusions. Integration of FNAB-Tg and FNAB-C, significantly improves the diagnostic accuracy in the evaluation of lymph nodes site of metastases of PTC, particularly in presence of non-diagnostic FNAB-C.
OC10
LOW T3 SYNDROME IS ASSOCIATED WITH GREATER MYOCARDIAL DAMAGE AND MORTALITY IN PATIENTS WITH ST-ELEVATION MYOCARDIAL INFARCTION

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Background. An altered thyroid hormone (TH) metabolism known as Low T3 syndrome (LT3S) is a frequent finding in patients with severe illness, including patient with acute myocardial infarction. Aim of this study is to evaluate the relationship between LT3S, myocardial damage, mortality and major adverse cardiac events (MACEs) in patients with ST-elevation myocardial infarction (STEMI).

Methods. 1007 patients (male: 74%; mean age: 66.1±12.5 years) admitted for STEMI and subjected to early reperfusion therapy were included in this study. TH levels were determined in all patients at admission. Myocardial injury was assessed by peak troponin I (TnI) levels. Brain natriuretic peptide (BNP) and 18±13 months follow-up was performed and cardiac mortality, all cause mortality, and MACEs used to describe myocardial dysfunction. An 18±13 months follow-up was performed and cardiac mortality, all cause mortality and MACEs (cardiac death, re-hospitalization for acute coronary syndrome and elective revascularization for angina) were reported.

Results. A LT3S (T3<1.7 ng/L) at admission was observed in 242 patients (24%). Subjects with LT3S had higher peak TnI (86.9±98.0 vs 72.9±79.7 ng/mL, P=0.02), basal BNP (515.4±1018.9 vs 242.9±449.6 ng/mL, P<0.0001), peak BNP (890.6±1494 vs 470.5±659.7 ng/mL, P<0.0001), and had a lower ejection fraction (42.4±10 vs 45.5±9%, P<0.0001). A significant increase in all cause mortality (Log-Rank 13.7; P=0.0002) and MACEs (Log-Rank 9.1; P=0.003) were also observed in patients with LT3S.

Conclusions. Patients with LT3S have a greater degree of myocardial damage and myocardial dysfunction ater STEMI and an increase in all cause mortality and MACE at follow-up. Further investigations are needed to clarify the potential role of a TH replacement therapy in this group of patients.

SY25
CEREBROSPINAL FLUID BIOMARKERS FOR ALZHEIMER’S DISEASE

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Amyloid β (Aβ), Tau and phosphorylated Tau (pTau) in cerebrospinal fluid (CSF) are established biomarkers for Alzheimer’s Disease (AD). Their main application is the differentiation of patients with AD from patients with other memory complaints. Combined in a logistic model, these biomarkers can distinguish patients with AD from patients with subjective memory complaints with 93.5% sensitivity and 82.7% specificity.

Different centres have reported different levels of the CSF biomarkers in the various disease states and multi-center combination of data sets showed a larger variation than datasets from individual research centers. Hence an initiative was launched for international quality control. Initially the variation of Aβ between laboratories was reduced significantly, i.e. from 30 to 17%. Currently, over 65 laboratories participate in the world wide QC program of the Alzheimer Association. Continuous surveillance of the performance of the CSF biomarker tests is imperative. Lot-to-lot differences have been observed that influence patient results and, therefore, reference values. Despite the absolute differences in results, the clinical value of different assays for CSF biomarkers appears to be comparable. This observation complicates comparison of datasets between research centers as well as multi-center investigations.

A serious drawback of the CSF markers is the fact that the sample can only obtained invasively. Establishing a blood test would be of paramount importance and might have a major impact on early diagnosis, as well as monitoring the course of disease or the effect of treatment. Apart from the search for blood based markers, proteomic studies are in progress to identify possible new markers, that overcome the disadvantages of the current markers.

Based on serial measurements CSF Aβ, Tau and pTau are considered not to be of prognostic value in patients with established AD. They have, however, been implicated in differential diagnosis of dementia and the progression of patients with mild cognitive impairment (MCI) to AD. Our findings implicate a different role for biomarkers in diagnosis and prognosis of MCI-AD. While amyloid markers can be used to identify MCI-AD, injury markers like Tau and pTau may predict rapid progression to dementia.

SY24
ADVANCES IN THE PATHOGENESIS AND DIAGNOSIS OF ALZHEIMER’S DISEASE

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Recent progress have been made on the pathogenesis of Alzheimer’s Disease (AD) including the identification of biomarkers facilitating an earlier diagnosis. However, if we consider the huge investments made over the past few decades by the public and private sectors, progress has been rather limited and translation of research findings into effective clinical treatments has been extremely limited—the current drug arsenal is mostly similar to that of the early eighteen! We will review here recent progress on genes possibly associated with AD, and their association with lifestyle events with a particular focus on the early features of the disease process. Recent findings in animal models will also be presented and discussed in term of relevance to some of the earliest features of AD. Supported by Canadian Institutes of Health Research (Canada).

SY26
MOLECULAR IMAGING TECHNIQUES IN NEURODEGENERATIVE DISEASES

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Modern molecular imaging has provided new exciting tool to investigate the brain and understand functional disturbances as well the time course of different pathological changes. Several neurodegenerative brain disorders are characterized by proteinopathies. Some similarity seem to exist for some diseases as Parkinson’s disease, Lewy body disease and Alzheimer’s Disease (AD) showing a continuum and similarity in pathology. AD is the most common neurodegenerative disorder when the first symptoms of subtle episodic memory disturbances the disease has most probably been on-going for several even decades. The current predominant hypothesis
for the cause of AD is related to dysfunction in brain of processing, deposition and clearance of amyloid-β (Aβ) proteins. The introduction of amyloid PET imaging with the radiotracer 11C-PiB ten years ago has provided new and valuable insight into the dynamic processes and time course of deposition of fibrillar Aβ in brain from preclinical to clinical stages of AD. Different amyloid PET tracers have been tested and one, florbetapir, has been recently approved for clinical use in US and Europe. PET studies have shown that the deposition of Aβ in brain precedes decline in regional cerebral glucose metabolism (18F-FDG PET), followed by impairment of neurotransmitter function and cognitive decline. Brain structural changes appears a quite late phenomena. By combining amyloid deposition with biomarkers for brain injury estimation of risk can be made of progression from mild cognitive impairment (MCI) to AD. Neurodegenerative processes are coupled to neuro-inflammatory reactions fundamental for defending the brain against injury. In a multi-tracer PET concept (11C-deuterium-L-deprenyl, DED has been used to measure reactive astrocytes. Elevated astrocytosis has been observed in MCI patients compared to AD and controls. High astrocytosis have also been observed in non-symptomatic mutation carriers from early-onset familial AD (eoFAD). Increased DED binding is measured by PET at detectable levels before significant deposition of fibrillar Aβ in subjects with high risk of developing AD which may indicate that some type of reactive astrocytes play a crucial role in early preclinical stages of AD.

OC11
APPLICATION OF QUANTITATIVE CLINICAL CHEMISTRY PROTEOMICS (qCCP) TO AMYLOID PEPTIDES, TAU PROTEIN, AND APOLIPOPROTEIN E IN HUMAN CEREBROSPINAL FLUID FOR ALZHEIMER’S DISEASE DIAGNOSIS

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Background: Recent improvements in mass spectrometry (MS) allow this technology to quantify with clinical grade analytical sensitivity and specificity, peptides and proteins in biological fluids. We believe that in some cases MS will represent a valuable alternative to traditional methods of immunochromatography for the quantification of proteins. We followed this path for the quantitation of biomarkers in Alzheimer disease (AD) which represents major cause of dementia. AD is associated with specific apolipoprotein E (ApoE) isoforms, and with alteration of cerebrospinal fluid (CSF) biomarkers. As a matter of fact, the decrease of amyloid peptides (Aβ) and the increase of Tau proteins in CSF are currently used for AD diagnosis. Many isoforms of these molecules exist and MS represent an interesting tool to quantify the diversity of the isoforms, and therefore, to improve AD diagnosis and follow-up.

Methods: For this purpose, quantitative targeted mass spectrometry was developed using a nano-LC-triple quadrupole Agilent 6490. Sample prefractionation (Solid Phase Extraction) trypsic digestion and sample clean-up were realised using an automated liquid handling robot (Agilent Bravo Assay Map plateform). Quantotypic peptides (Aβ1-40, Aβ1-42, tau, ApoE,...) were synthetized in light and heavy (13C and 15N) versions (Eurogentec) and used in calibration curve to evaluate the Limit Of Detection (LOD) and Quantification (LOQ). Experiments were run on series of biological samples from control and AD patients.

Results: Optimal Multiple Reaction Monitoring methods for the different analytes were developed. Detection of specific Apo E peptides resulted in a rapid method for e2/e3/e4 phenotyping.

Different isoforms of Aβ and Tau proteins were detected with sensibility compatible with pathophysiological variations. Correlation with immuno-detection methods and validation of the clinical relevance of the results are on-going.

Conclusions: The mass spectrometry detection of several isoforms of amyloid peptides, Tau protein, and apolipoprotein E in human cerebrospinal fluid represents an important achievement that opens new avenue for quantitative Clinical Chemistry Proteomics (qCCP). The perspective is exploit these results to improve phenotyping, diagnosis and follow-up of dementia.

OC12
REDUCED MITOCHONDRIAL COMPLEX II/III ACTIVITY RELATED TO THE RED BLOOD CELL FOLATE LEVELS IN PATIENTS WITH ALZHEIMER’S DISEASE

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Background: Folic acid-mediated one-carbon metabolism is essential in all cells, and mitochondria play a critical role in these pathways. This is reflected in human diseases associated with folate and homocysteine metabolism. Since mitochondrial dysfunction is accepted as a central role in the progression of neurodegenerative disorders like Parkinson’s Disease (PD) and Alzheimer’s Disease (AD). In this study we aimed to show the dysfunctions in mitochondrial energy metabolism and one-carbon metabolism to investigate the relationships between them in neurodegenerative disorders. Methods: Thirty eight AD, 14 PD and 25 healthy individuals as a control group were included to the study. Mitochondrial complex activities in the platelets, plasma folate, vitamin B12, total homocysteine (tHcy), urine methylmalonic acid (uMMA) and serum amino acids were measured in all groups.

Results: AD patients had significantly lower platelet Complex IV activity (with a mean reduction of 41%), Complex I+III activity (with a mean reduction of 47%) and Complex II+III activity (with a mean reduction of 56%). Also AD patients had declines in Complex II activity. Similarly, in comparison with the healthy controls, PD patients had significantly lower Complex I+III activity (with a mean reduction of 70%) and no significant differences were found between the other complex activities. In AD and PD groups folate and RBC folate levels were significantly lower and tHcy levels were significantly higher than in controls. Among serum amino acids, alanine, glycine, glutamate, glutamine, histidine, cystine, ornithine and proline were significantly higher in AD group whereas none of the amino acids were different in PD group compared with controls. In AD group there was a positive correlation between complex I+III activity and RBC folate concentrations whereas no correlation was found between reduced complex I+III activity and any parameter related to one carbon metabolism in PD group.

Conclusions: Mitochondrial dysfunction in AD might be as a consequence of impairment in folate mediated one carbon metabolism or vice versa.
LC-MS/MS offers highly attractive features regarding endocrine and metabolic testing. This includes high specificity of detection, capability of multi-analyte profiling, compensation of matrix effects by isotope dilution internal standardisation, no impact of anti-reagent antibodies, standardisation independent from reagent lots, and thus consistency of results over time and space with assay independent reference ranges. In the field of metabolic testing, neonatal screening for inherited metabolic diseases by flow-injection MS/MS is now standard in many countries; by simultaneous determination of a large number of analytes in one minute, very reliable identification of phenylketonuria but also detection of many less frequent, treatable diseases is achieved. Furthermore quantification of methylmalonic acid as the most reliable marker of cobalamin deficiency by LC-MS/MS is widely used now. Metabolomics, the concept of a comprehensive description of the small molecule patterns of sample materials might contribute to laboratory medicine with the introduction of new sets of markers which can be addressed by LC-MS/MS. In endocrinology, quantification of 25-hydroxyvitamin D is performed using LC-MS/MS in a continuously growing number of laboratories worldwide. Quantification of cortisol and androgens (testosterone, 17-hydroxyprogesterone, androstenedione) and of metanephrins in plasma is also implemented in a relevant number of laboratories now. Quantification of analytes such as oestrogens and aldosterone which are present in very low concentrations in serum is so far realized only in few institutions. Besides the low concentration range of analytes, the presence of isomers represents a particular challenge for LC-MS/MS in endocrinology. The quantification of proteo- and peptide hormones by LC-MS/MS is even more demanding compared to the quantification of small molecule hormones, however, first candidate reference methods have been described (e.g., for insulin and ghrelin). At present the application of LC-MS/MS in laboratory medicine is still restricted to rather specialized laboratories; however, complete automation finally leading to industrialized availability of highly convenient MS/MS-based routine analysers seems feasible today.
OC13

ISOTOPE DILUTION MASS SPECTROMETRY (IDMS) BASED DETERMINATION OF GROWTH HORMONE FOR RE-CALIBRATION OF IMMUNOASSAY IDS-ISYS

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Background: A growth hormone (GH) peak-level of less than 10 ng/mL in response to two different stimulation tests is biochemically defining growth hormone deficiency (GHD) in children. Variability of test results is partly attributed to the use of different commercially available GH assays. As a consequence, for many patients diagnosis of GHD is assay-dependent.

Methods: A recently developed mass spectrometry (MS) based method is offering an option to SI-traceable re-calibration of the commercial antibody-based assays linking them to the metrologically primary level. To this end, a representative set of patient sera was analyzed using both, isotope-dilution mass spectrometry and the IDS-ISYS immunoassay. A particular advantage associated with the MS approach is the capability of specifically quantifying the main (22 kDa) isoform by using an appropriate tryptic cleavage product (T6) next to measuring "whole GH" which is represented by a cleavage product common to all major isoforms (T12).

Results: Data obtained using the IDS-ISYS assay are in good correlation with both, MS results for "whole GH" as well as 22 kDa GH as expressed by regression equations y=1.086x-0.747 (r: T12 based 'whole GH'; R² =0.89) and y=1.228x +0.506 (r: T6 based 22 kDa GH; R² =0.92). This indicates an average bias of 9 percent of the iSYS assay with respect to "whole GH" as measurand, whereas 22 kDa GH is overestimated by iSYS by about 23 percent. The data fit significantly is improved by inclusion of growth hormone binding protein (GHBP) concentration as regression parameter, which is proof of susceptibility to GHBP levels of the iSYS antibody-assay.

Conclusion: It has been demonstrated here for the first time that, owing to close correlation of results, SI-traceable re-calibration of antibody-based GH assays is a promising way forward to increase reliability and comparability of clinical testing results. Basically, other commercial assays can be expected to be amenable to similar calibration.

OC14

ON LINE TLC-MALDI FOR THE CHARACTERIZATION OF NEUTRAL AND ACIDIC GLYCOSPHINGOLIPIDS: QUALITATIVE AND QUANTITATIVE ANALYSIS

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Background: Glycosphingolipids are a wide class of ubiquitous lipids, characterized by a great structural and functional variety. Importantly, altered levels of these lipids have been correlated with different diseases, as lipid storage disorders or cancer, suggesting their crucial role in health as potential diagnostic markers. To date, characterization and quantification are mainly based on often unspecific antigen-antibody reactions or by cumbersome radioactive labelling followed by thin layer chromatography and retention factor comparison with known standards. Unfortunately these approaches do not allow to fully identify the molecular structure of these lipids, especially in terms of differences in fatty acid chains.

Herein we set up an online analytical methodology which combines the ease of separation of HPTLC chromatography and the high resolving power and mass accuracy of MALDI-MS, directly performed on the HTPLC plate.

Methods: Total lipids from wild-type and overexpressing NEU3 sialidase C2C12 murine myoblasts were extracted with 20:10:1 (v/v/v) chloroform/methanol/water. The aqueous and organic phases were analysed by HPTLC, followed by MALDI-TOF and results compared to [3H]sphingolipids radiolabeled HPTLC. In order to set up the best conditions for matrix delivery to improve GSLs detection, different matrices solutions were tested. Quantitative analyses were conducted with serial dilutions of Gb3 and GM3 standards.

Results: Analysis by HPTLC-MALDI gave comparable sphingolipid profiles to those obtained by [3H]sphingosine radiolabeling. However, MS resolution allowed to identify several species with similar retention factor on the HPTLC plate, and that could not be resolved with the radiolabelling. Several sphingolipids that differed for their fatty acid chains could be distinguished. In particular, neutral GSLs (SM, Gb3, LacCer, GlcCer) and gangliosides (GM1, GM2, GM3, Gd1a) were identified as C16:0, C22:0, C24:1 and C24:0 chains. Quantitative analysis showed a linear trend comparable to radioactive measurements.

Conclusions: On line TLC-MALDI is an easy and high-throughput analysis for the qualitative and quantitative characterization of GSLs suggesting its use for their profiling with high specificity and sensitivity.

SY30

MICRORNAS IN THE SPOTLIGHT: UNDERSTANDING CANCER GENE DEPENDENCY

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Since the discovery of miR-15a and miR-16-1 deletions in CLL15, many laboratories around the world have shown miRNA dysregulation in all tumors studied, including the most common, such as lung, breast, prostate and gastrointestinal cancers. Such dysregulation, like the dysregulation of oncogenes and tumor suppressor genes, can be caused by multiple mechanisms, such as deletion, amplification, mutation, transcriptional dysregulation and epigenetic changes. As miRNAs have multiple targets, their function in tumorigenesis could be due to their regulation of a few specific targets, possibly even one, or many targets. A future challenge will be to identify all of the targets of the miRNAs involved in cancer and establish their contribution to malignant transformation. An additional challenge will be the identification of all of the miRNAs that are dysregulated by pathways that are consistently dysregulated in various types of human cancers. This point is of particular importance, as instead of focusing on specific alterations in protein-coding oncogenes or tumour suppressor genes — which may be difficult to treat — we could focus on their downstream miRNA targets. If these miRNA targets are crucial for the expression of the malignant phenotype and the cancer cells depend on their dysregulation for proliferation and survival, we can expect that the use of miRNAs or anti-miRNAs will result in tumor regression. Genomic analyses for alteration in miRNA genes or for copy
number alterations in various human tumors by deep sequencing is in progress but has not been completed. These studies could provide additional information concerning the involvements of miRNAs in cancer and in many other diseases. Over the past few years, we have observed a shift from conventional chemotherapy to targeted therapies, and miRNAs and anti-miRNAs will contribute extensively to the latter.

SY31
THE ROLE OF NON-CODING RNAs IN METASTASES: NOVEL DISCOVERIES AND FUTURE CHALLENGES
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The newly discovered differential expression in numerous tissues, key cellular processes and multiple diseases for several families of long and short non-codingRNAs (ncRNAs, RNAs that do not codify for proteins but for RNAs with regulatory functions), including the already famous class of microRNAs (miRNAs) strongly suggest that the scientific and medical communities have significantly underestimated the spectrum of ncRNAs whose altered expression has significant consequences in diseases. MicroRNA and other short or long non-codingRNAs alterations are involved in the initiation, progression and metastases of human cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of non-codingRNAs in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the processing machinery. MicroRNA and other short or long non-codingRNAs expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify non-codingRNAs that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer. Recent studies proved that miRNAs and non-coding ultraconserved genes are main candidates for the elusive class of cancer predisposing genes and that other types of non-codingRNAs participate in the genetic puzzle giving rise to the malignant phenotype. Last, but not least, the shown expression correlations of these new ncRNAs with cancer metastatic potential and overall survival rates suggest that at least some member of these novel classes of molecules could potentially find use as biomarkers or novel therapeutics in cancers and other diseases.

SY32
MicroRNAs AS PROMISING NOVEL TUMOR BIOMARKERS IN THE CLINICAL LABORATORY
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Changes in miRNA expression levels have been detected in many human tumor types, and recent studies have demonstrated the critical roles of miRNAs in cancer pathogenesis. MicroRNA profiling in most types of tumors has shown significant different miRNA profiles when compared to normal cells from the same tissues. Nowadays, there is increasing evidence that altered microRNA expression is associated with tumor progression and survival in many types of cancer. Very recently detection of cell-free miRNAs, circulating in plasma and serum has been shown in several studies. Altered expressions of miRNAs in plasma would provide potential blood-based biomarkers for the clinical laboratory. Circulating miRNA profiles have now been associated with a range of different tumor types, diseases such as stroke and heart disease as well as altered physiological states such as pregnancy. According to recent findings plasma miRNAs expression patterns could correctly discriminate between normal and cancer patient samples. Plasma levels of specific miRNAs were associated with short disease-free survival and overall survival, and are associated with poor outcome. In conclusion, circulating miRNAs obtained by noninvasive methods have a high potential to serve as highly specific and sensitive circulating tumor biomarkers in the clinical laboratory. However there is still a lot of work to be done before the establishment of circulating miRNAs as biomarkers in the clinical laboratory, especially towards the standardization of analytical methodologies used, the inclusion of internal and external controls in each assay, and the consensus towards normalization of these results.

OC15
AN ASSOCIATION BETWEEN MicroRNA-155 AND THE HP GENOTYPE IN PATIENTS WITH SICKLE CELL ANEMIA
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Background: Sickle cell anemia (SCA) is characterized by chronic inflammation with a variable immune response that depends on multiple genetic and environmental factors. Haptoglobin (Hp) is an acute-phase protein with immunomodulatory and antioxidant properties whose main function is to bind to free hemoglobin in the plasma and protect blood vessels from its oxidative effects. Two codominant alleles (HP1 and HP2) result in three main genotypes/phenotypes (Hp1-1, Hp2-1, Hp2-2), which correspond to proteins with distinct physical, chemical and functional characteristics. MicroRNAs (miRs) are post-transcriptional modulators of gene expression and their role in infection, inflammation and cell differentiation, proliferation and apoptosis has been investigated. miR-155 is involved in red blood cell differentiation and also plays an important role in inflammation and immunity; however, there is a dearth of information in the literature about the expression of these molecules in SCA. The aim of this study was to investigate the expression profile of miR-155 in granulocytes in SCA patients classified according to Hp genotype.

Methods: 12 patients of each Hp genotype (determined by allele-specific PCR), in steady state, were selected for the study. miR-155 expression profile was determined by q-PCR. Results: The miR-155 expression rate in granulocytes from patients with the Hp1-1 genotype was lower than the expression rate in Hp2-1 patients, which in turn was greater than that in Hp2-2 patients. The difference between the expression rates for Hp1-1 and Hp2-2 patients was statistically significant (P=0.002).

Conclusion: miR-155 is considered important for inflammatory activation of human myeloid cells and, when overexpressed in
CD14+ cells in peripheral blood, can lead to down-regulation of SHIP-1, an inhibitor of inflammation, and an increase in production of pro-inflammatory cytokines such as IL-6 and TNF-α. In agreement with this, our results suggest that miR-155 may be related to Hsp genotype and may play a role in the inflammatory response in SCA patients. Financial support: FAPESP/CNPq/CAPES.

OC16
GENE MUTATION IN MicroRNA TARGET SITES OF CFTR GENE: A NOVEL PATHOGENETIC MECHANISM IN CYSTIC FIBROSIS?
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Background: Cystic fibrosis (CF) is the most frequent lethal genetic disorder among Caucasians with one child in every 3000 newborns affected by disease. It depends on alterations of a chloride channel expressed by most epithelial cells and encoded by CFTR gene. Also using scanning techniques to analyze the whole coding regions of CFTR, mutations are not identified in up to 10% of CF alleles, and such figure increases in CFTR-Related Disorders (CFTR-RD). This suggests that other gene regions, i.e., the 3’UTR region of CFTR gene, may be the site of causing-disease mutations.

Methods: We set up a multistep analysis: 1) Analysis of 1500 bp of CFTR 3’UTR region to search for genetic variants in either CF patients with the F508del homozygous genotype and different clinical expression (n=20), CF (n=32) and CFTR-RD (n=43) patients with one or none mutation after CFTR scanning and in controls (n=50). 2) Identification of mRNA binding sites within the CFTR 3’UTR using software, i.e., TargetScan, miRBase, PITA. 3) Cloning of the 3’UTR of CFTR into pGL3- Control vector for luciferase assay to evaluate the activity of putative miRNA, using both miRNA mimic or Lentiviral particle pre-miRNA expressing. 4) Western Blot analysis to verify the CFTR protein level in cell transient transfected with miRNA mimics.

Results: We identified three SNPs, one of which located in a putative miRNA, using scanning technique to search genetic variants in the 3’UTR region of CFTR. These SNPs are able to down regulate the CFTR protein expression and the luciferase assay that this down regulation is due to the interaction of miRNA with the 3’UTR of CFTR gene. Further, this variant was peculiar of a CFTR-RD patient and the expression analysis demonstrated that such SNP increases the affinity for mir-509-3p and slightly decreases that for the mir-433.

Conclusion: These data show that at least two new miRNAs are able to regulate the expression of CFTR protein. But, more importantly, demonstrate that the presence of a SNP in the 3’UTR region can affect the binding of miRNAs likely leading to a pathological effect.

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SY33
THE INTRODUCTION OF A NOVEL BIOMARKER FOR PULMONARY EMBOLISM - A PARABLE
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The unraveling of the human genome and the expansion of genomics, proteomics and metabolomics have fuelled the development of novel biomarkers. New in-vitro diagnostics other forms of medical testing and can improve health care, bringing us closer to stratified and personalized medicine. At the same time, society is concerned about the never-ending increase in health care expenditure while other groups lament a creeping medicalization through the use of novel forms of testing. Increasingly, decision-makers, physicians and other users request more information than technical and analytical performance and diagnostic accuracy. Health care policymakers have long called on manufacturers to shift from a narrow technical or biomedical perspective to a wider one, one that considers whether the diagnostic technology improves final outcomes in typical patient populations. Before recommending the use of diagnostic tests and markers, and before deciding on their reimbursement, decision-makers and users now want to see evidence that testing actually improves outcomes in relevant patient populations, or that it enhances patient outcome, health care quality, efficiency and cost-effectiveness. Using the introduction and dissemination of a new test to detect pulmonary embolism as an example, we will illustrate how the landscape for the evaluation of medical tests is changing. Our presentation will be structured as a parable, a short allegorical story designed to illustrate or teach some truth.

SY34
MANUFACTURER’S AND REGULATORY PERSPECTIVES
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New in-vitro diagnostics tests are one of the keys to personalized medicine and improved patient care. Widespread implementation of a novel biomarker usually depends on availability of a commercial product. In this presentation, we look beyond the scientific development of a novel biomarker, to discuss the steps involved in placing an in vitro diagnostic product on the market. Development and commercialization of such devices are heavily controlled by regulators worldwide. Regulations aim to ensure that the technical performance and diagnostic accuracy. Health care expenditure while other groups lament a creeping medicalization through the use of novel forms of testing. Increasingly, decision-makers, physicians and other users request more information than technical and analytical performance and diagnostic accuracy. Health care policymakers have long called on manufacturers to shift from a narrow technical or biomedical perspective to a wider one, one that considers whether the diagnostic technology improves final outcomes in typical patient populations. Before recommending the use of diagnostic tests and markers, and before deciding on their reimbursement, decision-makers and users now want to see evidence that testing actually improves outcomes in relevant patient populations, or that it enhances patient outcome, health care quality, efficiency and cost-effectiveness. Using the introduction and dissemination of a new test to detect pulmonary embolism as an example, we will illustrate how the landscape for the evaluation of medical tests is changing. Our presentation will be structured as a parable, a short allegorical story designed to illustrate or teach some truth.

Acknowledgements: Grants from Regione Campania (DGRC 1901/09 and L. 548/03, 2005, 2006 and 2007) are gratefully acknowledged.
The ability of novel medical tests to improve patient outcomes is becoming more central in decisions about their market entry, clinical use, reimbursement and coverage. These considerations and new regulatory requirements affect the way novel medical tests are developed and evaluated. The evaluation of medical tests is more difficult and differs in many ways from the evaluation of therapeutics. One of the most important differences is that medical testing rarely improves health outcomes directly; biomarkers used for several different purposes (diagnosis, monitoring, prognosis, etc.) are often part of a more complex intervention; and most clinical outcomes follow from subsequent clinical management decisions guided by the test results. New biomarkers should be developed in response to unmet clinical needs. After identifying a link between the clinical condition and the biomarker, the subsequent essential components of medical test evaluation are: analytical performance, clinical performance, clinical effectiveness, cost-effectiveness and the broader impact of testing. The Test Evaluation Working Group of the European Federation of Clinical Chemistry and Laboratory Medicine has defined and tightly integrated these components into a dynamic evidence-based framework which clarifies the link and sequence between the various stages of test evaluation and describes the journey of a new biomarker in becoming a medically useful test in the research translation continuum. No new test should be subjected to tedious trials and released to the market if it is unlikely that the test will result in improved clinical actions and measurable outcomes. Therefore in our framework the clinical purpose and role of testing and the intended application of the biomarker in a well defined clinical pathway drive all stages of the test evaluation cycle and define the most appropriate study designs that have the potential to provide the highest level of evidence as proofs. The framework aims to support and improve the understanding of key stakeholders of the necessary steps to be taken when evaluating a test and promotes that larger and more costly studies are only initiated if there is prior evidence of sufficiently high quality of the test’s value.
SY38
VALUE OF THE LABORATORY IN CLINICAL MEDICINE
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Background: Systematic evidence for the contribution of the clinical laboratory to the overall assessment, diagnosis, and management of patients is not readily available. Establishing this evidence is vital to all promotional activities by the IFCC and other organizations involved in laboratory medicine. Evidence is unclear to support blanket claims that 60-80% of medical decisions are influenced by laboratory testing. There is a critical need for both a systematic review of the available evidence in the published literature as well as the initiation of new retrospective and prospective studies to more clearly establish this crucial evidence.

Methods and Results: The IFCC has recently established a new taskforce to evaluate the published evidence on value and impact of laboratory medicine on clinical outcomes and healthcare delivery, and if necessary propose new studies to more clearly establish this evidence. The major aims of this new Taskforce are to: a) Evaluate the available evidence supporting the impact of laboratory medicine in healthcare (a critical review of published literature); b) Develop the study design for new retrospective and prospective studies to generate evidence-based data to support IFCC promotional activities to the healthcare community and the public.

Conclusions: In this presentation, I will review the evidence supporting the key role of laboratory medicine in clinical outcomes with specific examples in the areas of clinical biochemistry, microbiology, pathology, hematology, and molecular diagnostics. The key role of the IFCC in promoting the visibility of the field among healthcare professionals, hospital administrators, and governmental regulators and funders will also be discussed.

SY39
DEMONSTRATING THE VALUE OF LABORATORY TESTS: A CLINICAL AND ECONOMIC PERSPECTIVE
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Introduction: The ability to identify patient sub-groups and better understand disease mechanisms has ushered in an era of developing new diagnostic tools and targeted drugs. New biomarker and diagnostic tests are becoming increasingly available to provide valuable information about which patients benefit from novel agents. This pairing of targeted compounds to patients with a high likelihood to be receptive to that treatment offers an alternative to the traditional broad-spectrum approach when treating disease. Translating excellence in science into effective treatments for patients is at the core of a scientific vision for “Personalised Healthcare” (PHC).

A prerequisite for pairing a therapy and a diagnostic test in clinical development is a very high level of assay quality. Only when a robust level of technical validation has been achieved, should an assay be clinically validated in pivotal studies. Only then can 'Fitting the treatment to the patients', add value to patients, physicians, insurers, and society. PHC rationale for society: From a healthcare insurer’s perspective, ‘fitting the treatment to the patients’ is imperative to optimize resource usage. Additionally, Patient compliance increases when the efficacy of a drug is clear to the person undergoing treatment, which is important to insurance payers. PHC also helps avoid unnecessary or disadvantageous treatment, reducing the risks of side effects and costs for their treatments. PHC today & in the future: The fields of cardiology, neurosciences and inflammatory diseases are examples of therapeutic areas that will potentially benefit from PHC in the future.

Conclusion: Tailoring treatments to specific patient sub-groups who share similar characteristics in their genetic makeup or in the molecular nature of the disease has proven to be highly successful in a few examples. Although this field is just emerging, and certainly is not without significant challenges ahead, we expect to see many more success stories in the future. The advent of new Companion Diagnostic (CDx) tests that provide essential information required to inform treatment decisions will further reinforce the value of the laboratory in improving the management of patients and efficiently utilizing healthcare resources.

SY40
ROLE OF SOCIAL MEDIA AND THE INTERNET IN EDUCATION
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Since the advent of the Internet, and in particular the development of the interactive version of the web, Web 2.0, use of Social Media has developed into a major strategy for businesses and organizations such as the IFCC to use for the purposes of Public Relations and Education. The early Internet ‘Web 1.0’ was a largely static environment which did not allow interaction between organizations and their customers and/or members and as such was mainly used as an information repository rather than a dynamic environment for the exchange of ideas and active marketing and education. Since the development of Web 2.0 we have seen a massive increase in web based traffic which could be loosely called ‘social networking’ which initially was mainly networking between individuals but more recently has developed into a major marketing resource allowing networking between organizations and individuals on the web. It follows then that by developing a Social Media presence on platforms such as Facebook, LinkedIn, Twitter and other social media sites organizations can use this networking for the purposes of marketing, public relations, and in the case of IFCC, education of members and other interested individuals across the globe.

SY41
PSA IN SCREENING FOR PROSTATE CANCER
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Population based screening of prostate cancer has proven to reduce its mortality with at least 20 %, while the reduction of symptomatic metastatic disease is over 30 %. This is of interest in countries that face a growing population of elderly male with increasing life expectancy. Serial PSA testing can however also lead to the diagnosis of and treatment of large number of indolent tumours (23-50% of detected cancers). Population screening likely produces a large benefit in quality of life in those few men diagnosed and treated, but will also cause a large number of men to know that they are having a cancer for many more years of their life. The best age to start screening is unknown, and might be dependent on risk factors like family history or genetic factors. The optimal interval for repeated
screening still has to be determined, but might be dependent on the individual level of PSA. Men with intermediate risk tumours appear to benefit most from screening. From a public health perspective, the associated morbidity may or may not be balanced by net health care benefits. To date, prostate cancer screening has yet to satisfy public health criteria for population based testing, leading many researchers to explore the efficacy of individual risk assessment for early detection of this disease. Risk assessment instruments based on PSA combined with information on prostate cancer provide mechanisms to avoid unnecessary prostate biopsies, and to reduce the potential for overtreatment in men with low risk for prostate cancer.

SY42
BIOMARKER STRATEGIES CURRENTLY BEING EXPLORED FOR PROSTATE CANCER
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Background: More sensitive and specific diagnostic testing that can reliably distinguish aggressive from indolent prostate cancers is urgently required. Measurement of prostate specific antigen (PSA) is integral to the clinical management of patients with prostate cancer, but its limitations for diagnosis and population screening are increasingly well-recognised. Many more men will be diagnosed with prostate cancer than will die of it and many of these men will never have needed to know they had the disease.

Method: Biomarker strategies currently being explored include the Prostate Health Index (PHI) in which results for PSA, free PSA, and a PSA precursor form [-2]pro-PSA are combined in an algorithm to provide an estimate of the risk of prostate cancer and the Prostate CAncer gene 3 (PCA3) urine test. An age-based screening strategy with PSA which combines age and the presence of common genes for prostate cancer so that only the highest risk men are screened has been modelled. Some men would start screening at 45 years, some at 60 years and some would never be screened. Means of improving PSA monitoring in patients with diagnosed prostate cancer are also being developed, with major focus on the interpretation of serial changes in the biomarker and the effective use of this information in routine practice.

Results: Results suggest that personalised approaches to screening could reduce the number of screens required by up to 50% and decrease the number of men diagnosed with prostate cancer by 18%, while also increasing the number of quality adjusted life years and significantly decreasing costs as compared with previously proposed screening strategies. More efficient models for post-treatment monitoring of prostate cancer patients, particularly those on active surveillance, are also likely to be cost-effective as well as more attractive to patients. Objective and rigorous evaluation of such strategies is essential before they can be introduced into clinical practice with particular attention paid to their effect on outcome.

Conclusions: Improving the diagnosis of prostate cancer and the monitoring of diagnosed patients post-treatment remains a high priority.

SY43
ADDED VALUE OF NEW TESTS FOR PROSTATE CANCER DETECTION
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Background: We compared urinary prostate cancer antigen 3 (PCA3), TMPRSS2:ERG gene fusion(T2:ERG) and the serum (-2)pro-prostate-specific antigen (p2PSA)-based prostate health index (Phi) for predicting biopsy outcome.

Methods: Serum samples and first-catch urine samples (after digital rectal examination, DRE) were collected from consented outpatients scheduled for prostate biopsy with PSA 0.5–20 µg/L. The PCA3 Score (PROGENSA PCA3, Gen-Probe) and T2: ERG Score (Gen-Probe) were determined. Measurements of serum PSA, free PSA, p2PSA (Beckman Coulter) were performed and percent free PSA (%fPSA) and Phi (p2PSA/IPSA + pPSA) determined.

Results: Of 246 enrolled men 110 (45%) were diagnosed with prostate cancer (PCa) and 136 men had no evidence of malignancy (NEM). 136 (55%) of all men underwent a first set of biopsies and 110 (45%) had ≥1 repeat biopsies. PCA3, Phi and T2:ERG differed significantly between PCa and NEM and these markers showed the largest areas under the ROC curve (AUCs) (0.74, 0.68 and 0.63, respectively). PCA3 had the largest AUC of all parameters albeit not statistically different from Phi. Phi showed somewhat lower specificities than PCA3 at 90% sensitivity. Combination of both markers enhanced diagnostic power with modest AUC gains of 0.01 to 0.04. Although PCA3 had the highest AUC in the repeat biopsy cohort, the highest AUC for Phi was observed in DRE negative patients with PSA in the 2-10 µg/L range.

Conclusions: PCA3 and Phi were superior to the other evaluated parameters but their combination gave only moderate enhancements in diagnostic accuracy for PCa at first or repeat prostate biopsy.

OC17
SERUM ISOFORM [-2]proPSA DERIVATES (%P2PSA AND PHI) SIGNIFICANTLY IMPROVES THE PREDICTION OF PROSTATE CANCER AT INITIAL BIOPSY IN A FPSA RANGE 2-10 mg/L: A MULTICENTRIC EUROPEAN STUDY
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Background. The current study is designed to test the sensitivity, specificity and accuracy of [-2]proPSA (p2PSA) and its derivatives in discriminating between patients with or without prostate cancer (PCa) and in identifying cases of clinically significant PCa within a prospectively collected, European, multicentric, large and contemporary cohort of candidates to initial prostate biopsy (IPBx) for suspected PCa. Methods. A detailed description of study design, setting, centres and patients, is available at http://www.controlled-trials.com ref. ISRCTN04707454. The study was designed according the STARD methodology in order to test the
Background: Different forms of serum prostate-specific antigen (PSA) and kallikrein-related peptidase 2 (hK2 or KLK2) have been suggested to improve prostate cancer diagnostics. We studied the presence of the different PSA and hK2 forms in prostate tissue samples from radical prostatectomy patients.

Results: Prostate cancer at initial biopsy was diagnosed in 264 (40.1%) of the overall population. Median PSA (5.7 vs. 5.8 ng/mL; P=0.942) and p2PSA (15.0 vs 14.7 pg/mL) did not differ between the two groups, conversely median fPSA (0.74 vs 0.95 ng/mL; P=0.001), %PSA (0.14 vs 0.17; P=0.001), %p2PSA (2.1 vs 1.6; P=0.001) and PHI (48.2 vs 37.9; p<0.001) did differ significantly between men with and without PCA. %PSA (P=0.021), %p2PSA (P=0.015) and PHI (P=0.012) were significantly associated with the presence of PCA at biopsy but not tPSA (P=0.705) and p2PSA (P=0.368).

In univariable accuracy analysis, %p2PSA (AUC: 0.67) and PHI (AUC:0.67) were the most accurate predictors of PCa and significantly outperformed PSA (AUC: 0.50; P<0.001), tPSA (AUC: 0.62; P<0.001) and p2PSA (AUC: 0.51; P<0.001), but not %fPSA (AUC 0.64; P>0.300). In multivariable logistic regression models testing the predictors of PCa at biopsy, p2PSA, %p2PSA and PHI significantly increased the accuracy of the base multivariate model by a 6.4%, 5.6% and a 6.4% extent, respectively (all P<0.001). Conclusions. In patients with a PSA between 2.0 and 10 ng/mL, %p2PSA and PHI are the strongest predictors of PCa at initial biopsies and are significantly more accurate than the currently used tests (PSA, %fPSA) in determining the presence of PCa at biopsy.
SY45
LABORATORY ASSESSMENT AND MONITORING OF THE NEW ORAL ANTICOAGULANTS
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The tests currently employed within most haemostasis laboratories to monitor anticoagulant therapy largely comprise the Prothrombin Time (PT)/International Normalised Ratio (INR) and the Activated Partial Thromboplastin Time (APTT). These are respectively used to monitor Vitamin K antagonists (VKAs) such as warfarin, and unfractionated heparin. Additional tests for assessing or monitoring unfractionated heparin include Thrombin Time (TT) and the anti-Xa assay, which can also be used to monitor low molecular weight heparin (LMWH). Several new antithrombotic (or anticoagulant) agents have recently emerged, or are in the final process of clinical evaluation. These novel drugs, including dabigatran, rivaroxaban and apixaban, theoretically not require laboratory monitoring; nevertheless, testing is useful in specific situations. Tests currently used to monitor VKAs and heparin are typically either too sensitive or too insensitive to these new drugs, and some methodological adjustments may be required to increase or decrease their sensitivity. Alternately, different tests may be better employed. Various expert guidelines are in development to help guide laboratory decisions regarding assessment of these new anticoagulants. In brief, the relative sensitivities for existing routine assays are: TT>APTT>PT (dabigatran), and PT>APTT (rivaroxaban). Accordingly, routine coagulation assays may play a role in the assessment of these agents when used as a part of a panel. Alternatively, the laboratory may opt to perform more specific testing, such as a Hemoclot or dilute TT for dabigatran and a specific anti-Xa assay for rivaroxaban. Whatever decision, the future coagulation landscape is likely to change, with either a reduced or possibly increased number of tests, the same kind of tests but perhaps performed differently, or conceivably different assay panels entirely. Specific laboratory guidance on the choice of the appropriate test to be ordered according to the drug being administered, as well as on appropriate interpretation of test results, will also be necessary. The current report reviews the current state of play and provides a glimpse to the possible future of the coagulation laboratory.

SY46
STANDARDIZATION AND CLINICAL UTILITY OF THROMBIN-GENERATION ASSAYS
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Thrombin generation (TG) and other global assays have gained much attention during recent years within the field of haemophilia due to mainly two reasons: 1) the bleeding phenotype in haemophilia does not correlate entirely to basal factor VIII or IX levels and 2) by-pass therapy i.e. recombinant activated factor VII and activated prothrombin complex concentrate cannot be monitored by currently available clotting factor tests. The hope is that global tests better can predict haemostatic outcome of the treatment. Thrombin generation tests have been available since many years. Some 20 years ago Hemker described a quantitative method to measure thrombin generation and described the parameter endogenous thrombin potential. With the advent of automatic assays the interest in the test has rapidly increased also among clinicians. However, there is still no consensus regarding standardization of the test. Preeanalytic handling of samples as well as activator constituents still create a problem when comparing results between different laboratories. Issues like tissue factor and phospholipid concentrations as well as presence of platelets need to be addressed. A working group within the ISTH SSC is addressing these issues and will soon come up with proposals. At single centers working with TG the test seems valid and reliable and results on clinical materials have been published. So far the cohorts have been small and not well controlled but results are promising. In haemophilia without inhibitors TG indicate a relationship with bleeding frequency. Pre-testing in vitro of plasmas from inhibitor patients with presence of by-pass agents may help to choose the most appropriate product for a specific patient. In a large study evaluating different factor VIII products for immune tolerance induction (ObstiT) TG can differentiate between products and concentrate lots in their capacity to induce thrombin in inhibitor plasma. Collaboration among international haemophilia centers will probably within few years better delineate the role of TG and other global tests for haemophilia treatment.

OC19
SIMULATION OF THE COST-EFFECTIVENESS OF GENETIC SCREENING FOR THE SUSCEPTIBILITY TO ORAL CONTRACEPTIVE ADVERSE REACTIONS
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Background: Several studies have shown a strong association between oral contraceptive (OC) use and venous thromboembolism (VTE). Various risk factors could explain this association, among these, mutations in the factor V Leiden and/or prothrombin F2 G20210A. Screening for these mutations could help to reduce the incidence of OC-induced VTE. The objective of this study was to determine the cost/effectiveness ratio (C/E) and the cost/utility (C/U) of screening options for the prevention of VTE in OC users.

Methods: A decision model was used to simulate the cost-effectiveness of various screening options for genetic risk factor. The virtual population consisted of 14 to 28 years old women who wanted to start taking OC, and whose age and BMI distributions were identical to the Canadian population. Model inputs were taken from an extensive review of the literature. The model considered three situations: no screening, universal screening and targeted screening. In total, 19 options were tested. Three outcomes (besides the estimated costs) were considered: 1) the number of VTE events, 2) the number of pregnancies, 3) QALYs.

Results: The most C/E and C/U options are a biochemical screening (APC-r) and biochemical screening (APC-r) combined with genetic screening (F5 G1691A and F2 G20210A), with overlapping confidence intervals between them. All other options are dominated (i.e. less cost/effective than the status quo). Moreover one option stands out as clearly unfavorable: systematic genetic testing for Factor V Leiden and F2 G20210A. Sensitivity analyses show that the results in term
of C/E are robust, on the contrary of C/U results. Conclusion: Genetic screening (FS G1691A and F2 G20210A) alone is not a favorable option for the prevention of thromboembolism in women wishing to take OC. However decision makers might consider an universal program of biochemical testing (APC-r).

OC20
WARFARIN PHARMACOGENETIC PREDICTION PERFORMANCE: CONTRIBUTION OF EXTENDED PANELS OF CYP2C9 AND VKORC1 GENETIC VARIANTS

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Background: Warfarin is a widely used oral anticoagulant with narrow therapeutic index and relevant interindividual variability in dosing requirements. We have recently published an algorithm based on clinical (body surface area and age) and genetics (CYP2C9 *2 and *3 alleles, -1639G>A VKORC1 SNP, CYP4F2 *3 alleles) variables (Zambon C, et al. Pharmacogenomics. 2011;12(1):15-25). The aim of this study was to verify if the use of an extended panel of CYP2C9 (*2,*3,*4,*5,*6,*7,*11,*12,*18) and VKORC1 (-1639G>A, +6009C>T,+6484C>T,+6853G>C,+7566C>T,+9041G>A) gene polymorphisms could significantly improve our capability in warfarin maintenance dose prediction.

Methods: CYP2C9, VKORC1 and CYP4F2 polymorphisms were retrospectively analyzed (Taqman chemistry and INFINITI®Autogenomics Analyzer) in 317 Italian patients (median age 74yrs, range 43-91yrs) under stable warfarin therapy (target INR=2.5 median weekly dose 31.25mg, range 6.25-80mg). Using stepwise regression and warfarin maintenance dose as dependent variable we developed 3 algorithms considering BSA, age and 3 different panels of polymorphisms: 1. CYP2C9 extended panel, VKORC1-1639G>A and CYP4F2 *3 2. CYP2C9 *2,*3, VKORC1 extended panel and CYP4F2 *3 3. CYP2C9 and VKORC1 extended panels and CYP4F2 *3 We compared these algorithms with our present algorithm (algorithm 4).

Results: The percentage of explained warfarin variability (R2adj%) was slightly different among the algorithms (55.6%, 53.1%, 55.3% and 53.4% for algorithm1,2,3 and 4 respectively). The Mean Absolute Error in the prediction of maintenance dose ranged from a minimum of 7.52±0.64 mg/week (algorithm 3) to a maximum of 7.80±0.63 mg/week (algorithm 4). Considering three classes of weekly warfarin maintenance dose (=26.25 mg, 26.25-43.75 mg, >43.75 mg) the predictive performance of the algorithms was statistically higher in intermediate than in low (Wald test z=2.54, P=0.011) and in high classes (z=2.11, P=0.035). Predictive performances were not statistically different within classes.

Conclusions: the analysis of an extended panel of CYP2C9 alleles might improve the predictive ability of our algorithm. Instead the use of high number of VKORC1 SNPs didn’t increase our capability in warfarin dose prediction.

SY47
EPIDEMIOLOGY OF INFLAMMATORY BOWEL DISEASE IN CHILDREN

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25-30% of patients with inflammatory bowel disease (IBD) are diagnosed in childhood. Recent epidemiological evidence has shown that there has been a rising incidence of paediatric IBD (PIBD) both in Europe and worldwide in recent decades, although the pathogenic mechanisms involved in this rise are yet to be elucidated. Although detailed genetic studies have not uncovered a distinct genotype in those with early-onset disease, a clear difference in clinical phenotype is observed. Those with PIBD present with or quickly develop pan-enteric disease in paediatric Crohn’s disease (CD) or pancolitis in paediatric ulcerative colitis, compared to those with adult-onset disease. Additionally, isolated ileal disease is very uncommon in paediatric CD (~5%), with colonic disease featuring frequently in those diagnosed with CD less than 10 years of age. There is currently a paucity of data with regard to treatment strategies in PIBD, which much of the therapeutic data extrapolated from adult studies. However, emerging evidence now supports the use of exclusive enteral nutrition in Crohn’s disease with this modality now becoming utilised in the adult population. With the rising incidence and extensive disease at diagnosis the disease burden in those with PIBD is high, with detrimental effects on growth, education and psychosocial well-being.

SY48
PATHOPHYSIOLOGY OF INFLAMMATORY BOWEL DISEASES

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The pathogenesis of inflammatory bowel diseases (IBD) is only partially understood; various environmental and host factors are involved. Genetics has been revolutionized in recent years by the advent of genome-wide association (GWA) studies with IBD being one of the most successful areas. So far, > 100 susceptibility loci (e.g. smoking and NOD2) have been discovered and replicated with a significant geographic variability. Both the innate and the adaptive immune systems appear to be disregulated. In CD, defective crosstalk between bacteria and host seems to play a key role; according to the gene discoveries in autophagy and innate immunity (associations with NOD2, IRGM, and ATG16L1 are specific to CD). These genes also play key roles in the homeostasis of a cell type that stands at the interface of host-microbial interaction – the Paneth cell by altering antimicrobial peptide production (so-called defensins). In contrast, the importance of barrier function, contributing to a balance that determines the pathogenic mechanisms involved in the development of UC. In addition, impaired IL10 homeostasis is the IL23/Th17 signaling with multiple genes identified as susceptibility loci (e.g. IL23R, IL12B, STAT3 and JAK2) affecting both innate and adaptive immune responses. Nevertheless, the interaction between mechanisms is more complex in vivo and influenced also by environmental factors (e.g. smoking and NOD2). There is a mutual interaction between gut microbiome, innate defense mechanisms and barrier function, contributing to a balance that determines
physiological or pathological inflammation. However, translation from bench to bedside is sometimes difficult as highlighted by the recent clinical trials failing to demonstrate a benefit by targeting the IL17 or CTLA4. In conclusion, recent advancement in basic research has significantly contributed to the understanding of IBD pathogenesis and it may ultimately lead to improved diagnostics, course prediction, identification of possible targets for therapeutic intervention and hopefully to a more optimized, personalized therapeutic armamentarium.

SY49
LABORATORY TESTING IN INFLAMMATORY BOWEL DISEASE

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IBDs comprise an heterogeneous group of chronic inflammatory disorders of the intestinal tract which onset is typically during young adulthood, although about 20-25% of patients are diagnosed during childhood. Faecal markers are useful to evaluate patients with symptoms of IBDs. The two most commonly used are calprotectin and lactoferrin, which are markers of “neutrophilic intestinal inflammation”. Calprotectin, a 36.5 kDa heterotrimer composed of one light (S100A8) and two heavy (S100A9) chains, represents 60% of granules of neutrophils. The sensitivity and specificity of fecal markers in detecting IBDs are 93% and 96% in adults, 92% and 76% in children. Fecal markers do not allow to distinguish CD from UC. In this setting the application of serological markers is advisable. Serological markers include antibodies towards bacteria and yeast’s components, and antibodies which recognize perinuclear neutrophil cytoplasmic antigens (pANCA). IgG and IgA antibodies anti the mannose residue from the phosphopептидоманнан of the cell wall of S. cerevisiae (ASCA) and pANCA IgG are established serum biomarkers for CD and UC respectively. Their sensitivity is 70-75% and their specificity is 80-85%. New serum biomarkers for CD diagnosis include antibodies anti-bacterial proteins (anti-I2, CBir, OMPc) and surface components (chitobioside ACCA, laminaribioside ALCA, and mannobioside AMCA) of microorganisms and human cells. Although as much as 80% hereditability in IBDs is still unexplained, in approximately 30% of CD patients mutations in NOD2 are found and the most commonly identified mutations are R702W, G908R and L1007fsinsC. Heterozygosity increases the risk 2- to 3-fold, whereas CD patients with colonic location (L2) revealed a significantly diminished prevalence thereof and PAB (P <0.05 respectively). Occurrence of anti-GP2 and ASCA IgA/IgG was significantly more prevalent in CD patients with age at diagnosis <16 years (P=0.0083, P=0.0028). Appearance of one or more anti-GP2 or ASCA IgA/IgG was significantly more prevalent in L3, B2, and A1 and less prevalent in L2. (P <0.05, respectively)

Conclusions: The novel CD-specific anti-GP2 IgG and IgA antibodies are associated with distinct disease phenotypes. They are more prevalent in patients with CD at a younger age, with ileocolonic location, and stricturening behaviour with perianal disease.

OC22
AUTOANTIBODIES DIRECTED AGAINST COMPONENTS OF PROMYELOCYTIC LEUKEMIA PROTEIN NUCLEAR BODIES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Background: Primary biliary cirrhosis (PBC) is a progressive, autoimmune liver disease. Some of patients have antinuclear antibodies (ANAs). Part of these ANAs targets promyelocytic leukemia protein (PML) nuclear body (NB) components such as Sp100 and PML. Novel protein identified using serum PBC patients designated as Sp140 is considered to be disease-specific. The aim of this study was to analyze the sensitivity, specificity and predictive values of this new anti-Sp140 antibody, as well anti-Sp100 and anti-PML antibodies in a well characterized cohort of PBC patients.
Methods: We studied 80 PBC patients, 55 pathological controls with primary sclerosing cholangitis, autoimmune hepatitis, rheumatoid arthritis and 30 healthy blood donors. The ELISA “in-house” test was established for the detection of anti-Sp140 antibody. The microtitration polystyrene plates coated with recombinant protein Sp140 were consecutively incubated with diluted sera, anti-human IgG antibody conjugated with horseradish peroxidase and with TMB. Optical density was read at 450 nm. Anti-Sp100 and anti-PML antibodies were detected by commercially available kits.

Results: Anti-Sp140 antibodies were present in 22 (28%) PBC patients, also in AMA negative cases. The results were negative in healthy sera and in all but one (patient with primary sclerosing cholangitis) pathological controls. The specificity of the test and positive predictive value were 99% and 96%, respectively. Anti-Sp100 antibodies were present in 27 (34%) PBC patients and anti-PML in 28 (35%). Anti-Sp140 coexisting with anti-Sp100 was found in 16/22 patients (73%) and with anti-PML in 14/22 (64%). Anti-Sp140 antibodies were found together with anti-Sp100 antibodies and with anti-PML antibodies in 8 (36%) cases.

Conclusions: Sp140 is a novel, highly specific PBC autoantigen. Our study identifies anti-Sp140 for the first time together with anti-Sp100 antibodies and with anti-PML antibodies. The microtitration polystyrene plates conjugated with horseradish peroxidase and with TMB. Optical density was read at 450 nm. Anti-Sp100 and anti-PML antibodies were detected by commercially available kits.

Results: Anti-Sp140 antibodies were present in 22 (28%) PBC patients, also in AMA negative cases. The results were negative in healthy sera and in all but one (patient with primary sclerosing cholangitis) pathological controls. The specificity of the test and positive predictive value were 99% and 96%, respectively. Anti-Sp100 antibodies were present in 27 (34%) PBC patients and anti-PML in 28 (35%). Anti-Sp140 coexisting with anti-Sp100 was found in 16/22 patients (73%) and with anti-PML in 14/22 (64%). Anti-Sp140 antibodies were found together with anti-Sp100 antibodies and with anti-PML antibodies in 8 (36%) cases.

Conclusions: Sp140 is a novel, highly specific PBC autoantigen. Our study identifies anti-Sp140 for the first time together with anti-Sp100 antibodies and with anti-PML antibodies. The ability to detect anti-Sp140 antibodies expands the diagnostic armamentarium of PBC-specific markers.

SY50
GENOMICS, EPGENOMICS AND TRANSCRIPTOMICS: BUILDING AN INTEGRATED VIEW OF DISEASE USING NEXT GENERATION SEQUENCING DATA
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The sequencing of the human genome was meant to revolutionise mechanistic understanding of disease, deliver better diagnosis and better therapy. The subsequent emphasis on GWAS studies has delivered numerous loci of relevance to disease, but has fallen short of those promises. Touching on examples spanning from basic science (our recent discovery of a new class of small non-coding RNAs) to translational science (biomarker discovery) I will dwell on some of the reasons that in my opinion underlie this failure. I will relate on my recent experience in establishing a translational genomics center situated within a hospital with the aim of eventually truly benefiting patients. By explaining our current setup and projects I will try to convey my view on how these failed promises still hold great potential for understanding disease, as long as a new, more integrated and patient-centric approach is taken. In this approach all components and functions of the genome (the histone code, DNA methylation, DNA sequence, transcription) need to be taken into account together and need to be treated as potentially heritable features. By viewing heritability of the genome in this way, we might begin to understand better the relationship between environment and disease, the partial heritability of complex disease, and, above all, the unique puzzle which makes up a specific disease progression and phenotype within the context of his/her environment and family, as opposed to a “blanket” diagnosis.

SY51
BIOINFORMATICS FOR NEXT GENERATION SEQUENCING: FROM DATA MANAGEMENT AND ERROR CORRECTION TO MEDICAL INTERPRETATION
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Next-generation sequencing (NGS) methods generate huge amount of data that can only be handled and analyzed by sophisticated computational approaches. Bioinformatic methods are therefore relevant for all phases of NGS experiments. Read simulators for the different NGS technologies support the planning and design of NGS studies. These programs generate reads from read sequence data and incorporate the typical sequencing error types and distributions. These simulated reads are widely used to evaluate the efficiency and correctness of computational data analysis pipelines, and to select the optimal sequencing technology for a given diagnostic problem. The real sequencing data are managed and organized through specialized computational tools. The main challenge consists in the very high data volumes and is addressed through specific compression algorithms as well as distributed storage and processing of raw sequence data. An important requirement for subsequent algorithms is the error reduction and correction of NGS sequence data. Different strategies for the removal of erroneous reads as well as the direct correction of particular error types, such as homopolymer indels or artificial amplification, allow for an efficient reduction of experimental errors in the raw NGS data. The reconstruction of the originally longer sequences from short reads is necessary in many projects. Sequence assembly tools implement alternative strategies such as mapping, de novo assembly, hybrid assembly or comparative assembly. The quantification of sequences requires additional computational approaches. These first determine the raw read counts per sequence position. These counts need to be corrected for NGS-method specific biases, and are normalized for the comparison between different samples. Statistical approaches that were originally developed for microarray-based sequence quantification needed substantial adaptations and improvements in order to be suitable for quantitative NGS. Bioinformatic tools and methods are crucial for the success of NGS-based projects. A substantial portfolio of tools has been developed during the last years. However, the rapid increase in NGS throughput will keep this area an active research field also in the next future.

SY52
MULTIGENE ANALYSIS IN THE MOLECULAR DIAGNOSTIC OF GENETIC DISORDERS
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In recent years, massive research efforts coupled with major advances in high-throughput technologies have greatly aided in the elucidation of the molecular causes not only of the majority of Mendelian disorders but also of a multitude of gene loci strongly associated with common, often age-dependent human diseases. In the case of the hereditary disorders it has become evident that genetic heterogeneity is widespread rendering traditional genetic diagnostics approaches based on Sanger sequencing of single genes mostly insufficient to diagnose the molecular defect in the patient. A prominent
example of genetic heterogeneity offers the group of retinal dystrophies with retinitis pigmentosa caused by mutations in probably more than 100 distinct genes. As an integral part of customized healthcare, molecular diagnostics is essential to tailor medical care to an individual. In particular, it is needed to secure a clinical diagnosis, to establish carriers status, and to allow sub-classification of a given disease state. Clarification of an individual’s genetic defect provides the basis for accurate evaluation of recurrence risk and paves the way for targeted treatment approaches. Available state-of-the-art technologies for high-throughput DNA analysis such as microarray-based resequencing and massively parallel sequencing are suited to address those needs of modern DNA testing. Such technologies have the capacity to yield sequence information of up to 600 Billion base pairs in a single analysis. Not surprisingly, the resulting depth of information and the required interpretation of the data pose enormous challenges to the analyst and the medical geneticist. A particularly demanding task necessitates the distinction of pathologically relevant mutations from a plethora of neutral sequence changes. Here, systematic approaches are needed to bring together available resources from bioinformatics, population-based information and functionally relevant data. The interpretation of such data will be complex as will be the task to communicate such a complexity to the patient.

OC23
NEXT GENERATION SEQUENCING IN RESEARCH AND DIAGNOSTICS OF GENETIC CARDIOMYOPATHIES

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Background: Hypertrophic cardiomyopathy (HCM) is the most frequent genetic cardiovascular disease worldwide and is the first cause of sudden cardiac death in young people. HCM molecular bases are highly heterogeneous, over 30 causative genes having been reported to date. Here, we report a next generation sequencing procedure associated with DNA sequence capture that was able to sequence simultaneously 202 cardiomyopathy-related genes. Methods: The study was performed on 3 HCM patients and on their genomic pool. Totally, 202 genes were selected and a custom sequence capture array was designed for target enrichment of all their coding regions, bounded by 500 nt at each end. The size of our target was 3.9 Mb. Each selected patient was individually analyzed. Briefly, each DNA sample was enriched using one custom array, and then sequenced with the GS FLX System. In this way, we were able to obtain an average of 203 Mb/sample, being equivalent to 647,888 sequencing reads/sample with an average read length of 327.8 bp. A data analysis and annotation pipeline was developed and used to prioritize the identified genetic variants. Results: We identified a large number of variants in each of the three HCM patients analyzed. Computational filtering revealed some variants that may be involved in the pathogenesis of HCM: a missense mutation in MYH7 and a non-sense variant in INS-IGF2 (patient 1); a splicing variant in MYBPC3 and an indel/frameshift variant in KCNQ1 (patient 2); and two concomitant variations in CACNA1C (patient 3). Sequencing of the pool of the three DNAs produced similar results, which indicates that this cost-effect approach is feasible. Conclusions: Our procedure allows the simultaneous analysis of a large number of genes thus obtaining a molecular diagnosis also in those patients for which traditional screening was not informative. In addition, it can identify mutations in other genes that, acting as phenotype modifiers, could be responsible for clinical variability. Thus, reducing time and costs and increasing the sensitivity of molecular testing, we could implement routine HCM molecular diagnostics and obtain a model easily applicable to other genetic diseases.

OC24
WHAT WE DON’T KNOW ABOUT THE GENETIC BASIS OF BRUGADA SYNDROME

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Background: Brugada syndrome (BS) is cardiac arrhythmic hereditary disorder that can lead to sudden death with a prevalence of 1:5,000 in Caucasian population, affecting mainly male patients in their 3rd-4th decade of life. BS is inherited as an autosomal dominant trait however to date genetic bases have been only partially understood. Most mutations are located in the SCN5A gene, encoding the alpha-subunit of the Na+ cardiac channel, but more than 70% BS patients remain genetically undiagnosed. In our project we expect to increase the proportion of genetically diagnosed BS patients, identifying new BS-related genes and causative variants by high throughput sequencing analysis. Methods: We analysed 100 BS patients for a panel of 158 candidate genes, mainly encoding ion channels, structural proteins, modulators of ionic flow, regulatory proteins. We performed the design of 2320 exons corresponding to 498,094 nucleotides using the eArraytool developed by Agilent. We used the Agilent Sureselected target enrichment protocol and the sequencing was performed on Illumina GA Ixl. To manage the sequencing data, we developed an automated bioinformatics pipeline based on BWA aligner, which is able to map reads versus the hg19 reference with great accuracy. Results: For each sample, at least 2 million paired-end reads was successfully mapped. More than 98% of the target sequences were covered with more than 230X mean depth by gene and the 90% of the bases are sequenced above a 50X depth. We detected 225 new missense variants in 70 genes of our panel: 2 of them are in the splice-site donor in two different cardiac voltage-dependent ion channels and 30f them cause stop codon. Two of them are localized in regulatory proteins of the cardiac excitability and the other one has an important role in the cardiac action potential. All these mutations are privates. We detected also some missense variants in the same genes in different patients with the same phenotype and it could suggest their important pathogenic role. Conclusions: Our preliminary results suggest that some of investigated genes could have pathological role in the BS onset that is never been considered until now.
A recent systematic review of randomised controlled trials of point-of-care testing (POCT) for HbA1c found insufficient evidence of the effectiveness of POCT for HbA1c. What can we learn from this review? The reviewers concluded that there needed to be further development of the research methodology, particularly in (i) employment of common outcome measures in all studies, (ii) the stratification of patients according to baseline HbA1c, (iii) clear definition of current standard of care (control) and POCT supported care (experimental) processes, (iv) confidence that the results are discussed with the patients at the time they become available, and (v) ensuring that the analytical performance meets the required quality standards. Crucially (a) it was not possible to pool data from several of the studies, and (b) there was evidence that results were not discussed with patients during the clinic visit in the POCT arm of the study. The latter is one of the key attributes for the use of POCT in the management of long term conditions. The issue of stratification of patients according to baseline HbA1c relates to two issues (i) linking risk stratification to specific treatment protocols, and (ii) enabling pooling of data according to risk. The two key outcome measures in such studies should be an agreed outcome measure [or surrogate] of clinical effectiveness – in this case HbA1c measured in the laboratory, and evidence of a change in practice, e.g. treatment intensification. If an economic analysis is to be performed then there needs to be identification of the costs associated with both arms of the study, and for true cost effectiveness - long term follow up.

Interestingly there were two large observational studies that did show a significant fall in the mean HbA1c levels with the introduction of POCT for HbA1c. However again there was little definition of the care processes, or any stratification of patients in regard of treatment or pooling of data. The conclusions drawn from analysis of this review are that (a) patients should be stratified for treatment and follow up, (b) treatment protocols should be transparent, and followed, (c) the POCT care process must show evidence of action, and (d) evaluation must be of a “test-and -treat” intervention.

**SY54**

**DETERMINING WHAT WORKS BEST: THE LABORATORY MEDICINE BEST PRACTICE INITIATIVE**

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**Background:** The Laboratory Medicine Best Practices (LMBP) program applies evidence-based medicine principles for developing recommendations on laboratory medicine topics. Topics are selected for systematic review based on relevance with the Institute of Medicine’s healthcare priorities of safety, timeliness, effectiveness, efficiency, equity, and patient-centeredness.

**Methods:** The LMBP A6 methodology includes the elements of ASK, AQUIRE, APPRAISE, ANALYZE, APPLY and AUDIT. Asking involves question formulation by specifying the Practice, Indicator, Control and Outcome. Acquiring evidence includes searching literature data bases and locating unpublished studies. Appraisal of evidence is conducted by 2 trained reviewers using a standard rating tool. Analyzing the evidence includes use of meta-analytic techniques as appropriate. Evidence based recommendations for Application to laboratory practice are developed. Assessing the impact of LMBP recommendations is also a component.

**Results:** Systematic reviews were performed on topics involving hemolysis, critical value notification and positive specimen identification. For reducing sample hemolysis in emergency units, straight needle collection was compared with IV starts finding a reduction in hemolysis rates [risk ratio = 0.16 (95% CI=0.11-0.24)]. Use of automated notification and customer service call centers for reporting critical values showed a standard difference in means (d=0.42; 95% CI=0.2-0.62), while studies reporting improved service with call centers showed OR=22.1; 95% CI=17.1-286. Use of barcoding was favored for reducing patient specimen and laboratory testing identification errors. The mean odds ratio for use of barcoding systems was 4.39 (95% CI: 3.05-6.32) and for use at point-of-care testing 5.93 (95% CI: 5.28-6.67).

**Conclusions:** For reducing hemolysis rates in EDs, straight needle venipuncture instead of IV starts is effective. Call centers are effective in improving the timeliness of critical value reporting but evidence for use of automated notification systems is not sufficient for or against a best practice. Barcoding is effective for reducing patient specimen and laboratory testing identification errors in diverse hospital settings and is recommended as an evidence-based best practice.

**SY55**

**TRANSLATING EVIDENCE INTO PRACTICE: SUCCESSES AND CHALLENGES**

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In the United States (U.S.), considerable attention is presently focused on the importance and impact of evidence-based laboratory medicine (EBLM). With President Obama re-elected, providers are ramping up efforts as they prepare for more radical healthcare reform. Numerous professional and governmental organizations are dedicating more resources towards assessing the comparative effectiveness of diagnostic interventions as well as focusing on the development of clinical practice guidelines (CPGs) and laboratory medicine practice guidelines (LMPGs). While many of these guidelines have great potential to impact use of laboratory services, translating the recommendations into real changes in practice remains a challenge. At our academic medical center (AMC) outside of Chicago, effective strategies have facilitated the implementation of EBLM developed guideline recommendations. These efforts involve multidisciplinary teams of clinical faculty and staff in pathology working collaboratively with physicians and staff from other departments. Our case studies that will be presented involve adherence to and implementation of key EBLM grounded practice changes. In triage of chest pain patients, selected changes have contributed to a 40% reduction in the ‘door-to-balloon’ time for AMI patients also resulting in 100% of AMI patients receiving percutaneous coronary intervention within 90 minutes of arrival. Increased availability of lactate measurements at the point of care with a critical value driven algorithm is currently being implemented for rapid triage and identification of patients in our early sepsis management protocols. A pilot study is underway.
evaluating use of 2-D barcode label systems for accurate administration of medications and laboratory specimen identification. Over three years, evidence-based major changes to our blood management program have led to more appropriate utilization of blood products, reduction of waste and decreased expenses. In one year, red blood cell transfusions per discharge decreased by 11% while plasma transfusions per discharge decreased by 23%. Our department will continue to identify actions which lead to evidence-based practice changes that improve the quality of services, use of resources and patient care.

SY56
EVIDENCE-BASED INITIATIVES FROM NEW ZEALAND
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The New Zealand (NZ) Guidelines Group has produced many excellent documents in areas including cardiovascular risk screening and type 2 diabetes that have received favourable international recognition. Better guidelines, however tend to be longer which may paradoxically render them less useful, particularly to those who need them most. It is also clear that there is a wide gap between what is actually achieved in clinical practice compared with recommendations. There is also inconsistency between guidelines, few clinicians access them and they are only as good as the primary studies upon which they are based. In particular, there is a paucity of good outcome data in laboratory medicine. In NZ, we have attempted to harness best practice through the development of decision support tools at the point of request, eg for D-dimer with a view to generating evidence-based, patient specific comments. Coupled with this, is a focus on better assessments of pre-test probability and also reporting of likelihood ratios to improve test interpretation. More importantly, at a local level, we have developed Health Pathways, which are locally agreed, evidence-based best practice guidelines, although also mindful of locally available resources.

SY57
LABORATORY ISSUES IN CLINICAL GUIDELINE DEVELOPMENT
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Background: Correct information provided by guidelines may reduce laboratory test related errors during the pre-analytical, analytical and post-analytical phase and increase the quality of laboratory results.

Methods: Twelve clinical practice guidelines were reviewed regarding inclusion of important laboratory investigations. Based on the results and the authors' experience two checklists were developed: one comprehensive list including topics that authors of guidelines may consider and one consisting of minimal standards that should be covered for all laboratory tests recommended in clinical practice guidelines. Number of topics addressed by the guidelines was related to involvement of laboratory medicine specialists in the guideline development process.

Results: The comprehensive list suggests 33 pre-analytical, 37 analytical and 10 post-analytical items. The mean percentage of topics dealt with by the guidelines was 33% (median 30%, range 17-55%) and inclusion of a laboratory medicine specialist in the guideline committee significantly increased the number of topics addressed. Information about patient status, biological and analytical interferences and sample handling were scarce in most guidelines even if the inclusion of a laboratory medicine specialist in the development process seemingly led to increased focus on e.g. sample type, sample handling, and analytical variation. Examples underlining the importance of including laboratory items are given.

Conclusions: Inclusion of laboratory medicine specialist in the guideline development process may increase the focus on important laboratory related items even if this information is usually limited. Two checklists are suggested to help guideline developers to cover all important topics related to laboratory testing.

SY58
GUIDELINE FROM THE EUROPEAN ATHEROSCLEROSIS SOCIETY: CLINICAL VIEW ON LABORATORY CHECKLIST AND ISSUES
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Background: Lipid testing is an important and integrated part of cardiovascular prevention strategies. The European Atherosclerosis Society (EAS)/EFLM Collaborative Project was initiated in 2012 for making joint guidelines.

Method: The 2011 EAS/ESC guideline for management of dyslipidemia was evaluated using the EFLM WG-Guidelines checklist of laboratory issues necessary to ensure good quality and application of in-vitro diagnostic test results.

Examples underlining the importance of including laboratory resources and patient care.

Results: Characteristics of the target population are well described and even incorporated in the risk score calculation (age, gender); patients with cardiovascular disease, diabetes, and chronic kidney disease are automatically assigned for "very high-risk" treatment. Test indication is clear for all tests: risk calculation (total cholesterol, HDL-cholesterol) vs. therapeutic target (LDL-cholesterol, apolipoprotein B). Sensitivity, specificity, and predictive values of the tests are missing. With the exception of a recommendation for fasting blood sampling, important pre-analytical issues are lacking. Analytical performance goals are not described. Concrete requirements of standardization are also missing. Direct LDL methods are recommended in cases of invalid LDL calculation. Well-known accuracy problems of direct LDL- and HDL- assays are not mentioned. There are no warnings about analytical interferences, except for the rare hyperglyceroolemia in triglyceride assays without glycerol blanking. Test reporting units (mg/dL or mmol/L) and conversion factors are provided. Risk-related cutpoints and therapeutic target values are clearly given, with gender-specific cutpoints for HDL-cholesterol. Biological interferences are summarized for triglycerides only. Intra-individual variation data are given for total cholesterol and triglycerides with a recommendation for repeat testing. Testing frequency is described in detail for follow-up of lipid-lowering therapy.

Conclusion: The EAS/ESC guideline includes some but not all pre-analytical (test request) and post-analytical (test interpretation) issues that are relevant to clinicians, but more analytical recommendations should be taken into account for medical decisions in cardiovascular risk management.
CARDIAC TROPONIN: THE SENSE OF MORE SENSITIVE ASSAYS

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Cardiac troponin (Tn) is the biomarker of choice for diagnosis of myocardial injury. The guidelines recommend the use of the 99th percentile upper reference concentration of cardiac troponin - estimated in a healthy population - as the diagnostic cut-off for acute myocardial infarction (AMI). The problem is that for troponin T and especially for troponin I a broad range of upper reference limits is employed, complicating the diagnostic process. Major causes are lack of troponin I assay harmonization and non-standardized selection of individuals in healthy reference populations. The 99th percentile upper reference limits should be estimated according to the guidelines. However, by studying the results of about 45 different papers, we showed that none of the reference populations used in the evaluated studies completely meet the guidelines. Forty percent of the studies collected less than the advised minimum of 300 subjects. Many studies did not report their inclusion criteria. It appeared that lower 99th percentile values are found when more stringent selection criteria are applied. Higher troponin cut-offs are found in men and elderly subjects, suggesting sex and age related cut-offs. These results indicate that better implementation of guidelines is required when estimating upper reference limits. With the introduction of the more sensitive methods the number of persons with measurable cTn concentrations increases, and as a consequence the number of false-positive AMI diagnosis. Methods used to exclude false positives are reference change values or relative or absolute cardiac troponin changes in time. These tools need further improvement and validation. Apart from diagnostic purposes high-sensitive troponin methods are also suitable for prognostic purposes, making reliable estimation of the cut-off values even more important. It is clear that extensive knowledge on cut-off values used by the different laboratories is required. Several audits to evaluate the implementation of guidelines with respect to above mentioned aspects were performed and a new one will be performed, all under auspices of the EFCC/EFLM. The results will be discussed.

CURRENT GUIDELINES ON CARDIAC MARKERS: HOW SHOULD THEY BE INTRODUCED AND HOW SHOULD THE IMPLEMENTATION BE EVALUATED

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There are a range of guidelines for the diagnosis and management of cardiovascular disease. The significant change that has occurred is that the use of cardiac biomarkers has become an integral component of these patient diagnostic and management pathways in a manner that would have been completely unexpected 20 years ago. Laboratory diagnostics has moved to the central stage of cardiac diagnosis. When assessing how well the laboratory utilises and conforms to these guidelines it is necessary to know the following. Is the laboratory aware of the guidelines existence? Does the laboratory incorporate the recommendations from the guidelines into their standard operating procedures and decision thresholds? Does the laboratory have dialogue with its clinical users to ensure that the clinicians understand the appropriate use of biomarkers and the strengths and limitations of their use for clinical decision making in patients with suspected cardiovascular disease? Assessment of the laboratory understanding and introduction of current guidelines has been investigated by performance of serial audits under the auspices of the EFCC/EFLM with the assistance of national societies of clinical biochemistry. It has taken the form of a web-based questionnaire to assess overall understanding by laboratory professionals of the implications of the current guidelines for laboratory practice. The most recent audit is targeted specifically at high sensitivity troponin assays and B type natriuretic peptide. The two previous audits highlighted a lack of independently verified data for decision thresholds for cardiac biomarkers and inadequate engagement between the laboratory and the clinical community. The current audit further explores the present state-of-the-art. Since the initial two audits, there have been minor amendments to one of the key guidelines, universal definition of myocardial infarction, and publication of a new recommendation on the use of high sensitivity troponin measurement in patients presenting with suspected acute coronary syndromes. It remains to be seen how far the reports of the previous audits have modified behaviour and being most recent clinical guidelines have been translated into routine laboratory practice. On behalf of the EFLM WG-CM

WHAT’S DIABETES IN 2013

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In this talk I will explore three features of variation, which impact our management of diabetes. First, genetic variation. Second, variation in disease biomarkers. Third, variation in therapeutic responses, which leads on to the concept of personalized medicine. Evolution strives through natural selection to maximize fitness and thereby enhance reproductive success. Since natural selection selects optimum genes to optimise survival, genes, by implication, must vary. Without genetic variation there could be no genetic selection. Genes adapt when the environment changes. But genotypes are only as good as the environment allows them to be. A genotype adapted to one environmental niche may be maladapted to another, or even maladapted to the same niche should that change. Common modern human diseases are largely associated with good genes, which behave badly in our modern environment. That variation in gene-environment interactions has resulted in variation in biomarkers used for diabetes diagnosis (e.g. HbA1c) as well as individual variation in the risk of complications associated with diabetes. The range of drugs now available to treat diabetes is also associated with a range of responses to each therapy so that there is a move to personalize medicine based on individual needs, heterogeneity in the causes of diabetes and variation in individual responses to drugs used to treat diseases. The benefits of each given therapy varies substantially across a population, but also from individual to individual and from one drug to another. Finally, mortality rates can vary and have varied substantially over the recent centuries, even over the last century. Many of these benefits in the 20th century are not noticeably linked to medical advances but benefit more from the study of epidemiology and political responses to epidemiological discoveries.
Background: HbA1c has a small intra-individual biological variation, also in diabetic subjects, and is well accepted as an integrated measure of circulating glucose levels, tracking well in individuals over time. In 2009, on the basis of epidemiological studies on microvascular complications, the American Diabetes Association proposed HbA1c as one method for diagnosing diabetes, and this proposal was endorsed by the World Health Organization in 2011.

Methods: I have investigated several contributions published in this area since 2009, some of them being new experimental studies, some other being retrospective or longitudinal studies, some other in the form of meta-analysis or reviews.

Results: In the majority of the contributions a comparison between different diagnostic criteria is the main focus, and is now clear that the criteria cannot be used alternatively, since different subjects are diagnosed with type 2 diabetes in middle-aged as well as in older subjects. Among the pre-analytical confounding factors ethnicity and age has been well investigated, and significant disparities have been found among different groups, although no ethnic differences in the association of HbA1c with retinopathy were found. Pregnancy, hemolytic anemia, chronic renal insufficiency and liver cirrhosis are the conditions which most frequently appear to give false low values of HbA1c, and iron deficiency due to various reasons is certainly the most common cause of false high HbA1c values. Other biochemical and physiological phenomena (red cell life span, glucose transport across the red cell membrane and deglycating enzyme activities) may be relevant in defining to the so-called fast or slow glycators phenotypes.

Conclusions: There is uncertainty regarding the use of HbA1c for diagnosing diabetes. It appears more and more evident that using both HbA1c and glucose tests to diagnosing diabetes is convenient and efficient, since HbA1c alone is adequate for screening but too insensitive for the diagnosis of diabetes. Important issues still to be solved concern which glycemia parameter is the best predictor of micro- and/or macro-vascular complications, and if the use of HbA1c as an additional diagnostic measure will improve the clinical outcome of type 2 diabetic patients.

Background: For over 10 years, controversy has reigned regarding the adequacy of point-of-care glucose measuring devices (often called meters) for use in hospitals. In the United States the Food and Drug Administration has made clear that none have been cleared with an indication for use in protocols in which glucose is measured by meters in comparison with other devices. As an alternative to clinical trials, we have undertaken simulation modeling of glycemtic control in which the imprecision and bias of the glucose measurement procedure are variables.

Results: These studies have shown that both the rate of hypoglycemia and glycemic variability are adversely affected by errors in measurement of glucose. These studies also have provided suggestions of the allowable total error of the measurements, which includes inherent meter error, operator error, and error associated with preanalytical and postanalytical steps of the process. On-going studies are defining the effect of frequency of measurement of glucose on this allowable error.

Conclusions: These studies are important as it is now possible to measure glucose several times per hour rather than, at best, hourly with meters. Devices with such capability for higher frequency of measurement can also provide alarms to indicate impending hypoglycemia, thereby offering potential to decrease the frequency of hypoglycemia even if their analytical performance is no better than that of comparison devices.

Background: Gestational diabetes mellitus (GDM) is a potentially serious condition that affects many pregnancies and carries risk for the mother and the neonate. The current recommendation is to perform GDM screening before 28 weeks of gestation by an oral glucose tolerance test (OGTT). The aim of this study was to determine the usefulness of hemoglobin A1C (A1C) as a diagnostic tool for GDM compared with the traditional criteria based on glycemias measurements.

Methods: This was a study of diagnostic test accuracy. We evaluated the status of carbohydrate metabolism by performing OGTT and A1C in Brazilian pregnant women attending prenatal visits at a university tertiary care hospital in southern Brazil. A1C, OGTT, and clinical history were evaluated. GDM was defined according to the American Diabetes Association (ADA) criteria (fasting, 1-h, or 2-h plasma glucose concentrations ≥5.1, ≥10.0, or ≥8.5 mmol/L, respectively) or World Health Organization (WHO) criteria (fasting or 2-h plasma glucose ≥7.0 mmol/L or ≥7.8 mmol/L, respectively). Presence of anemia, variant hemoglobin and chronic renal disease were excluded. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic performance of A1C.

Results: A total of 262 pregnant women (mean age 30 years, mean gestational duration 26 weeks) were enrolled and 83 (31.7%) were diagnosed with diabetes (40 by ADA criteria and 43 by WHO criteria). Based on ROC curve analysis, and considering OGTT as the reference criterion, the cut-off point obtained by the point with the best equilibrium between sensitivity and specificity (100%-to-100% diagonal) was A1C value of 31 mmol/L (5.3%). The sensitivity and specificity for this cut-off point were 67.7 % and 61.6 %, respectively. The cut-off points of A1C of 40 mmol/mol (5.8%), 41 mmol/mol (5.9%) and 42 mmol/mol (6.0%) presented specificities of 98.2%,
99% and 100%, respectively. Our results showed that 38.5% of GDM cases would be diagnosed by using the cut-off point of A1C ≥40 mmol/mol (5.8%) solely.

Conclusions: A1C test presented low sensitivity but very high specificity for GDM diagnosis when compared to traditional criteria. A1C test, solely or in combination with OGTT, may be a very useful diagnostic tool in GDM.

**OC26**  
**LOGISTIC REGRESSION ANALYSIS FOR IDENTIFYING TYPE 2 DIABETICS WITH POOR RESPONSE TO SULFONYLUREA THERAPY**

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Background: Sulfonylureas are used as first-line therapy drugs in type 2 diabetes; however, therapy failure occurs in approximately 30% of patients. We hypothesize that at least one part of the cause lies in genetic factors. Sulfonylureas are insulin secretagogues that act by binding to pancreatic beta cells sulfonylurea receptor (SUR-1) encoded by two genes: ABCG8 and SUR-1 exon 33. Biochemical parameters were determined on automatic analyzer Olympus AU 2700 (Olympus, Hamburg, Germany). Based on the HbA1c concentration, patients were divided into two groups: patients with HbA1c concentration over 53 mmol/mol Hb were classified as patients with poor outcome. All variables are analysed using univariate regression analysis. Statistically significant variables were included into multivariate regression model.

Methods: The study included 251 unrelated type 2 diabetics on sulfonylurea therapy. Polymorphisms were detected using polymerase chain reaction - restriction fragment length polymorphism. Biochemical parameters were determined on automatic analyzer Olympus AU 2700 (Olympus, Hamburg, Germany). Based on the HbA1c concentration, patients were divided into two groups: patients with HbA1c concentration over 53 mmol/mol Hb were classified as patients with poor outcome. All variables are analysed using univariate regression analysis. Statistically significant variables were included into multivariate regression model.

Results: Univariate regression analysis identified following variables as statistically significant: fasting glucose (OR (95% CI) = 1.60 (1.37-1.88)); postprandial glucose (1.28 (1.17-1.40)); SUR1 exon 16 polymorphism (1.58 (1.06-2.36)) and SUR-1 exon 31 polymorphism (0.51 (0.33-0.77)), while age, gender, BMI and lipid concentration didn't differ significantly across subgroups. After inclusion into stepwise multivariate regression model, fasting and postprandial glucose and SUR-1 exon 31 polymorphism remained significant (1.45 (1.22-1.74); 1.14 (1.03-1.27) and 0.48 (0.30-0.80), respectively). Percent of correctly classified cases was 74.10%. Conclusion: Type 2 diabetics with high fasting and postprandial glucose and wild type allele of the SUR-1 exon 31 (Arg1273Arg) polymorphism are more likely to have poor glycaemic control expressed as high HbA1c concentration.

**SY64**  
**NON INVASIVE APPROACHES FOR THE GENETIC ANALYSIS OF THE FOETUS**

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Background: Since 1997, it is known that cell-free foetal DNA is present in the plasma of pregnant women. Such circulating foetal DNA is present at a mean fractional concentration of approximately 15% and is detectable from the first trimester of pregnancy.

Methods: Since 2008, much interest has been focused on the use of massively parallel sequencing for the analysis of foetal DNA in maternal plasma.

Results: With this development, the robust non-invasive prenatal detection of foetal trisomy 21 has been achieved. This application has been validated by a number of large scale clinical studies from multiple groups. As a result, non-invasive prenatal tests for trisomy 21 and a number of other foetal chromosomal aneuploidies have been used clinically in a number of countries since 2011. There are also recent developments in the use of this technology for twin pregnancies. Furthermore, the application for maternal plasma DNA sequencing for the non-invasive prenatal diagnosis of monogenic diseases has also been described. In addition, since 2010, three papers describing the non-invasive prenatal determination of the fetal genome have been published.

Conclusions: These rapid developments suggest non-invasive prenatal genetic analysis will likely play an increasingly significant role in future prenatal care. It is thus timely for stakeholders to start exploring the ethical, social and legal implications of such developments.

**SY65**  
**BIOCHEMICAL MARKERS FOR EARLY PREGNANCY SCREENING FOR PRE-ECLAMPSIA**

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Preeclampsia (PE) affects 2 to 5% of pregnancies in developed countries and may reach a much higher prevalence in other parts of the world. It is still a major cause of maternal and perinatal morbidity in particular when delivery occurs before 34 weeks’ gestation (GA). Implementation of preventive measures such as the prophylactic use of aspirin early in pregnancy may reduce the prevalence of preeclampsia in women considered at high risk and diminish morbidity and mortality in both the affected mother and her child. Until recently, only maternal history and risk factors were available to identify women at higher risk with equivocal predictive values (low detection rate, high false positive rate). The emergence of Doppler ultrasonography parameters (e.g. uterine artery pulsatility index) and biochemical markers related to angiogenesis, placental function, inflammation and endothelial dysfunction has led to more complex risk-prediction models based on multivariable algorithms. Various models will be reviewed which include maternal characteristics and history (e.g. ethnicity, familial and personal history of PE, chronic hypertension, diabetes, mean arterial blood pressure, body mass index), candidate biomarkers such as placental growth factor (PIGF), soluble Fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF), pregnancy-
associated plasma protein-A (PAPP-A), hCG combined or not to biophysical markers such as uterine artery pulsatility index (PI). So far, early pregnancy prediction algorithms combining maternal characteristics, biophysical (PI) and biochemical markers have reached in one specific large cohort detection rates of over 90%, 80%, and 60% for early-onset (GA<34), intermediate-onset (GA<37) and late-onset (GA: term) PE respectively at a false positive rate of 10%. However these very encouraging results have not yet been reproduced with a similar performance in other independent studies. Before concluding to the transferability of such models, there is a clear need for investigating the performance of the most promising predictive models in cohorts from different populations and healthcare environments to demonstrate the clinical utility of the screening procedures.

SY66
ANTENATAL SCREENING FOR DOWN SYNDROME
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Down Syndrome screening over the past 20 years has developed from the measurement of second trimester maternal serum biochemical markers such as AFP, hCG, UE3 and Inhibin-A, through to the first early trimester Combined test utilising the ultrasound measurement of Nuchal Translucency and maternal serum free beta-hCG and PAPP-A. Such changes have allowed detection rates and false positive rates to improve from the 30%/5%/achievable using maternal age, through the 70%/5%/achievable using the second trimester Quad test, to the 85-90%/5%/achieved by the Combined test. The focus of national screening programs in recent years has been to move to the Combined test, whilst at the same time aspiring to reduce the false positive rate and Invasive Testing rate to 2-3%. This lecture will outline these developments and suggest ways in which the Combined test can reach these new targets. New models of pregnancy care based on early first trimester screening for multiple conditions will also impact on future screening services and this will be briefly discussed.

OC27
MATERNAL VITAMIN B12 LEVELS DURING THE FIRST TRIMESTER OF PREGNANCY AND INFLUENCE IN NEWBORN SCREENING RESULTS
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Background. During pregnancy, vitamin B12 concentrations may drop physiologically, and concentrations below reference values may not alter certain parameters. The reference range used for our general population is 254-1320 pg/mL. The use of non-pregnant values may not be appropriate for assessing vitamin B12 status during pregnancy.
Methods. Serum vitamin B12 concentrations were evaluated in 204 pregnant women during the first trimester to calculate the reference interval (LOCI®, Dimension Vista, Siemens Healthcare Diagnostics). To assess the effects of the variables BMI, vitamin supplements (including type and dosage), dietary intake, parity and smoking habits on vitamin B12 serum concentrations, stepwise multiple linear regression models using backward elimination were used. Other factors including the newborns' birthweight, and expanded newborn screening results (propionylcarnitine levels, C3/C2 and C3/C16 ratios) were considered.
Results. The women participating in the study ranged in age from 15 to 46 (mean 30.0, SD6.11). The mean serum concentration of vitamin B12 was 502.4 pg/mL (SD 142.81). The reference range calculated was 272.7-837.8 pg/mL. Vitamin B12 concentrations were significantly lower in smokers, and in women with low meat consumption. The other factors did not have any significant effect. Newborns of mothers with lower vitamin B12 levels presented lower birthweight and higher propionylcarnitine levels, with higher C3/C2 and C3/C16 ratios.
Conclusions. The reference interval for serum vitamin B12 concentrations obtained is narrower than the one currently used for our general population. Smoking seems to have a negative effect, and meat consumption a positive effect on vitamin B12 levels. Mothers with lower vitamin B12 concentrations during the first trimester of pregnancy seem to have children with lower birthweight and higher propionylcarnitine levels.

OC28
A 1H-NMR STUDY OF BRONCHOPULMONARY DYSPLASIA IN VERY LOW BIRTH WEIGHT INFANTS: THE CONTRIBUTION OF THE METABOLOMIC APPROACH
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Background: Bronchopulmonary dysplasia (BDP) is an important cause of mortality and morbidity in preterm infants; its prevalence in very low birth weight (VLBW) infants receiving mechanical ventilation ranges between 4.2% and 40%. The etiopathogenesis of the disease recognize as determinants the use of supplementary surfactant, the extreme prematurity, lack of angiogenesis, and infections. However, the molecular pathways triggering BPD are still undefined. Recently, the development of BPD has been correlated with an alteration of the metabolism. Metabolomics, the latest of omics disciplines, has been successfully used elsewhere in neonatology, pharmacology, toxicology, etc. Due to the ability to identify metabolic abnormalities aims to identify metabolite patterns with high specificity and sensitivity, each of them associated with certain conditions. The aim of this experiment was to analyze the urine metabolic profiles of VLBW infants affected by BDP and to compare with those obtained in a group without BDP (controls), by using the Nuclear Magnetic Resonance (NMR) coupled with mathematical models.
Methods: Urine samples were collected from 6 VLBW infants with BDP and 11 controls. Children were matched for birth weight and gestational age. Before analysis, 540 µL of urine sample were treated by adding 60 µL of phosphate buffer (1.5M, pH7.4). Urines were analyzed using 1H-NMR Varian. 1H-NMR spectra were subjected to multivariate analysis using
SIMCA-P+ (Umetrics, Sweden).
Results: Spectra were aligned, binned, normalized and analyzed using a OPLS-DA (Orthogonal Partial least squares discriminant analysis) model. The mathematical model was able to discriminate between the group of BDP infants and controls with a very high significant statistical value R2X=0.613; R2Y=0.823. The most significant metabolites discriminating the separation between the two classes belonged to the aliphatic and aromatic regions. In addition, among these metabolites, creatinine, hippurate, and urea have been well recognized.
Conclusion: These preliminary results seems to be promising for the characterization of the BDP urine profile, as well as for the identification of predictors biomarkers characterizing the condition which may help for the treatment of this type of diseases.

SY67
LABORATORY TESTING IN BONE DISEASE: AN INTRODUCTORY OVERVIEW
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The key laboratory measurements in the evaluation of metabolic bone disease are the measurement of calcitropic hormones such as parathyroid hormone and vitamin D and the measurement of bone turnover markers. The assay features of biochemical markers of bone turnover (BTM) have markedly improved in the past few years (Vasikaran 2011). The most sensitive and specific markers of bone formation include serum bone alkaline phosphatase, total osteocalcin (including the intact molecule and the large N-Mid fragment) and the N extension peptide of type I collagen (PINP) assay. Among the various markers of bone resorption, measurements of the urinary excretion N- and C- related telopeptides (NTX and CTX respectively) and of serum CTX are the most sensitive and specific ones. Bone markers can be used to predict the rate of bone loss in postmenopausal women. BTM can be used to assess the risk of fractures in borderline cases. The prediction of fracture risk by BTM independently of BMD level has been confirmed in 3 other independent studies. Bone markers can be used to monitor the efficacy of antiresorptive therapy such as hormone replacement therapy, raloxifene and used to monitor the efficacy of antiresorptive therapy such as bisphosphonates. We and others have shown that the short term (3 to 6 months) decrease of bone turnover is significantly correlated with the long terms (2 years) increase in BMD of the spine. In addition, the decrease of urinary NTX and CTX is associated with the risk of vertebral fracture in osteoporotic women treated with risedronate. Similar studies in patients under alendronate or raloxifene show that the short term decrease of bone turnover markers is correlated with the risk of subsequent vertebral and non vertebral fractures. While the increase in BMD over 2 to 3 years explains up to 25% of the efficacy of bisphosphonates to reduce the risk of vertebral and non vertebral fractures, the rapid (3 to 6 months) reduction of bone turnover markers explain up to 70% of their fracture efficacy. Thus, with adequate cut-offs, individual patients can be monitored with bone markers earlier than with DXA. Finally, bone turnover markers can be used to identify response within individuals and so to encourage compliance.

SY68
HOW USEFUL IS THE LABORATORY IN BONE AND JOINT DISEASES IN CLINICAL PRACTICE?
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Diagnosis and monitoring of bone and joint diseases include a number of clinical, morphological and biochemical aspects. As a new emerging field, biochemical markers of bone turnover and joint tissues are able to shed light on the individual degenerative, metabolic and inflammatory conditions of the skeleton. Targets of biochemical analysis of bone and joint status are enzymes and proteins or their respective metabolites. The usefulness of these biochemical markers has been widely recognized during the past years and increased significantly with the technical improvement of biochemical methods and knowledge of new compounds of bone and joint environment. Many of these markers have been established but only few of them have entered daily clinical routine applications. Many options for characterization and diagnosis of metabolic bone and joint diseases but also therapy decisions and monitoring may be improved by these biomarkers.

SY69
THE NEED FOR STANDARDISATION OF BONE MARKER ASSAYS
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The incidence of metabolic bone disease is highest amongst the elderly and so with the ageing of the population, clinical interest in diagnosis and prognosis of osteoporosis and estimation of fracture risk has increased. Biochemical markers of bone turnover (BTM) offer a means for assessing two major clinical questions. Can baseline levels of BTM predict the rate of bone loss or future fracture risk? Can BTM be used to monitor the response to treatments for osteoporosis? Assays for numerous BTM are readily available on automated clinical chemistry analysers and point-of-care devices; however there is still considerable debate as to their clinical utility. A position paper published by the IOF-IFCC-IOF Working Group on Bone Marker Standards for Osteoporosis concluded that there were insufficient high quality data to provide clinical guidelines for their use in individual patients because their clinical value is limited by inadequate appreciation of the sources of variability, by limited data for comparison of treatments using the same bone marker and inadequate quality control. This paper recommended that in future clinical trials serum β-CTx be used to assess bone resorption and serum PINP be used to assess bone formation. A consequence of these recommendations is the need to standardize or harmonize the assays for these BTMs as appropriate. Similar conclusions were reached by the National Bone Health Alliance commenting on specific requirements for the USA. A second working group was established (IOF-IFCC WG-Standardisation of Bone marker Assays) in January 2012 to undertake these projects. Preliminary data from an external quality assurance scheme suggest there is an approximate 30% bias between the two automated serum sCTx assays while for the 3 major serum PINP assays, although considered to measure different forms of PINP, actually provide harmonised results. Current studies are underway to extend the data on the comparability of patient results for the two major serum CTx assays. Further strategies will then be developed to harmonise these assays.
following which the serum PINP assays will be investigated.

OC29
DIFFERENCE BETWEEN TOTAL AND INTACT ASSAYS FOR N-TERMINAL PROPEPTIDE OF TYPE I PROCOLLAGEN (P1NP) DETERMINATION IN RENAL IMPAIRED PATIENTS

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Introduction: The amino-terminal propeptide of type I procollagen (P1NP) circulates as different forms: the larger intact trimeric and several fragment monomers. In healthy individual, the circulating P1NP is predominantly the trimeric intact with almost non-detectable monomers. Under certain conditions, especially in renal impaired patients, the proportion of monomeric form is elevated. Intact P1NP assay measures the trimeric propeptide while Total P1NP assay measures both trimeric and monomeric forms. In this study we compared these two assays in renal impaired patients.

Methods: 84 serum specimens from CKD Stage 3 to 5 not on dialysis and 125 specimens from Stage 5 Dialysis patients were analyzed with the IDS-iSYS Intact P1NP and Roche Elecsys Total P1NP assays.

Results: In CKD not on dialysis subjects, the observed ranges for Total P1NP and Intact PNp were 8.5 – 822.8 ng/mL and 8.2 – 146.5 ng/mL, respectively. The correlation between the Total P1NP and GFR was r = -0.3373 (P = 0.0017) and between the Intact P1NP and GFR was r = -0.4183 (P = 0.0172). In Stage 5D subjects, the observed ranges were 18.4 – 2192.0 ng/mL for Total P1NP and 16.3 – 641.5 ng/mL for Intact P1NP. Their Passing Bablok regression was Total P1NP = 3.68 x Intact P1NP - 64.4.

Conclusion: The Total P1NP values were much higher than Intact P1NP confirming the Total P1NP assay measures both trimeric and monomeric forms. The significant correlation between the Total P1NP and GFR indicated the Elecsys Total P1NP assay might not be suitable for renal impaired patients; in these patients, the IDS-iSYS Intact P1NP is preferred.

OC30
KIDNEY TRANSPLANTATION AND OSTEOPOROSIS, STUDY OF GENETIC SUSCEPTIBILITY: INFLUENCE OF VITAMIN D RECEPTOR (VDR) AND ESTROGEN RECEPTOR (ESR1) GENES IN RELATION TO BONE LOSS AND FRACTURE RISK

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Background: Prevalence of osteoporosis in renal transplant patients is 17-49% for lumbar spine (LS) and 11-56% for femoral neck (FN) 22.5% developed some type of fracture in the five years following transplantation. Osteoporosis is genetically complex, the identification of the genes involved in its development is made difficult by its multifactorial nature. The “B” and “F” alleles of Vitamin D Receptor gene(VDR-BsmI: “BB”, “Bb”, “bb” genotypes; VDR-FokI: FF, Ff, ff Genotypes) are associated with lower bone mineral density (BMD). The “xx” genotype of the Estrogens Receptor gene(ESR1X-XbaI: “XX”, “Xx”, “xx” genotypes) predisposes to a bone mass reduction and the “P” Allele (ESR1P-Pvull: PP, Pp, pp genotypes) is associated with having a greater benefit from hormonal therapy. We aim to assess the influence of genetic polymorphisms in bone loss measured by BMD in a group of kidney transplant recipients.

Methods: We studied 139 kidney transplant recipients from a reference Hospital(92 male, 47 female, average age 50 and 48 years respectively). Polymorphisms genotyping: amplification of 5 regions of the human genome and detection of the amplified product (Clinical Arrays® MetaBone, Genomica), BMD: at pre-transplantation (pre), 6 months (6m), 1 year (1y) and 2 years (2y) post-transplant by Dual-Energy X-ray Absorptiometry, at LS and FN, measuring T-score and Z-score.

Results: There’s a BMD decrease in LS (P < 0.05) and FN (P < 0.001) 6 m after transplantation. “BB” patients have lower LS BMD pre (P = 0.023) and after 2y (P < 0.05). Patients with “xx” genotype have lower FN BMD 6m (P = 0.009) and 1y (P = 0.028) and in LS after 2y (P < 0.05). Patients with “P” allele have more FN BMD 6m (P = 0.02) and those with “F” allele have more LS BMD 1y (P = 0.02, so both “P” and “F” allele could provide protection against BMD loss. Calculation of risks: “xx” genotype leads to 3.069 - fold of having lower FN BMD 6m(ExpB=3.069) and “PP” genotype leads to 0.423-fold odds of having lower FN BMD 6m(ExpB=0.423).

Conclusion: Genotyping based on extended panels of several SNPs markers might identify groups of people at high risk of osteoporosis. Thus, the study of polymorphisms of genes related to bone metabolism, may be useful in the preventive treatment of kidney transplant recipients particularly susceptible to bone disease.

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SEPSIS: EPIDEMIOLOGICAL AND CLINICAL ASPECTS

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Sepsis is now defined as an infection associated with one organ dysfunction (this was previously called severe sepsis, and sepsis was referred to as infection). Several recent studies have explored the epidemiology of sepsis in different patient populations. Comparisons among these studies are difficult due to differences in definitions and in organizational factors among hospitals and ICUs, but some important data can be derived: First, sepsis affects 20-30% of ICU patients with 0.5- 3 cases per 1000 of the general population; second, the most common sites of infection are the lung and abdomen, and Gram-positive organisms are no longer the predominant causative agents; third, hospital mortality rates remain high at between 30 and 35%; fourth, in addition to increasing short-term mortality, sepsis is associated with increased morbidity and long-term mortality and with high costs; finally, although mortality rates may have decreased slightly in recent years, the incidence of sepsis is increasing so that overall deaths from sepsis are also increasing. The treatment of sepsis can be considered under 3 headings: Eradication of infection, hemodynamic resuscitation, and modulation of the sepsis response. With no new effective immunomodulatory strategies currently available in the pipeline, the clinical focus must be on early diagnosis to enable rapid initiation of antimicrobial therapy and optimal resuscitation strategies. However, diagnosis can be difficult without specific (bio)markers to better characterize the disease, and, in terms of optimal therapy, many questions remain unanswered. For example, evidence clearly supports the benefits of early
appropriate antibiotic therapy, but the optimal duration of antimicrobial therapy is less clear. Adequate fluid administration is known to be important but which fluid and to which endpoints is less certain. Norepinephrine is now considered the first-line vasopressor but which hemodynamic endpoints to target and whether we should also monitor the microcirculation remains unclear. The place of vasopressin and its derivatives is also still unsettled. Further study is needed to provide answers to these questions as research continues to try and identify effective immunomodulatory strategies.

SYT1
NEW CONCEPTS IN PRO-/ANTI-INFLAMMATORY RESPONSE IN SEPSIS
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Septic syndromes (systemic inflammatory response associated with infection) remain a major although largely under-recognized health care problem and represent the first cause of mortality in intensive care units. It is well known that sepsis deeply perturbs immune homeostasis by inducing first an initial tremendous systemic inflammatory response. However, novel findings indicate that sepsis initiates a more complex immunologic response that varies over time, with the concomitant occurrence of both pro- and anti-inflammatory mechanisms alternately predominating. After a short pro-inflammatory phase, septic patients enter a stage of protracted immunosuppression. This is illustrated in those patients by reactivation of dormant viruses (CMV or HSV) or infections due to pathogens, including fungi, which are normally pathogenic solely in the immunocompromised host. These alterations might be directly responsible for worsening outcome in patients who survived initial resuscitation as nearly all immune functions are deeply compromised. Both arms of immunity, innate and adaptive, are indeed markedly suppressed (including enhanced leukocyte apoptosis, lymphocyte anergy and deactivated monocyte functions). This lecture will attempt to integrate these new facts into an up-to-date account of immunoo-inflammation during sepsis pathophysiology. It will be focused on immune dysfunctions described so far in septic patients, on the mechanisms sustaining this immune failure, on the monitoring of the pro-/anti-inflammatory balance rapidly changing over time and on new promising therapeutic avenues emerging from those recent findings.

SYT2
EMERGING BIOMARKERS FOR TARGETED AND TAILORED THERAPY IN SEPSIS
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Biomarkers for sepsis have been tested in the clinical arena for their usefulness in the diagnosis of sepsis, but also as prognostic markers, and to guide therapy such as antibiotic treatment duration. All six clinical studies testing this latter concept have concluded that serum procalcitonin levels decrease over time was a useful marker to stop antibiotic treatment safely, cutting down the duration by a mean of 4-5 days in patients with severe sepsis and septic shock. This was not accompanied by a resurgence of the primary bacterial infection, nor by an increased mortality rate. This approach based on procalcitonin-guided therapy has the advantage over empirical rules to tailor the duration of the antibiotic treatment on the response of a given patient and/or a given infection type. A potential benefit for reducing treatment duration is the decrease of the risk of emergence of multi-drug resistant bacteria in the patient and within the intensive care unit, the decrease of potential toxicity related to antibiotics, and also to reduce costs related to prolonged antibiotic therapy.

OC31
A NEW RAPID, EASY AND COST-EFFECTIVE METHOD THAT IDENTIFIES UNKNOWN PATHOGENIC MICROORGANISMS WITHIN 3 H FROM SAMPLE COLLECTION

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Background: As current blood culture methods require at least several days, empirically selected antibiotics are instead administered until the pathogenic microorganisms are identified. Though mass spectrometry is can be utilized as a rapid identification method of pathogens, it requires the blood culture process, so it takes more than 10 hours after sample collection. We developed a novel rapid, easy, and cost-effective method that identifies unknown pathogenic microorganisms within 3 hours after collection of patient samples.

Methods: To detect pathogenic bacteria by PCR with high sensitivity, we developed a novel “eukaryote-made” Taq polymerase, which is free from bacterial DNA contamination (J Clin Microbiol. 2011 Sep; 49(9):3316-20). We also developed a novel method to identify the species of pathogenic microorganisms using 7 primer sets, real-time PCR, and original web-based identification software. After PCR is performed, 7 amplicons are obtained. Next, the combination of melting temperature (Tm) is acquired. These 7 Tm values, when mapped on two dimensions, create a “fingerprint” of a specific bacteria. Comparing the shape of the mapped Tm values to the shapes in the database, the pathogenic bacteria is identified. We named this method “Tm mapping method”. Results: We performed blind tests to evaluate its accuracy and specificity. Using the DNA samples of 107 kinds of bacterial species, the novel method gave the correct answer almost perfectly (106/107). Using the 140 bacterial colonies of 51 kinds of bacterial species, the identification rate was 94% (5 kinds of bacteria were not included in the database of our identification software). Using 20 patient samples (blood, amniotic fluid, aqueous humor, and artificial valve of the heart) of infectious diseases, the novel method gave the correct answer (30/30) within 3 h.

Conclusions: Our group developed this system for practical use without any dedicated equipment. To use this method from anywhere easily, we also developed the identification software that can be available on the web. We are now planning to perform the comparative studies between it and conventional microbial testing with more patient samples, as well as repeatedly evaluate its accuracy and specificity.
Background: Sepsis, a leading cause of death in critically ill patients, is the result of complex interactions between the infecting microorganisms and the host responses that influence clinical outcomes. Optimal management requires early goal-oriented therapies and may benefit from individualized circulating biomarkers for early risk stratification. We evaluated the prognostic value of presepsin (sCD14-ST), a novel marker of bacterial infection.

Methods: This is a nested case-control study of the multicenter, randomized ALBIOS trial that enrolled patients with severe sepsis or septic shock in 100 ICUs in Italy. We selected 50 survivors and 50 non-survivors at the time of ICU discharge (21±18 days) matched for age, sex, center and time of enrolment after the presence of inclusion criteria. EDTA-plasma samples were collected at days 1, 2 and 7 after enrolment to assay presepsin (immune-chemiluminescence assay PATHFAST Presepsin, URL 320 pg/mL, CV 5%, Mitsubishi Chemicals) and procalcitonin (PCT, Elecsys BRAHMS Cobas® PCT, URL 0.046 ng/mL, CV 8.8%, Roche Diagnostics) in a central laboratory.

Results: Clinical characteristics were similar in the 2 groups, but non-survivors had a worse SOFA score at day 1. Patients with higher baseline presepsin had worse SOFA score, lower mean arterial pressure and diuresis. Early presepsin (d1) was higher in decedents (2268 [1145-4305] pg/mL, median [Q1-Q3]) than in survivors (1184 [855-2158] pg/mL, P=0.001) while PCT was not different (18.5 [3.3-45.7] vs. 10.8 [2.6-46.4] ng/mL, P=0.31). Presepsin decreased over time in survivors but remained elevated in non-survivors (974 [674-1927] vs. 1184 [855-2158] pg/mL, P=0.001) while PCT was not different (18.5 [3.3-45.7] vs. 10.8 [2.6-46.4] ng/mL, P=0.31). Presepsin decreased over time in survivors but remained elevated in non-survivors (974 [674-1927] vs. 1184 [855-2158] pg/mL, P=0.001) while PCT was not different (18.5 [3.3-45.7] vs. 10.8 [2.6-46.4] ng/mL, P=0.31). Presepsin decreased over time in survivors but remained elevated in non-survivors (974 [674-1927] vs. 1184 [855-2158] pg/mL, P=0.001) while PCT was not different (18.5 [3.3-45.7] vs. 10.8 [2.6-46.4] ng/mL, P=0.31).

Conclusions: We provide first evidence in a multicenter clinical trial that early presepsin measurement provides relevant prognostic information in patients with severe sepsis or septic shock and may be of clinical importance for early risk stratification.

Work on modification of ISO15189 "Medical Laboratories-Requirements for quality and competence" started immediately after the first edition of 2003. After much debate and an enquiry, it resulted in an FDIS in August 2012, which was accepted with a 100% vote. The third revised edition of ISO15189 can be expected in the first months of 2013. Important changes are: clarification in terminology, adding headings to chapters, which makes reading easier, stressing difference between verification for well described methods and validation for home brew ones. As in the earlier versions the whole process from request to result, including interpretation, was maintained. Much attention was paid to making it less prescriptive. All quality goals should be related to requirements in favor of treatment of patients. The patients and their clinical needs are in the middle. ISO15189 is a combination of ISO17025 with its focus on competence in testing, and ISO9001 with its focus on quality as expected by the customer. In the new edition chapters have as headings: resolution of complaints, identification and control of nonconformities, corrective actions, preventive actions, continuous improvement, control of records, evaluation and audits with as subheadings: review of request, user feedback, risk assessment, quality indicators and review by external organizations. The ISO TC212 WG decided to start with a revision of ISO/TS22367:2008 "Medical laboratories-reduction of error through risk management and continual improvement". It underlines the approach which should be followed in implementing ISO15189. Corrective actions related to identified and preventive actions related to potential nonconformities, errors and mistake, shall be taken by the management. There is a need for classification, and use of risk analysis is promoted. Examples are given of the Failure Mode and Effect Analysis, supplemented with hazard or criticality analysis. In the new regulation for CE marking of ivd’s their classification is dependent on patient safety risk. In fertility laboratories risk analysis to prevent harm to the patients is actually instituted. It is expected that this approach will be used in a modified form in all kinds of medical laboratories. Thus focus on patient safety.
issues on laboratory management in the Arab region

G. Shannan

The Arab Federation of Clinical Biology, AFCB represents considerable parts of the Middle East and North Africa with 11 countries of the region are members of this organisation. The population of AFCB countries is around 300 million inhabitants with more than 4000 - 5000 practicing laboratory medicine in the public and private sectors. Practicing Clinical Chemistry and Laboratory Medicine in AFCB Region varies from country to country; as well as the regulations which govern laboratory medicine practice. In this paper we will shed some lights on the current practice of Laboratory Management in AFCB region with discussion about the level of implementations; in addition to the legislative, financial and/or scientific obstacles which have prevented some countries of the region from prioritising Laboratory Management Practice. The laboratory Management concept has not been fully implemented in any of the AFCB countries; however, there are various levels of implementation in the Arab Region. In some countries only basic practice of Quality Assurance have been considered while in other countries advanced practice of laboratory management including accreditation have been implemented. The current practice with its pros and cons will be discussed including the differences from country to country. The role of legislation in shaping the future of Laboratory Medicine and the challenges to unify these legislations will be discussed. Quality issues will be a major target of any new developments. The implementation of quality standards and Accreditation Programme and the assistance required from various national and international organisations will be presented. The region faces some major problems with manufacturers of laboratory equipment and instruments due to the size of the market which in some instance is not so attractive in comparison with the European and American Markets. We feel that the manufactures are not so interested in investing in such countries to provide proper after sale service. The training of Laboratory Medicine in AFCB region is a key issue which should be addressed and a plan of action should be established to improve the quality of training to reach the international standards.

performance; d) timeliness and possible utilization as a measure of laboratory improvement

A model of quality indicators (MQI) has been consensually developed by a group of clinical laboratories according to a project launched by a Working Group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The model includes 57 QIs related to key processes (34 pre-, 7 intra- and 15 post-analytical phase) and 3 to support processes. Therefore, the scope of harmonization in laboratory medicine is more far-reaching than method harmonization and should cover a wider range of topics, namely all steps of the “brain-to-brain loop”. Valuable QIs, covering all steps of the TTP, valuable QIs represent a key step in the journey towards quality and patient safety in laboratory medicine.

EUROMEDLAB 2013 - SCIENTIFIC SESSIONS

OC33

USE OF STANDARDISED ALGORITHMS WITH CLINICAL DECISION RULES AND D-DIMER TESTING IN CLINICAL PRACTICE TO AID IN THE DIAGNOSTIC WORK-UP FOR SUSPECTED VENOUS THROMBOEMBOLISM: A STUDY IN 7 EUROPEAN COUNTRIES

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Background: It has been shown that the need for radiologic imaging can be reduced by about 1/3 if standardized algorithms consisting of clinical decision rules (CDRs) and D-dimer tests are used in the diagnostic work-up for patients with suspected venous thromboembolism (VTE). The aim of this study was to explore if such algorithms are used in different European countries.

Methods: Two case histories related to diagnosis and exclusion of suspected pulmonary embolism (Patient A, high pretest probability) and deep venous thrombosis (Patient B, low pretest probability) were sent to physicians working with VTE patients. The physicians were asked about the estimated probabilities of VTE before and after D-dimer testing, if clinical decision rules were used to assess the probabilities and when to use D-dimer and/or radiologic imaging.

Results: Altogether, 547 physicians from 7 different European countries responded, after exclusion of 78 that did not regularly diagnose VTE. For patient A, most physicians (83%) suggested a high pretest probability or likely diagnosis of PE. Still 10% of them would request D-dimer alone, 69% both D-dimer and radiologic imaging and 19% radiologic imaging alone. If the D-dimer was negative in this patient, 16% suggesting a high probability even would regard PE as excluded. For patient B, the majority (81.6%) of the physicians suggested a low pretest probability or unlikely DVT diagnosis. D-dimer alone was requested by 60.8% of physicians, while 17.4% of clinicians would request D-dimer and radiologic imaging in parallel and 11.4% only radiologic imaging. CDRs were always or often used by 31.5% of physicians. The pretest probability estimates in percent varied substantially for both patient A and B, regardless of the use of CDRs.

Conclusions: Exclusion of VTE by negative D-dimer results might underdiagnose VTE in high probability patients and puts the patients into significant risk. Applying radiologic imaging without or in parallel with D-dimer testing in low probability VTE cases imply extra burden on the health care system, and could be avoided if standardized algorithms were followed. Results from this study suggest that standardized algorithms are not systematically followed by a substantial amount of physicians.
OC34
ALERT VALUE REPORTING IN PRIMARY CARE SETTING: A NEW STRATEGY FOR PATIENT SAFETY

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Background: In contrast to critical value reporting, alert value reporting would not allude to a result that may imply a life-threatening situation, but would indicate that an early diagnostic/therapeutic action would improve the patient’s management and quality of life. Objectives are to introduce the “alert value reporting” concept, to propose a list of chemistry and hematology alert limit tests that can be chosen for that strategy, to show how this notification procedure can be designed and established, and finally to evaluate the effectiveness and physicians’ satisfaction regarding the proposed approach.

Methods: Since laboratory requests were made on-line and the reports sent electronically from the laboratory information system to the patient’s electronic medical record, General Practitioners (GP) daily habit of manually checking each laboratory report in order to look for test results that might necessitate a re-appointment was altered. A method was devised that would automatically alert the GP to a result that needed a follow up and alert value reporting concept was established. A list of chemistry and hematology alert limit tests to be used for the strategy was agreed upon between laboratory professionals and GPs. Next, a retrospective 12-month study involving more than 1 million laboratory tests was made to check how many of these alert values would had been communicated if this theoretical alert values had been applied. A prospective analysis of every reported alert value during 6 months was carried out to assess the intervention effectiveness and the requesting physician’s satisfaction with the new strategy.

Results: The alert value reporting was successfully executed. Regarding the 6 months period prospective analysis in a single primary care center, 4309 requests were received. Among those, there were 154 alert values (3.5%). 30 of them (19.5%) motivated an anticipation of the patient next appointment. 90% of physicians considered alert value reporting as an interesting strategy.

Conclusions: The alert value reporting concept was introduced and notification procedure designed. Alert value reporting strategy motivated changes in patient’s management. Further studies are needed to test the contribution to patient safety and decision-making.

EW002
THE NEW ERA OF HCV TREATMENT AND MONITORING

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Hepatitis C virus (HCV) infection is a global health problem affecting around 3% of the population worldwide, with chronic infection potentially leading to the severe consequences of cirrhosis and liver cancer. Over 250,000 people are dying from HCV-related liver disease each year. While chronic infection with HCV can be cured with effective drugs, only a fraction of those who could benefit are receiving therapy, highlighting the need for effective screening programs. The first breakthrough came when HCV was identified in 1989, allowing development of blood screening tools that have virtually eliminated HCV transmission via blood transfusion. Today, powerful immunoassays as well as molecular tests are available for diagnosis of all HCV genotypes and treatment monitoring of patients infected with HCV. For those patients who have acquired HCV by whatever means, interferon-based therapy paired with ribavirin has been the standard of care. Recently, new direct acting antiviral agents (DAAs) have been developed which substantially improve cure rates. Once patients are receiving treatment, HCV RNA monitoring indicates how well they are responding. Response-guided therapy is allowing physicians to shorten, extend or stop therapy based on the HCV RNA levels, and to ensure that patients have not relapsed during post-treatment follow-up. During this symposium we will give an overview on current trends in HCV diagnosis, treatment and treatment monitoring and will discuss how new treatment strategies might impact the future of HCV management.
**EW 003**

**CLINICAL IMPLICATIONS OF HBSAG QUANTIFICATION IN PATIENTS WITH CHRONIC HEPATITIS B**

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The quantification of serum hepatitis B surface antigen (HBsAg) has been shown to help the management of patients with chronic hepatitis B virus (HBV) infection. Median serum HBsAg levels differ significantly during the natural history of HBV infection, progressively declining from immune tolerance to inactive phase. The combination of an HBsAg <1000 IU/mL and HBV DNA <2000 IU/mL at a single time point accurately identifies true inactive carriers. During antiviral treatment, HBsAg levels decline more rapidly in patients under peg-interferon (Peg-IFN) than in those under nucleos(t)ide analogues (NUC), and in responders to peg-IFN compared to non-responders suggesting that a response-guided therapy in both HBsAg-positive and -negative patients treated with Peg-IFN could improve to cost-effectiveness of this therapeutic approach. Given the low rates of HBsAg clearance on NUC therapy, new studies are needed to test whether Peg-IFN and NUC combination fosters HBsAg decline in long-term responders to NUC, are being explored.

**EW004**

**GENETIC TESTING FOR RISK OF CORONARY HEART DISEASE: FACT OR FICTION?**

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Currently, estimation of an individual's risk of future coronary heart disease (CHD) risk is achieved using risk algorithms that include only conventional risk factors (CRFs) such as age, BMI, blood pressure, smoking status, family history and plasma levels of lipids. What is the potential of improving risk prediction by adding data from an individual's genetic make-up? Since many SNPs can be cheaply determined simultaneously in a single sample (for example using DNA obtained from a buccal swab) this will not have major cost implications. Meta-analyses have identified 8-10 "candidate" genes with common functional variants that influence CRFs (e.g. APOE on plasma lipids) and that have statistically robust but modest effects on CHD (Relative Risk: 1.1-1.4), although none of these singly have clinical utility when added to a risk algorithm. Genome Wide Association Studies have now identified > 30 SNPs associated with CHD risk, and the top priority now is the determination of the functional variation at each locus, their causal mechanism for CHD, and the accurate estimation of their risk effect in prospective studies, in different ethnic groups and in men and women. Although the effect sizes of these SNPs is also similarly modest, recent data and modelling suggests that, in combination, these will have at least moderate clinical utility, primarily in risk stratification and treatment prioritisation in individuals at intermediate risk. Here we present data demonstrating this in a group of 3000 healthy middle aged men (NHPS-II) followed prospectively for >18 years with >300 CHD events. Additional studies are necessary to examine both the acceptability and the cost effectiveness of any such DNA-based additions to CRF-based algorithms. However further research is needed to explore different ways to present such genetic risk information to individuals, so as to find approaches that minimise a sense of falsafism and maximise motivation for behaviour change.

**EW005**

**GENE-GENE INTERACTIONS CAN MODIFY THE EFFECT OF A SINGLE-NUCLEOTIDE-POLYMORPHISM ASSOCIATED WITH BLOOD PRESSURE LEVELS**

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Background: Although numerous genetic studies have been performed, only 0.9% of blood pressure phenotypic variance has been elucidated. This phenomenon could be partially due to epistatic interactions. Our aim was to identify epistatic interaction(s) associated with blood pressure levels in a pre-planned two-phase approach.

Methods and results: In a discovery cohort composed of 3,600 French individuals, we found rs6046A allele in SELE associated with decreased blood pressure levels (P = 3.7x10-3) and rs535ST allele in SELE associated with decreased diastolic blood pressure levels (P= 5x 10-3). Both variants interacted in order to influence blood pressure levels (P ≤0.048). This interaction was replicated with systolic blood pressure in 4,620 additional European individuals (P=0.03). Similarly, in this replication cohort, rs6046A was associated with decreased blood pressure levels (P ≤8.5x10-4). Furthermore, in peripheral blood mononuclear cells of a subsample of 90 supposed healthy individuals, we found rs6046A positively associated with NAMPT mRNA levels (P ≤9.1x10-5), suggesting an eventual involvement of NAMPT expression in blood pressure regulation. Confirming this hypothesis, further transcriptomic analyses showed that increased NAMPT mRNA levels were positively correlated with ICAM1, SELL, FPR1, DEFA1-3 and LL-37 genes expression (P ≤5x10-3). The last two mRNA levels were positively associated with systolic blood pressure levels (P ≤0.01) and explained 4% of its phenotypic variation.

Conclusion: These findings reveal the importance of epistatic interactions in blood pressure genetics and give new insights for the role of inflammation in its complex regulation.

**EW006**

**AUTOMATED SOLUTION IN A MULTI-DISCIPLINARY LABORATORY FOR BETTER PROCESSES AND CLINICALLY EFFECTIVE SERVICE**

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Background It is widely accepted that nearly 60% of medical decisions are dependant upon patients' laboratory diagnostic results and it is therefore clear that clinical diagnostics play a critical role in helping to save lives and improve patient care. To remain effective, clinical laboratories must be able to turn tests around quickly without compromising accuracy. More so, laboratory staff must be utilized in a way that keeps them focused on examining and managing test results and away from laborious manual tasks that do not directly impact the delivery of critical healthcare data. Facing a shrinking labour force and budgets, clinical laboratories are increasingly turning to advanced automation systems to help them meet the demand for in vitro diagnostic testing. Laboratory automation systems not only deliver workflow and efficiency improvements, but have also been proven to reduce error rates and redirect staff time to better use.

Methods: NHS Tayside recently merged its Biochemical Medicine, Immunology, and Haematology departments into a single Blood Sciences Department serving a population of...
470,000. As part of this transformation, the health care system adopted the Siemens Healthcare Diagnostics Aptio\textsuperscript{TM} Automation solution to facilitate the integration of a multi-disciplinary workload onto a single track with a Lean approach to sample management.

Results: The system is currently processing over 6,000 tubes per day.

Conclusions: This paper describes NHS Tayside's decision process around its eventual acquisition of an automation system as well as the metrics it used to measure anticipated outcomes. Additionally, future key performance indicators are discussed.

EW007
CENTRALLY MANAGING POINT-OF-CARE TESTING TO IMPROVE PATIENT CARE AND STANDARDISE PROCEDURES

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Background: The Royal Free London NHS Foundation Trust is known worldwide for clinical excellence. The need to demonstrate compliance across a range of point-of-care testing (POCT) services was a major driver for upgrading to version 4 of Siemens Healthcare Diagnostics' RAPIDComm\textsuperscript{®} Data Management System. Achieving Clinical Pathology Accreditation (CPA) for POCT services is extremely important for the Trust. The RAPIDComm system facilitates record keeping, remote troubleshooting, quality assurance monitoring, operator management and auditing, all of which are key to meeting regulatory requirements.

Methods: Accreditation standards require comprehensive maintenance, patient, and quality assurance records for all POCT devices. The RAPIDComm system allows maintenance tasks to be scheduled when due and then recorded as complete and we have gone paper-free with our maintenance records. Patient safety is paramount; The system enables our governance arrangements for POCT to be implemented effectively. An ADT feed from our hospital information system facilitates positive patient ID at the analyser. By using RAPIDComm operator management capabilities, we can be sure that only authorised, competent users have access to our POCT devices. Ensuring quality of patient results; Internal quality control data can be monitored and device performance compared easily. Using the freetext comment feature allows us to comprehensively document any corrective or preventative actions taken, providing traceability and a full audit trail.

Results: The RAPIDComm system has significantly improved our workflow. Daily analyser checks can be performed centrally via the status screen and reagent levels and expiry dates can be observed. This allows the POCT team to plan routine maintenance effectively. Ascertainment in advance those consumables to be replenished prevents unnecessary journeys to and from the laboratory. Remote device monitoring allows us to proactively troubleshoot issues before they become a problem. We have realized increased analyser uptime and more effective use of staffing resources; allowing more time for training clinical users and auditing.

Conclusions: The RapidComm system has become a vital member of our point-of-care testing team, helping us to record-review-act.

EW008
CURRENT AND FUTURE LABORATORY REQUIREMENTS IN A CONSOLIDATING MARKET

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In the United Kingdom the National Health Service (NHS) spends over £2.5b each year on pathology services, equating to 4% of the overall budget. In 2008, the “Report of the Second Phase of the Review of NHS Pathology Services in England” was published, which stated that consolidation was necessary for pathology services to enable them to respond to future challenges, system and workforce reform. The report is focused on three main themes: improving quality and patient safety; improving efficiency, and identifying the mechanisms for delivering change. The review estimated up to £500m (20%) per year could be realised by consolidating pathology services. The following organisations agreed at CEO level to set up a Consortium, for the management of pathology services with a goal of 20% cost reduction: • Isle of Wight NHS Trust (IOW); • Portsmouth Hospitals NHS Trust (PHT); • University Hospital Southampton NHS Foundation Trust (UHS). In April 2011 a project team was set up with the aim of developing an outline business case (OBC) that described the formation of a Consortium that would change the configuration of pathology services. The preferred model for blood sciences was to have a hub laboratory for the outpatient (OPD) and primary care work based at PHT with essential services laboratories (ESLs) on the IOW and UHS sites. A specialist laboratory for referral work would be at UHS. In 2007 the biochemistry departments jointly procured the Beckman Coulter Power Processor system with DxC 800 and Dxl 800 analysers. Within biochemistry the workload was IOW 198k (2,494k), PHT 956k (8,163k) and UHS 886k (6,210k) samples (tests) per year. In the future configuration the workload will shift towards the hub; IOW 198k (2,494k), PHT 1,541k (11,870) and UHS 300k (2,503k). To facilitate this transfer the current analytical platforms are to be refreshed with a combination of AU5800, AU680, Dxl 800 and Dxl 600 systems. This will enable the hub laboratory to process a larger workload and allow further consolidation of tests from other analytical platforms. At the time of writing the full business case for the formation of the Consortium was being finalised and it is anticipated that the Consortium will be formed in April 2013.

EW009
ENHANCING EFFICIENCY IN A HUB AND SPOKE ENVIRONMENT

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Until 2005 Modena area Public Health System consisted of a network of 9 hospitals where six hospital local Clinical Pathology laboratories provided the analytical and diagnostic services serving 700,000 people. Laboratory workload ranged from 680,000 to 3,000,000 test/year, mostly of specialized test in outsourcing. Aims: To improve the quality of health service, a new area hospital (approximately 600 beds) started its activity in June 2005, together with the hospital re-organization, also the Clinical Pathology system has been re-organized. The main aim of project was to enhance patient services in terms of access-equity to the diagnostic service, to reach in home satisfaction for all the requested tests increasing types and complexity of tests performed and to improve the efficiency in
a Clinical Governance policy. Project state of the art: A central laboratory, named BLU (Baggiovara Unified Laboratories), has been created serving all Modena area. The new network laboratory system was based on a “Hub” laboratory (BLU) plus 3 other laboratories and about 30 points of care, dealing solely with hot tests, as “Spoke”. The Core Lab, section of the area laboratory, is based on two automated mirror lines performing chemistry and immunochemistry analyses plus an automated line devoted to hematology and coagulation. Further in BLU laboratories new specialized activities were developed as toxicology, pharmacology molecular biology, etc. Results: Now Core Lab deals daily with about 4,000 in-outpatients and 30,000 tests plus the activity in emergency due to requests of 4 hospitals (1,100 beds). There is not an emergency laboratory area as all tests are performed on the same automation lines with different priority. In 2011 we have met some of the expected project goals: the volume of activities incremented of about 53% (7.3 millions in 2005 vs. 11.2 in 2011 on department basis), the Core Lab located human resource decreased of about 25% nevertheless the increase in activities. The human resources saved in Core Lab reorganization allowed us to start up new laboratory diagnostic activities with a consequent strong increase in laboratory value production (24.7 euro millions in 2005 vs. 39.9 in 2011, plus 60%).

EW010
WORKFLOW OPTIMIZATION WITH ISLANDS OF AUTOMATION
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In today’s market for laboratory medicine in Germany a fairly high-consolidated group of providers face an increasing demand for tests. Demographic changes, medical innovation and increasing numbers of younger patients with chronic illness have all contributed to this increase. In contrast, the healthcare budget remains limited and the continuous reforms and austerity efforts of the government have led to significant cuts in reimbursement (i.e. almost 15% cut for the 1st quarter 2011). The Core Lab is dealing daily with about 5,000 in-outpatients and 30,000 tests plus the activity in emergency due to requests of 10 hospitals (1,100 beds). There is not an emergency laboratory area as all tests are performed on the same automation lines with different priority. In 2011 we have met some of the expected project goals: the volume of activities incremented of about 53% (7.3 millions in 2005 vs. 11.2 in 2011 on department basis), the Core Lab located human resource decreased of about 25% nevertheless the increase in activities. The human resources saved in Core Lab reorganization allowed us to start up new laboratory diagnostic activities with a consequent strong increase in laboratory value production (24.7 euro millions in 2005 vs. 39.9 in 2011, plus 60%).

EW011
AUTOMATED DETERMINATION OF ALDOSTERONE AND RENIN: CLINICAL INFORMATION AND METHODOLOGICAL ISSUES
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The prevalence of primary aldosteronism (PA) among patients with hypertension is significant. PA is associated with major adverse cardiovascular outcomes and an early diagnosis of PA is needed to initiate the appropriate treatment and to improve the patient’s prognosis and quality of life. The aldosterone to renin ratio represents a reliable marker for the screening for PA. Accurate measurements of aldosterone and renin are therefore important for the screening and diagnosis of PA and for the investigation of other diseases related to the renin-angiotensin-aldosterone axis. The reference methods for aldosterone and renin determination are still based on radioimmunoassay. However, such methods are time consuming, labor intensive and lack appropriate standardization. Recently, new potential game changers such as automated methods and mass spectrometry assays have been developed and are now candidate for routine use. Nevertheless, several barriers remain to be broken before a broader community use of these new assays. Their analytical accuracy, such as functional sensitivity and cross-reactivities, as well as their clinical reliability will have to be determined. Furthermore, laboratorians and physicians will need to be educated and trained for their appropriate use and interpretation. Lastly, the cost-effectiveness of these emerging assays will have to be determined with large transversal studies.

EW012
ALDOSTERONE TO RENIN RATIO: INCREASING DEMAND FOR PRIMARY ALDOSTERONISM SCREENING
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Primary aldosteronism (PA) is characterized by inappropriately high aldosterone and suppressed renin levels. It is caused by autonomous hypersecretion of aldosterone from either an adrenal adenoma or from uni- or bilateral hyperplasia of the adrenal glands. The chronic aldosterone excess leads to increased blood pressure and electrolyte imbalances. However, it has recently been recognized that the “classical” hypokalemic form is less frequent, and screening for PA should not be restricted to patients with hypokalemia. PA is associated with increased cardiovascular morbidity and mortality not only in comparison to healthy subjects, but also in comparison to other causes of hypertension. This has been demonstrated for the frequency of strokes, myocardial infarctions, atrial fibrillations and left ventricular hypertrophy. PA is also associated with other comorbidities like depression. PA is more frequent than previously assumed: In recent years, changes in the screening methods used and the more widespread availability of aldosterone and renin measurements have led to a significant increase in the number of cases detected. Population-based studies revealed that a significant proportion of patients with hypertension exhibit an elevated aldosterone to renin ratio potentially is related to PA. Furthermore, systematic
screening in large cohorts of hypertensive patients revealed that up to 20% of patients with hypertension suffer from PA. The diagnosis is more frequent in specific patient groups which therefore must be screened for the disease. Patients with hypertension stage II or above, drug resistant hypertension, hypertension plus hyponatremia, cardiovascular events at young age and patients with hypertension in the presence of an adrenal incidentaloma. Screening for and early detection of PA are important because the correct diagnosis can lead to specific treatments: If PA is caused by an adrenal adenoma, surgery can cure or at least ameliorate the disease. In case of bilateral hyperplasia, pharmacological blockade of the aldosterone receptor through mineralocorticoid receptor antagonists is a rational treatment modality.

EW013 HOW THE USE OF HIGHLY SENSITIVE TROPONIN ASSAYS IS CHANGING OUR DAILY PRACTICE
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The Universal Definitions of Myocardial Infarction (MI) of years 2007 and 2012 endorsed cardiac troponins (cTn) as the preferred biomarkers for the diagnosis of MI. The so-called “conventional” cTn assays were those measuring the cTn concentration corresponding to the 99th percentile (p99th) with an analytical imprecision higher than the recommended. Many authors showed that detectable cTn concentrations, regardless the imprecision they were measured, were associated with an increased risk of future adverse outcomes. “High sensitive” cTn (hs-cTn) assays were developed to detect very low cTn concentrations with the recommended imprecision. Compared to conventional assays, hs-cTn assays show an improved imprecision at the p99th value and an increased proportion of detectable hs-cTn concentrations in healthy subjects. Hs-cTn assays are changing the clinical practice in those centers which introduced them in routine. In MI, hs-cTn concentrations increase above the p99th value time before the required with conventional assays. The possibility of properly measuring very low hs-cTn concentrations permits to calculate biological variation, a key value for calculating what increase/decrease in hs-cTn should be considered as clinically significant. This would led to a clear differentiation between patients with increased hs-cTn by MI or with chronic hs-cTn increases caused by some cardiac and non-cardiac conditions (arrhythmia, renal and heart failure, pulmonary embolism, myocarditis, etc.). All these are advantages facilitating a more rapid and specific diagnosis of MI. However, hs-cTn is now detectable in many otherwise healthy subjects and some population studies are showing that detectable hs-cTn concentrations, even those below the p99th value, are associated with increased risk of adverse outcomes. These results challenge the current knowledge, but require confirmation before changing the current risk evaluation in supposedly healthy subjects. In conclusion, high-sensitive cTn assays are providing increasing information that helps to improve the management of patients and could open a new era in the diagnosis, prognosis and therapy of cardiovascular diseases, particularly those of coronary ischemic origin.

EW014 THE BENEFITS OF HIGH-SENSITIVE TROPONIN T FOR THE EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION
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Background: Patients with acute chest pain or other symptoms suggestive of acute myocardial infarction (AMI) account for about 10% of all emergency department (ED) consultations. Electrocardiography and cardiac troponin complement clinical assessment and form the cornerstones of the AMI diagnosis according to the universal definition. A limitation of former generation cTn assays is a delayed increase of circulating levels which mandates serial sampling for 6 hours. Delays in diagnosing disease (“rule-in”) holds back prompt use of evidence based therapies. Delays in excluding disease (“rule-out”) interferes with evaluation of alternative diagnoses and contributes to expensive overcrowding in the ED. The recently developed sensitive and high-sensitivity cardiac troponin (hs-cTn) assays have enabled measurement of cTn concentrations not reliably detected with prior generations of tests. The aim of this presentation will be to highlight an one-hour algorithm for rapid rule-in and rule-out of AMI using hs-cTnT levels

Methods: In a prospective, observational, multicenter study enrolling consecutive patients presenting with acute chest pain, we derived and validated algorithms on how to best apply hs-cTnT data (including absolute changes) either alone or in conjunction with other clinical information. Blood samples were collected at presentation and after 1,2,3 and 6 h in a blinded fashion. The final diagnosis was adjudicated by 2 independent cardiologists using all information. All patients also received long-term follow up.

Results: The algorithm is able to accurately help clinicians to quickly separate three groups of patients: 1) those with a extremely high negative predictive value for AMI (100% in both the derivation and validation data set) ready for early discharge, 2) a group with intermediate risk in need for regular observation and serial blood sampling in the ED, and 3) those with a very high likelihood for AMI (about 80%) who in general are candidates for immediate coronary angiography. The detailed results will be presented and discussed.

Conclusions: Validated algorithms will help clinicians to best use the advantages provided by the hs-cTnT assays in the early rule-in and rule out of AMI.

EW015 TOWARDS A BETTER UNDERSTANDING OF BIOMARKER GUIDED HEART FAILURE CARE: THE PROTECT STUDY
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Background: The increasing incidence of heart failure (HF) places an enormous economic and clinical burden on health care systems. Significant opportunities exist in developing therapeutic approaches to improve outcomes, maintain safety, and reduce cost. It is unclear whether standard HF treatment plus a goal of reducing N-terminal pro-B type natriuretic peptide (NT-proBNP) concentrations improves outcomes compared with standard management alone.

Methods: A total of 151 patients with HF resulting from left ventricular systolic dysfunction (LVSD) were treated with HF treatment by standard-of-care (SOC) management or treatment with a goal to lower NT-proBNP ≤1000 pg/mL over 10 months.

Results: A significant reduction in the primary endpoint of total
cardiovascular events was seen in the NT-proBNP arm, compared to SOC (58 versus 100 events; P=.009; odds ratio = 0.44; P=.02), with particular improvements in rates of worsening HF and HF hospitalization (both P <.003). Compared to SOC, NT-proBNP guided patients had greater improvements in quality of life and NT-proBNP guided patients also had greater improvements in echocardiographic parameters. Elderly patients treated with NT-proBNP guided care had substantial benefit (1.76 events per patient versus 0.71 events per patient, P=.03), and NT-proBNP guided care was substantially cost-saving. There was no increase in treatment-related adverse events due to NT-proBNP guided care.

Conclusions Natriuretic peptide-guided HF care results in substantial improvement in cardiovascular event rates, quality of life, echocardiographic parameters, and is cost-saving. Results will be discussed in context of other biomarker guided HF studies.

EW016
THE CLINICAL RELEVANCE OF URINARY SEDIMENT EXAMINATION
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Urinary sediment examination is an integral part of urinalysis. When performed with proper methodology, equipment and knowledge of particles and profiles, urinary sediment examination can supply relevant information in a wide spectrum of clinical conditions, some of which are describe here.

In isolated microscopic hematuria, the examination of erythrocytes morphology make it possible to distinguish hematuria of glomerular origin from non-glomerular hematuria, which arises from the urinary excretory system. This differentiation is important to decide whether the patient requires a nephrological or a urological workup.

In glomerular diseases, urinary sediment examination can identify different profiles such as “minor urinary changes”, “nephritic profile”, “nephrotic profile”, “nephritic and nephrotic profile”, which derive from variable combinations and numbers of erythrocytes, leukocytes, renal tubular epithelial cells (RTEC), fatty particles, and cast subtypes. The identification of these profiles, influences clinical and therapeutic decisions. In acute kidney injury, it is possible to separate pre-renal insult from acute tubular necrosis, based on the absence or presence in the urinary sediment of RTEC, RTEC casts and/or granular casts. Again, this information helps in guiding the clinical and therapeutic approach.

In kidney transplant recipients, the search of “decoy cells” (i.e., cells with typical morphological changes) is used to identify the reactivation of polyomavirus BK, a diagnosis which leads to the reduction of immunosuppressive treatment.

In urolological disorders, a typical profile characterized by isomorphic erythrocytes, leukocytes and transitional epithelial cells (either superficial or deep) is found. This can be used to monitor the course of the underlying disease.

In urinary tract infections, urinary sediment examination shows leukocytes and bacteria, a finding which often leads to the request of urine culture. Moreover, it can be used to monitor the course of the infection during antibiotic treatment.

Today, automated urine microscopy is replacing manual microscopy in many laboratories all over the world. It is paramount importance that automated instruments be able to identify the most important particles and the main urinary profiles.

EW017
AUTOMATED URINALYSIS: EXPERIENCE IN 50000 SAMPLES
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Background: In our lab, urinalysis was performed using manual dipstick testing and classical microscopy until 2010. On the 09/10/2010, decision to automate urinalysis was taken after the conclusions of an internal audit. The system that was chosen was the Sedimax in combination with the AutonMax for the following reasons: total automation of the testing process, possibility to view images, low cost, low number of reviews using classical microscopy, and positive review of the system in the literature.

Methods: The Sedimax coupled with AutonMax system was evaluated with 336 samples that were compared with classical microscopy and manual dipstick. For validation, reproducibility assessment was performed using Bio-Rad controls, repeatability was performed on patient samples and trueness was assessed with the comparison of 86 samples with classical microscopy. Following validation, the Sedimax combined with AutonMax were implemented in our lab on the 17/01/2011.

Results: From 01/2011 to 11/2012, a total of 55296 samples were examined on the Sedimax. The software performed very well for the identification of white and red blood cells, non-pathological casts, squamous epithelial cells, non-squamous epithelial cells, bacteria as well as calcium oxalate, uric acid and triple phosphate crystals. Other elements like cystine and bilirubin crystals, as well as erythrocytic casts could not be recognized by the software version in use at the time, but could easily be identified on the images and notified on the report as a comment. Urinalysis automation allowed saving significant time for the medical laboratory technologists, especially during evenings and nights on call. Turnaround time (TAT) was also significantly improved. The digital microscopy platform proved to be especially interesting for training, continuous education and traceability.

Conclusion: Urinalysis automation with the Sedimax combined with the AutonMax proved to be reliable and had positive outcome regarding TAT, technologist time and overall quality. Future works will include the implementation of the latest version of the crosscheck software that allows comparing the sediment results with the dipstick results. This could allow increased standardization and additional workload reduction.

EW018
EXPERIENCES OF SCREENING FOR URINARY TRACT INFECTIONS WITH THE SEDIMAX AUTOMATED DIGITAL IMAGE ANALYSER: ROVIGO EXPERIENCE
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Background. Urinary tract infections (UTIs) are among the most common bacterial infections and urine samples constitute a large proportion of the specimens processed in a microbiology laboratory. Urine culture is time and labour consuming but it can produce up to 80% negative results. We evaluated the sediMAX urinary analyser to screen for positive samples in order to reduce the number of urines requiring culture and to rapidly identify patients with UTIs.

Methods. Two thousand and forty seven urine specimens from hospitalized patients and outpatients were collected during a...
6 months period. Samples were analysed in parallel by routine culture and by the sediMAX instrument, adopting a single set of cut-off values of both leukocytes and bacteria. Results were evaluated first considering the entire population and then dividing patients into subgroups based on their gender, age and inpatients/outpatients status.

Results. The sediMAX compared to culture showed a global sensitivity of 96%, a specificity of 74%, a positive predictive value (PPV) of 56% and a negative predictive value (NPV) of 98%. High NPVs were observed among all the subgroups. Compared to the global population investigated, samples collected from male and female hospitalized patients revealed high sensitivity (98%) but lower specificity (61% and 48% respectively), mainly due to concomitant antimicrobial treatment. Samples collected from female outpatients showed higher sensitivity (97%) but lower specificity (72%), mostly related to the large amount of false positive results observed in urine of pregnant women. Compared to female samples, specimens collected from male outpatients revealed lower sensitivity (90%) but higher specificity (89%), probably because bacterial cut-off values were not appropriate.

Conclusions. The sediMAX has demonstrated to be a suitable screening system, able to rapidly detect true negative urine specimens and to reduce the number of samples candidate for culture. For some patient groups the sediMAX instrument was able to identify with good probability patients with a concomitant UTI. Clinical data management, coupled with patient-specific cut-off values, could further improve the performance of the sediMAX for the screening of UTIs.

EW019 EXPERIENCES OF SCREENING FOR URINARY TRACT INFECTIONS WITH THE SEDIMAX AUTOMATED DIGITAL IMAGE ANALYSER: DESIO EXPERIENCE

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Background: Urine bacterial culture constitutes the cornerstone for the diagnosis of urinary tract infection (UTI). Rather time consuming and expensive, this procedure would benefit from prior screening of urine for the presence of bacteria and leukocytes, particularly since approximately 80% of urine cultures are negative. In a previous study we demonstrated that the automated urine sediment analyzer, sediMAX, rapidly excludes negative bacteriuria samples from further processing by culture. In the present study, we performed a similar experiment focusing on the utilization of autoverification rules to further improve screening with sediMAX.

Methods: One thousand and thirty-two consecutive midstream and catheter sterile urine specimens were evaluated with the sediMAX over a 2-month period using urine culture as the reference method. The autoverification rules utilized were obtained from the screening experience gained in the previous study.

Results: Of the 1032 urine samples, 826 (80.0%) were culture negative and 206 (20.0%) were culture positive. The autoverification rules requested operator reclassification of sediments in which debris could be erroneously classified as bacteria. This approach determined an improvement of performance (sensitivity, 99.0%; specificity, 85.3%; negative predictive value (NPV), 99.4%; positive predictive value (PPV), 62.7%; false negative rate (FNR) of 1.0%; and false positive rate (FPR), 37.3%). The false positive samples were evaluated and it was found that the most frequent categories of patients within this group were those with ongoing antibiotic therapy (40.4%) and pregnant women (37.2%). The initial autoverification rules were then modified according to the characteristics of these two categories further improving screening performance (specificity, 94.1%; PPV, 81.8%; FPR, 5.9%), and reducing the number of samples to be processed for culturing by 70%.

Conclusions: The utilization of autoverification rules improved screening with sediMAX, easing both costs and workload by reducing the number of unnecessary urine cultures performed.

EW020 REVIEWING THE ROLE OF LEAN MANAGEMENT FOR ERROR REDUCTION IN THE PRE-ANALYTICAL PART OF TTP

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This presentation will describe a system of Lean Management and Daily Accountability Meetings which underpin the Continuous Quality Improvement approach in Bolton. Together with real time data collection and analysis and root cause analysis of defects, this approach gives control of process quality to the staff who perform the work. The system provides an integrated set of planning, measurement and problem solving tools to help the work group focus on daily performance measurement and improvement; improve effectiveness of supervisory communication; encourage staff improvement ideas; and define and monitor improvement objectives and Key Performance Indicators (KPI). Essential to the effectiveness of these meetings are the visual display boards which show metrics for the work area related to KPIs; process control boards (PCB) which show the actual real time performance against planned performance; and Continuous Improvement (CI) sheets which staff use to record observed defects and improvement ideas. The root cause of any issue identified might not always be possible to determine at the daily meeting. It may require the collection of more metrics or it may require a more detailed problem solving event, in which case a planned time out will be required with support from trained facilitators.

Benefits from this system of Daily Accountability Meetings include improving employee autonomy and commitment through involvement; improved perceptions of management through a tie-in to the vision and strategy of the organisation; productivity is improved and the focus on quality leads to reduced costs.

The systems described in this presentation are, by and large, just common sense. You already know how to do them; you might already be doing something similar or have done so in the past. The difference in a Lean transformation is that there is a structure and a discipline to applying this common sense. Plus a determination to make this ‘the way we do things here’, to be relentless in the pursuit of continuous improvement and (albeit unattainable) perfection. Examples will be given of error reduction and quality improvements in the pre-analytical phase using this methodology.

EW021 ROLE OF INSTRUMENT DESIGN FOR ERROR REDUCTION IN THE ANALYTICAL PROCESS

C. Grandone

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The analytical part of the total testing process is the ‘core’ of the laboratory. With automation, improved laboratory technology, assay standardization and well defined rules for assessing
laboratory quality the error in this testing phase can be reduced. In this presentation the critical role of instrument design from the early research state to the final product and its role in improving overall quality of results will be reviewed. Setting quality requirements early in the instrument design process allows to also set sub-system goals for quality, from sample delivery, optics, temperature control, timing, carry-over and more key instrument parts which will have an influence on the quality of the final test result. Long before the final instrument will be available on the market the performance is tracked starting already with first instrument prototype. Any results outside 5 standard deviations for the planned testing menu is documented, investigated and followed-up right from the beginning as outlier or defect. By the end of the feasibility phase the defect rate is significantly reduced by focusing on the subsystems potentially responsible for these outliers. During the next development phase, the design control phase, internal and external validation data are included in this assessment and this will be continued also after instrument is available on the market.

In conclusion following good manufacturing practices and monitoring overall quality including sub-system quality from early development to final analyzer reduces variability and ensures instrument performance within established specification.

EW022
ROLE OF THE SIX SIGMA QUALITY SOLUTION IN TTP

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A plan for Analytical Quality Management is presented and “sigma-tools” are identified to support the achievement of quality in laboratory testing processes. The objectives are to make quality measurable by defining requirements for intended use and to make quality manageable through optimized control procedures. The plan includes steps to (1) define goals for intended use, (2) select measurement procedures, (3) validate method performance, (4) implement analytic method and system, (5) formulate a “sigma QC” strategy, (6) select SQC procedures, (7) develop an analytic QC Plan, (8) implement the analytic QC Plan, (9) verify attainment of intended quality, (10) measure quality and performance, (11) monitor failures, and (12) make improvements to the testing process.

The Six Sigma concept of “tolerance limits” is consistent with quality requirements in the form of Allowable Total Errors (TEa). Method performance in terms of imprecision (CV) and inaccuracy (bias) can be estimated from method validation experiments. Then a “sigma-metric” can be calculated as (TEa-Bias)/CV to characterize quality on the sigma-scale. A Method Decision Chart can be used to provide a graphical assessment of method performance and to facilitate a judgment on the acceptability of performance.

The sigma-metric can also be used to formulate a Total QC strategy. Appropriate SQC procedures (control rules, total number of control measurements) can be designed using the QC Selection Tool found in CLSI C24A3 or by using a Chart of Operating Specifications that is similar in form to the Method Decision Chart. Priorities for controls in a risk management based QC Plan can also be related to the sigma performance of the testing process. One of the advantages of the QC Plan is the capabilities of integrating pre-analytic and post-analytic controls together with analytic controls. For analytic control, high sigma methods can rely on SQC procedures with fewer individual controls, whereas low sigma methods require maximum SQC effort as well as maximum deployment of individual controls that focus on specific failure-modes.

EW023
IMPORTANCE OF REFERENCE INTERVALS FOR THE INTERPRETATION OF ANTI-MUELLERIAN HORMONE IN CLINICAL PRACTICE

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Anti-Müllerian Hormone (AMH), a member of the transforming growth factor beta family, is produced by the preantral and small antral follicles of the ovary. Serum concentrations of AMH reflect the number of these follicles as well as the primordial pool and, as such, can be used to provide an estimate of “ovarian reserve”.

The age related decline in the primordial follicle pool/ovarian reserve has been well described, based on cross sectional observation of follicle numbers. However recent studies, using AMH as a marker of functional ovarian reserve, have allowed modelling of changes in the rate of active follicular recruitment that underlie this decline. Furthermore studies of AMH during childhood, adolescence and adult life, examined in the context of human fertility, gives new insights into follicular development. AMH** is increasingly used in clinical practice to quantify ovarian reserve and tailor controlled ovarian stimulation programmes but it is yet to realise its full clinical potential. Due its relationship with functional ovarian reserve there are indications that AMH may be useful in predicting the age of menopause and possibly the reproductive lifespan in young women. The latter indication may be important in determining whether fertility preservation strategies are needed in women undergoing chemo- or radiotherapy, but also for women planning when to have their families. Finally there is also interest in the use of AMH in the diagnosis of menstrual disorders and polycystic ovarian syndrome.

The need for reference intervals and clear decision levels for AMH is becoming increasingly important. Whilst these are relatively well defined for use in fertility clinics more research is required for the wider clinical applications of AMH particularly in girls and young women below the age of 25.

**Note: Beckman Coulter’s AMH Gen II ELISA kit does not have any clinical claims.

W024
IMPACT OF PROSTATE HEALTH INDEX ON THE MANAGEMENT OF PATIENTS WITH PROSTATE CANCER

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Prostate cancer (PCa) screening programs using the prostate specific antigen (PSA) remains controversial due to a considerable number of unnecessary prostate biopsies and the detection of low volume, non-aggressive prostate cancers leading to overdagnostic and overtreatment. Recent results demonstrated that a molecular isoform of free PSA (IPSA), the [-2]proPSA has a higher specificity for the detection of PCa as compared with PSA or fIPSA. Beckman Coulter recently developed the “Prostate Health Index” (phi) which combines IPSA, IPSA and [-2]proPSA (phi=[-2]proPSA/PSA)x[1/PSA]. A multinational, multicenter prospective European study (PROMTheUS, http://www.controlled-trials.com, ISRCTN04707454) was conducted to evaluate the clinical performance of phi for the detection of PCa on 1026 patients who underwent initial or repeat biopsy (IPBx and RPBx respectively). In patients with IPSA between 2 – 10 ng/mL, who underwent IPBx, phi was the best predictor of the
presence of PCs at the biopsy with an area under the ROC curve of 0.7 as compare to tPSA (ROC AUC 0.5). In multivariable logistic regression models testing the predictors of PCs at biopsy, p2PSA, %p2PSA and phi significantly increased the accuracy of the base multivariate model with tPSA, IPSA and %IPS, to predict PCa presence, by a 6.4%, 5.6% and a 6.4% extent, respectively (all P <0.001). The Spearman’s rho coefficient analysis demonstrated a significant relationship between Gleason score, %p2PSA (rho: 0.245; P <0.001) and phi levels (rho: 0.276; P <0.001). A significant number of biopsies could be avoided with a negligible number of cancers missed using phi at sensitivity of 90% (phi =28) and at the best balance of sensitivity and specificity (phi=41). In a subset analysis consisted of a nested case-control, %p2PSA and phi are more accurate than the reference standard tests (tPSA, IPSA and %IPS) in predicting PCs in men with positive family history of PCa and in men younger than 60 years of age. In conclusion in patients with a tPSA between 60yrs, %p2PSA and phi are the strongest predictors of PCa at initial biopsy, and are significantly more accurate than the currently used tests (tPSA, %IPS) in determining the presence of PCs at biopsy.

EW025
NEW SOLUTIONS FOR TOTAL AUTOMATION OF MOLECULAR DIAGNOSTICS TESTING
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With increasing labour shortages in laboratory medicine, molecular diagnostics instrumentation will need to evolve toward central lab-like automation. In a report, Piper Jaffrey described a recipe for success in the molecular market as requiring a solid core technology, instrumentation, and menu. Few platforms today meet those requirements. The new Beckman Coulter molecular diagnostics platform will automate the entire real-time PCR procedure utilizing a proprietary extraction methodology and innovative design for consumables to provide real “sample to answer” capability, for a diverse, clinically relevant menu. The benefits of this technology applied to quantify ribonucleic (RNA) and deoxyribonucleic acid (DNA) in human plasma will be reviewed. As an example, HCV RNA viral load (VL) monitoring is a well-established diagnostic tool for managing chronic hepatitis C patients. HCV RNA VL results are used to make treatment decisions with the goal of therapy to achieve an undetectable VL result. Therefore, a sensitive assay with high specificity for quantifying HCV RNA across genotypes is critical. The BEC HCV assay design achieves clinically relevant performance across six HCV genotypes. Qualification of system design elements will also be overviewed such as utilization of a true internal process control, assay reagents pack offering extended on board shelf life and streamlined workflow for QC.

*product in development

EW026
EVALUATION OF QUANTITATIVE-LOOP MEDIATED ISOTHERMAL AMPLIFICATION (Q-LAMP) IN THE FOLLOW-UP OF TRANSPLANTED PEDIATRIC PATIENTS
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Bone marrow transplantation (BMT) and solid organ transplantation (SOT) have evolved to become the preferred treatment options for a number of malignancies and end-stage organ dysfunctions, especially in children. Despite the success of transplantation procedures, infections in transplant recipients remain the leading cause of death in this population. Although CMV, Epstein-Barr virus (EBV), HSV-1, HSV-2, VZV, and HHV-6 are well recognized for their potential oncogenicity in transplant patients, because at risk for induction of uncontrolled cellular proliferation due to normal immune surveillance “machine”, mostly specific cytotoxic T cell mechanism, is compromised.

In immunocompromised individuals EBV is associated with disorders with high rates of morbidity and mortality. The spectrum ranges from benign B-cell hyperplasia resembling IM to malignant lymphomas. Allograft organ transplant recipients, especially children with pre-transplantation EBV seronegativity, are at particular risk for the development of post-transplantation lymphoproliferative disease (PTLD) during immunosuppressive therapy.

The clinical microbiology laboratory plays an essential role in the diagnosis and management of infection during all phases of the transplantation process. Quantitative EBV DNAemia is routinely performed to monitor EBV Viral loads by using quantitative PCR methods. Recently a new quantitative assay based on Loop-mediated isothermal amplification (LAMP) as rapid detection of specific nucleic acid EBV sequences demonstrated optimal sensitive with good quantitative correlation to conventional quantitative PCR.

Laboratories, that join transplant programs will be called upon to support needs in term of TAT and optimal diagnostic strategies, from early detection of EBV replication to a high positive predictive value assay, also in respect to economic climate of cost containment.

EW027
PRE- AND POST-NATAL DIAGNOSIS OF CONGENITAL CYTOMEGALOVIRUS INFECTION
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Recent development of advanced serological tests allow us to identify pregnant women with primary cytomegalovirus (CMV) infection who are at higher risk of transmitting CMV to their fetus. Given the high risk of mother–fetus transmission and fetal damage, prenatal diagnosis is recommended, between 20-21 gestation’s weeks and at least 6-8 weeks after the onset of maternal CMV infection.

In this work we studied a cohort of 790 pregnancies at risk of in utero CMV transmission; 796 amniotic fluid (AF) samples were tested by real time PCR assay and virus isolation.
symptoms like mild muscle weakness and pain with slight myocites damage and varies from subtle nonspecific. Clinical presentation of rhabdomyolysis depends on severity of surgical operations mainly orthopedic and aneurysm of groups as a result of patient’s position during long lasting. Blood tests are of a great importance in diagnosis and specificity of the urine PCR assay were 100% (95% CI, 95.8 to 100). The gold standard for the diagnosis of congenital CMV infection in newborns remains viral isolation in the urine and/or saliva within the first two weeks of life. But also urinary and saliva molecular testing is a reliable, rapid and convenient method to diagnose congenital CMV infection. In this work we compared real-time PCR assays of urine specimens with rapid culture of urine specimens obtained at birth. A total of 101 of 199 newborns were congenital CMV infected. Of 199 newborns screened with the use of the urine PCR assay, 98 were negative for CMV and the remaining 101 infants had positive results on both culture and PCR assay. The sensitivity and specificity of the urine PCR assay were 100% (95% CI, 95.8 to 100).

EW028
THE LABORATORY CONTRIBUTION TO DIAGNOSIS AND MANAGEMENT OF RHABDOMYOLYSIS
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Rhabdomyolysis first described as crush syndrome during the World War II appears as a breakdown of muscle cells and leakage of their contents to extracellular fluid and circulation. Etiological causes can be divided into several groups and among them: mechanical trauma, including extensive blunt injury and electric shock; muscular activity including different intensity physical exercise in trained as well as untrained persons and status epilepticus; toxins including carbon monoxide, alcohol, hemlock herbs that the quails consume; medications including statins; ischaemia of large muscle groups as a result of patient’s position during long lasting surgical operations mainly orthopedic and aneurysm of abdominal aorta. Clinical presentation of rhabdomyolysis depends on severity of myocites damage and varies from subtle nonspecific symptoms like mild muscle weakness and pain with slight coloration of urine up to acute renal injury. That is why laboratory tests are of a great importance in diagnosis and management. The first finding is often (but not always) brownish urine showing positive “blood” on dipstick with no erythrocytes in sediment, what is caused by myoglobin. Very typical is high serum creatine kinase (CK) and its heart isoenzyme (CKMB) with normal ratio. Activity of aspartate aminotransferase (AspAT) and lactate dehydrogenase (LDH) are also increased. At first stage increase of serum potassium is a result of its liberation from broken cells but it may aggravate with deterioration of kidney function. Myoglobin is the most significant, but usually not performed as a first line laboratory test. Its serum concentration can reach values above 100 000 ng/ml (normal range about 100 ng/mL). In several critically ill patients mainly in postoperative and intensive care units persistent elevation of serum myoglobin is one of indications for dialysis. As the acute renal injury is the most serious complication of rhabdomyolysis the careful monitoring of renal function by laboratory tests should be done. Serum CK and myoglobin may also be successfully used in monitoring statin therapy.

EW029
UMBILICAL CORD BLOOD ANALYSIS BY AUTOMATIC HEMATOLOGY ANALYZERS
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Background: Umbilical cord blood (CB) is as important source of hematopoietic progenitor cells and also reflect the hematologic status of neonates. Several morphologic and immunophenotypic characteristics of CB are different from those of adult peripheral blood. The performances of hematology analyzers have been studied mostly on adults’ peripheral blood. Moreover, the current use of their state-of-art parameters seems to be limited. We evaluated the performances of ABX Pentra DX 120 (Horiba Medical, Montpellier, France) and Sysmex XE-2100 (Sysmex, Kobe, Japan) in CB specimens. We questioned whether their data are interchangeable and reliable in CB and whether their specific parameters for hematopoietic progenitor/im mature cells, double matrix by ABX Pentra DX 120 and hematopoietic progenitor cell (HPC) by Sysmex XE-2100, are reflective of CD34+ cells.

Methods: The routine complete blood cell parameters were evaluated in a total of 200 CB specimens. The white blood cell differential and nucleated RBC (NRBC) counts were compared with manual differential. Double matrix by ABX Pentra DX 120 and HPC by Sysmex XE-2100 were compared with flow cytometric CD34+ cells. Results: Most of the parameters, except mean corpuscular hemoglobin concentration, showed acceptable correlation between ABX Pentra DX 120 and Sysmex XE-2100. The difference of white blood cells (WBC) and platelets between ABX Pentra DX 120 and Sysmex XE-2100 tended to increase as WBC and platelets increased. The difference of mean corpuscular volume (MCV) tended to decrease as MCV increased. Sysmex XE-2100 did not report WBC differentials in five specimens. Both analyzers showed acceptable correlation with manual differential for neutrophils, lymphocytes, and eosinophils. Mononuclear cells (MNC) by ABX Pentra DX 120 better correlated with manual count than MNC by Sysmex XE-2100. Double Matrix better correlated with CD34+ cells than HPC. NRBC by Sysmex XE-2100 better correlated with manual count than NRBC by ABX Pentra DX120.

Conclusion: The parameters from ABX Pentra DX 120 and Sysmex XE-2100 were mostly interchangeable and reliable in CB specimens. The double matrix by ABX Pentra DX 120 can be a valuable option to evaluate the quality of CB for further utilization in therapy and transplantation.
APS is an auto-immune disease characterized by thrombotic complications in both arteries and veins as well as pregnancy-related complications in combination with the presence of so-called antiphospholipid antibodies in plasma of these patients. It is now generally accepted that these auto-antibodies are not directed against negatively charged phospholipids but towards plasma proteins bound to these phospholipids. The most prominent antigen in APS is beta2-Glycoprotein 1 (β2GPI), a plasma protein with affinity towards anionic phospholipids. Three assays are available to detect the presence of these auto-antibodies: lupus anticoagulant, a prolongation of a clotting assay and two ELISAs with cardiolipin or beta2-GPI as antigen. Many studies have identified lupus anticoagulant as the assay that correlates best with the clinical manifestations that characterize the syndrome. The auto-antibodies that cause lupus anticoagulant are gain-of-function antibodies, when they bind to beta2-GPI, the affinity of beta2-GPI for anionic phospholipids increases to such an extent that the beta2-GPI-antibody complex can compete with clotting factors for the binding to anionic phospholipids. Addition of extra phospholipids can neutralize the effects of lupus anticoagulant and this ‘conformation’ step has become the hallmark to identify the presence of lupus anticoagulant. It should be noted that the anionic phospholipids used in clotting tests are poorly characterized, and the sensitivity of different clotting assays for lupus anticoagulant is different. Moreover, the auto-antibodies that cause lupus anticoagulant are a heterogeneous population of antibodies. To circumvent that the presence of lupus anticoagulant is missed, the official guidelines require that two clotting assays should be performed that are based on two different activation principles. In this lecture the mode of action on how the auto-antibodies can induce a prolongation of clotting times will be discussed.

Evidence that the abnormality of the screening test is due to the presence of circulating anticoagulants and not the deficiencies of coagulation factors [mixing (patients and normal plasma) study] and the third requires that circulating anticoagulants are directed to negatively-charged phospholipids combined with plasma proteins and not to specific coagulation factors (confirmation study). The rational of the screening is that LA binds and neutralizes the phospholipids included in the test reagents and therefore prolong the clotting time of the APTT/dRVVT. The rational of the mixing procedure is that any coagulation factor deficiency is (nearly) corrected upon mixing equal amounts of patient and normal plasma. Thus, normalization of the mixture clotting time suggests a coagulation factor deficiency as the causes of the prolongation observed in the patients plasma, whereas a persistent prolongation of the mixture clotting time suggest the presence of circulating anticoagulants. Finally, the rational of the confirmation procedure rests on the fact that repeating the screening test on patient plasma upon increasing the concentration of phospholipids is able to quench the effect of LA on these moieties and therefore the clotting time will be normalized, but only if the cause of prolongation was due to the presence of LA.
comparison and methodological and interpretational recommendations are needed.

EW033 CURRENT STATUS AND FUTURE OUTLOOK OF IT SOLUTIONS

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Laboratory medicine faces many challenges in managing information including the request-report cycle for central laboratory and point of care testing, advice to clinicians on the selection and interpretation of investigations, communication with other laboratories, external quality assurance, management of organisational information within the laboratory as well as the support of accountability for both regulatory and financial purposes.

For central laboratory testing key task is computerisation of the request-report cycle. Increasingly this will be done using the clinical information systems the clinicians have selected and implemented, rather than as an extension of the laboratory information management system. Computerised reporting is widespread but there are many residual problems, including integrity of information and ensuring that reports are seen by clinicians.

In Oxford we have used the clinical information systems in primary care and now receive over 85% of our requests by computer messaging, issue no paper reports and are making our initial steps into using decision-support rules on the requesting system in front of the clinicians. In secondary care a new electronic patient record system is used. Paper reports have been stopped. Specimens are checked against patient wristbands using barcodes.

Laboratory Information Systems are being squeezed between two developments: the introduction of computerised requesting and reporting on clinical information systems and the in-laboratory systems which control and are supplied with analysers. It is possible that smaller and simpler laboratories will not need a LIS in the near future.

All laboratories need information on costs and workloads. The information needed for clinical reports can also be reused for research and development. The workload from primary care is now so high that it can be used for population surveillance. Modern data-mining techniques that are widely used in retail are likely to be similarly productive in Laboratory Medicine. Process efficiency and patients safety can be further improved using disease management systems and improved knowledge management centered all around the patient care.

Developments in all of these areas will be discussed and demonstrated.

EW034 DECISION-MAKING IN UNIVERSITY CORE LABS AND THE SUPPORT OF IT

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Background: Nowadays a lot of University Hospitals are planning to invest in centralization and standardization of laboratory activities. Beside the constructional, personnel and analytical challenges there is an increasing demand of implementing a consistent, integrative IT-solution that supports maximum consolidation and workflow optimization.

Method: In 2011 the University Hospital of Goettingen placed a European wide tender for a total laboratory automation solution including an integrated IT concept.

Key requirements for the IT-Solution were the support of existing IT-Systems, the flexibility to support Core Lab, integration of 3rd party analysers, different Laboratory informationsystems (LIS), purchasing procedure, suppliers relationship, accounting “pay per result contracts” and stock-optimization through RFID-tracking-system. Metrics tools like TAT, performance, efficiency and workload measurements the automated generation and management of physician driven information by case tools and/or Business Intelligence Systems played an important role for decision.

Results: The concept of the leading bidder was able to fulfill most of the tendered challenges. Now our Lab will be able to succeed in optimization the most common workflow’s pain points of a University Lab like: •Reception of the tests - preanalytic: labeling, aliquoting, centrifugation; •Handling of exceptions through auto-validation features; •Easy interpretation and acceleration of the workflow; •Delays and delivery time in diagnosis and treatment as impediments to optimal patient care, particularly in high-volume patient care environments; •Avoiding discrepancies and effects due to an inaccurate information.

Conclusion: As the main topics in the UMG-Lab have been improved, the investment in a modern Lab-IT-Solution can not only accomplish and satisfy nowadays requirements but also support the value and return of investment. So a modern Lab will benefit from Increasing the progress and the improvement in the Lab operations through timely decision making.

EW035 MAXIMIZING STABILITY OF VENOUS BLOOD SPECIMENS

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Background: Guidance is required to ensure blood sample integrity during the preanalytical phase, especially to comply with ISO 15189 requirements. Informations such as type of tube, temperature conditions & delays before centrifugation need to be known. We studied the pre-analytical stability of 81 analytes based on the variables of delay before processing, storage as whole blood or serum/plasma, the storage temperature and the type of tube the sample was stored in.

Methods: The mean difference between assays for samples from 10 subjects was calculated with the samples being kept between sampling and analysis: up to 24 h for biochemistry, coagulation and hematology, and up to 72 h for hormoneology. This difference was compared to the acceptable limits derived from the analytical and the intra individual biological variation (RICOS).

Results: Most of the analytes investigated remained stable up to 24 h under all storage conditions prior to centrifugation. However, some analytes were significantly affected either by delay, tube type or temperature, such as potassium, inorganic phosphorus, magnesium, LD, glucose, lactate, mean corpuscular volume, mean corpuscular hemoglobin, activated partial thromboplastin time, insulin, C-peptide, PTH, osteocalcin, C-telopeptide and ACTH.

Conclusions: This study may be useful to help defining acceptable delay times and storage conditions when a short time between sample collection and processing is not possible.
EW036
MAXIMIZING STABILITY OF URINE SAMPLES

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Urine specimens should be stabilised before analysis based on the specific need of each requested test, rather than trying to do all tests from the same non-preserved specimen. For regional analysis, preservation at least overnight (preferably for 3 days) must be considered. Specifications for transportation of most common urine tests are to be included in regional protocols, as given in the examples below:

Urine Bacterial Culture And Particle Counting: A new era of regional analysis is possible by using documented preservative tubes for storage at +20 °C and transport for 1-3 days. A separate primary tube for microbiology investigations is recommended to avoid carry-over in automated instruments. Demanding bacteria may survive in non-additive tubes only, usually with a defined maximum storage time at +4 °C.

Chemical Test Strip Measurement: Use of a non-preservation tube is possible with storage at +4°C for a maximum of 3 days. A combined preservative tube can be used at +20 °C up to the specified period of time only, because sensitive oxidative reactions on the test pads are easily biased.

Urinary Albumin Creatinine-Ratio: A non-preservation tube can be used for single-voided samples within 7 days after collection at +20 °C. Preserve at +4 °C for a maximum of 30 days if necessary.

Urinary Protein (24 hour collection): A non-preservation collection container and aliquotting tube can be used for 1 day after a home or hospital collection at +20 °C. Replace the test with albumin creatinine-ratio of morning specimen if possible (not a myeloma patient or tubular proteinuria).

Urinary Electrolytes (24 h collection): Calcium may precipitate within two days if stored at +20 °C without acidification. Use pre-made 10 mL bottles of 6 mol/l HCl solution to be added into a 24-hour collection container after the first voided portion. No preservatives are needed for sodium or potassium at +20°C for 1 month.

EW037
ENSURING STABILITY THROUGH TRANSPORTATION

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Blood drawing, a primary requisite of in vitro diagnostics, is the most vulnerable step of the testing process. An appropriate venipuncture is essential to obtain a quality specimen, wherein mishandled or incorrect procedure can produce unsuitable results. Appropriate time. These are the areas in which a quality improvement should be done by 3-6 times gentle inversion. Primary blood tubes should never be mixed to prevent cross-contamination of additives. The specimen should hence be sent to the laboratory in a suitable time frame and under the best environmental conditions, with no injury.

EW038
THE USE OF QUALITY INDICATORS TO IMPROVE MEDICAL LABORATORY QUALITY

J. Barth
Leeds General Infirmary, Leeds, UK

Clinical laboratories have an important role in improving patient care. The past decades have seen enormous changes with unpredictable improvements in analytical performance, range of tests and capacity to manage large volumes of work. At the same time, there has been a dramatic fall in the rate of laboratory errors. However, there is now a growing awareness that the testing process includes the time before samples reach the laboratory and after reports have been printed and that these areas need to be included in the quality assessment of the total testing process.

Laboratory quality should include a focus on patient safety and clinical effectiveness. Services should be patient centered, timely, efficient and equitable, and finally, should be molded to ensure optimal outcomes. There is a need to define quality indicators that will ensure there is appropriate choice and selection of tests, use of the appropriate assay standardization and the correct interpretation of the assay results at the appropriate time. These are the areas in which a quality laboratory can, and should, now involve itself. This presentation will describe the process of development of Quality Indicators in the UK.

EW039
QUALITY INDICATORS: EXPERIENCE OF A LARGE LABORATORY

L. Sciacovelli
Department of Laboratory Medicine, University-Hospital of Padova

Background: The International Standard ISO 15189:2007 (IS) requires the implementation of quality indicators (QIs) as a part of quality improvement procedures but it does not define: what has to be defined and how has to be managed; who has to collect data; the times for data collection; the quality specifications (QS) for each QIs. The aim of the present study is to report the experience of our Department of Laboratory Medicine of University-Hospital of Padova that defined a set of quality indicators and realized a reporting system to manage them.

Methods: QIs have been defined on the basis of IS
EARLY BIOCHEMICAL SCREENING FOR FETAL ANEUPLOIDY IN THE FIRST TRIMESTER

N. Tørring

Århus Universitetshospital-Skejby, Denmark

Background: Screening for foetal trisomy 21 in the first trimester includes analysis of the serological markers pregnancy-associated plasma protein A (PAPP-A) and free beta human choriongonadotropin (free $\beta$hCG). The recent launch of the PAPP-A and free $\beta$hCG assays on the Roche Cobas and Elecsys platforms, we investigated their clinical and analytical performance in samples from gestational weeks 8+0 to 14+0.

Methods: We conducted a multicentre study based on serum data collection, the procedures for data analysis and the staff awareness about the need of improving actions and of QIs revision (introduction, deactivation, correction). The results analysis demonstrated that processes under the control of the laboratory have improved much more than those that require a close cooperation between the laboratory and the care teams.

Conclusion: QIs are becoming an indispensable tool to monitor and improve all activities of the total testing process. The effectiveness of QIs depends on the reporting system used for data collection. The periodic data analysis allowed us to understand the trend of performance over time (improvement or worsening), to know the need of improving actions and of QIs revision (introduction, deactivation, correction). The results analysis demonstrated that processes under the control of the laboratory have improved much more than those that require a close cooperation between the laboratory and the care teams.

Conclusions: The Roche Elecsys® free $\beta$hCG and PAPP-A assays apply with the standards for biochemical assays for prenatal screening set by the Fetal Medicine Foundation, with low assay imprecision and a high clinical performance of prenatal screening for fetal trisomy in the first trimester.

PREECLAMPSIA AND ANGIOGENIC FACTORS: FUTURE PERSPECTIVES IN CLINICAL MANAGEMENT

H. Stepan

University of Leipzig, Germany

The pathogenesis of preeclampsia is still not completely known; however, in the recent decade, there have been tremendous research efforts leading to impressive results highlighting the role of a disturbed angiogenic balance as one of the key features of the disease. Soluble fms-like tyrosine kinase 1 (sFlt-1), induces a preeclampsia-like phenotype in experimental models and circulates at elevated levels in human preeclampsia. Although preeclampsia seems to be a clearly defined disease, clinical presentation, and particularly the dynamics of the clinical course can vary enormously. The only available tools to diagnose preeclampsia are blood pressure measurement and urine protein sampling. However, these tools have a low sensitivity and specificity regarding the prediction of the course of the disease or maternal and perinatal outcome. The sFlt-1/PIGF ratio can now be determined by a rapid, automated immunoassay and can differentiate between preeclampsia and other hypertensive pregnancy disorders. The ratio can indicate the severity and progression of the disease and gives hereby a short term prognosis that is useful for clinical management. Thus, the sFlt-1/PIGF ratio has developed from an aid in diagnosis to a robust biomarker for prediction and risk stratification. Targeted therapies to stabilize the clinical manifestations and prolong pregnancy in preeclampsia do not exist. Removing sFlt-1 from circulation may benefit women with early-onset preeclampsia, since maternal sFlt-1 levels are closely linked to the severity of the symptoms and of the disease. A recent pilot study has demonstrated that negatively charged dextran sulfate cellulose columns adsorb sFlt-1 in vitro. In women with preterm preeclampsia and elevated circulating sFlt-1 levels, apheresis treatment reduces circulating sFlt-1 levels in a dose-dependent fashion. Extracorporeal sFlt-1 apheresis lowered circulating sFlt-1, stabilized blood pressure, and reduced proteinuria without apparent adverse events to mother and fetus. This approach opens the horizon to a real therapeutic intervention and supports further clinical studies with apheresis technique.

CERVICAL CANCER SCREENING AND DIAGNOSIS: HOW TO ANSWER TODAY’S CHALLENGES

M. Sideri

European Institute of Oncology, Milan, Italy

Several population studies have established that tests for human papillomavirus (HPV) have higher sensitivity than cytology in detecting high grade cervical intraepithelial lesions (CIN3) and that combined HPV and cytology testing has high negative predictive value for CIN3. The randomised trials found that the increased sensitivity for CIN3+ is not merely overdiagnosis as there is a correspondingly lower incidence of CIN3+ in the future. This in turn favourably affects invasive screening requirements, on the evidences reported in literature and from the project “Model of Quality Indicators” (MQI) of IFCC Working Group “Laboratory Errors and Patient Safety”. Data have been collected by means of a computer system specifically created and QS have been defined taking into account the level of performance achieved (state of the art). Moreover, the laboratory participates in the MQI in order to analyze its data in a benchmarking process.

Results: Sixty-six QIs have been defined: 35 concerning pre-, 11 intra- and 10 post-analytical phase; 5 to monitor the activities of point of care testing (POCT); 5 related to support processes. The periodic data analysis allowed us to understand the trend of performance over time (improvement or worsening), to know the need of improving actions and of QIs revision (introduction, deactivation, correction). The results analysis demonstrated that processes under the control of the laboratory have improved much more than those that require a close cooperation between the laboratory and the care teams.

Conclusion: QIs are becoming an indispensable tool to monitor and improve all activities of the total testing process. The effectiveness of QIs depends on the reporting system used for data collection. The periodic data analysis allowed us to understand the trend of performance over time (improvement or worsening), to know the need of improving actions and of QIs revision (introduction, deactivation, correction). The results analysis demonstrated that processes under the control of the laboratory have improved much more than those that require a close cooperation between the laboratory and the care teams.

Conclusions: The Roche Elecsys® free $\beta$hCG and PAPP-A assays apply with the standards for biochemical assays for prenatal screening set by the Fetal Medicine Foundation, with low assay imprecision and a high clinical performance of prenatal screening for fetal trisomy in the first trimester.
cancer incidence. Increased sensitivity has two important clinical outcomes: reduced mortality and elongation of screening interval; this latter implies better compliance to screening and lower costs. While the negative predictive value of HPV testing has a direct and easy understandable clinical outcome, the clinical meaning of HPV positivity does open a new perspective in cervical cancer prevention: from a system based on disease diagnosis and treatment to a system of risk stratification and risk reduction interventions. HPV positive women are stratified according to at least one of these discriminators: age; grade of pap smear positivity; HPV typing; molecular markers of oncogenic transformation; colposcopic appearance of the cervix; HPV vaccination status. Based on these characteristics optimal management strategy is selected among the followings: three years, one year or six months follow up; colposcopy and biopsy; excision of the transformation zone. In this way molecular markers are going to play a central role in the risk stratification exercise that is today the cervical cancer screening system.

EW043 METHODS FOR THE RAPID DETECTION OF SYNTHETIC CANNABINOIDS

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Physicians Choice Laboratory Services, LLC, North Carolina, USA

Background: We have implemented an ELISA screening and semi-quantitative method targeted at illicit recreational drugs. This test can be followed by liquid chromatography mass spectrometry (LCMS) confirmation for specific compound identification and quantitation. In these assays, we have demonstrated analytical sensitivity and specificity for three main classes of newer recreational drugs. From urine samples, we can detect parent and metabolite for synthetic cannabinoids, methcathinone and derivatives, along with kratom and kava. In the U.S., the Drug Enforcement Administration has placed synthetic cannabinoids and two bath salt drugs as Schedule I Controlled Substances. In the rehabilitation arena, practitioners need as much information regarding the patient’s compliance with a treatment program as possible.

Methods: Patient urine samples were collected and tested for a broad panel of prescription drugs and drugs of abuse. These samples were blinded for this study. Randox Enzyme-Linked Immunosorbent Assay (ELISA) kits were utilized for semi-quantitative analysis of urine samples for synthetic cannabinoids. Preparation of the plates followed the standard instructions provided with the kit. The ELISA plate was loaded on a Biotek reader for spectrophotometric detection. A standard curve was included on the plate for quantitation. Transition ions were determined for the triple quadrupole mass spectrometer specific to each ion of interest. Multiple target compounds were interrogated, including several JWH, RCS4 and AM cannabinoids. Urine samples were subjected to base hydrolysis and dilution prior to MS detection.

Results: Comparison of ELISA sensitivity and specificity, included 25 samples positive for synthetic cannabinoids reflexed to a mass spectrometry confirmation method. 25 negative samples were reflexed for MS analysis, and high correlation was observed. The enzyme cross-reactivity was >50% for 11 synthetic cannabinoid compounds. The MS confirmation method, scanned for four of the most cross-reactive synthetic cannabinoid compounds. A high correlation was observed. With a combined analytical platform consisting of both screening and confirmation techniques, rapid and quantitative results can be obtained to facilitate high quality patient care.
patients with positive HCV antibodies because of broad availability and superior sensitivity compared to HCV core antigen assays. General screening of populations with the highest risk of chronic hepatitis C (“baby boomers”) may be useful to enhance the rate of diagnosis of patients. Respective programs have been initiated in several countries. With the approval of HCV protease inhibitors telaprevir and boceprevir, a new standard treatment has been established for patients with HCV genotype 1 infection. However, triple therapies in combination with pegylated interferon and ribavirin are associated with complex rules for determination of optimal treatment durations in responders and stopping rules for non-responders. This response-guided treatment of chronic hepatitis C is managed with frequent measurements of HCV RNA before and during antiviral therapy. Recently, significant differences between commercially available HCV RNA assays have been observed and must be taken into account for proper management of treatment. Early results of phase 2 studies with newer direct antiviral agents for IFN-containing and IFN-free treatment regimens indicate the likelihood of further improvements in efficacy as well as simplification of treatment management.

Conclusion: The primary current challenges regarding HCV infection are establishment of diagnosis in affected patients and proper application of complex rules for triple therapies with NS3 protease.

EW047 EFFICIENT MANAGEMENT OF THE HEMATOLOGY LABORATORY ACTIVITY

J. Naegelen

Beckman Coulter Marketing Manager Global Product Management, Krefeld, Germany

Exalab laboratory is a group of 30 private laboratory sites located in Southwest of France. Since March 2012, Exalab has consolidated the hematology activity on its Core Platform in Le Haillan, with an integrated hematology solution, HematoFlow, from Beckman Coulter (BC). In the decision process, the lab wanted to achieve the following objectives: to improve TAT, to save time, to increase standardization of processes, and quality of results. The laboratory has built its organization in routine and expert islands around BC discrete automation with the Automate 1250. All samples received are checked and sorted by the Automate system and then directed to the DxH 800 hematology analyzers. Tubes w/o differential abnormalities are archived with other redirected to the HematoFlow™ with a blood smear if necessary. The abnormal Differentials are now checked by a new method using automated flow cytometry analysis using CytoDiff* reagent, the unique HematoFlow multicolor reagent. This process was previously a manual task in the laboratory requiring reviewing blood smears using a microscope. All information and data management is automatically retrieved by the data manager, REMISOL, from which the biologists consult and validate the results. The HematoFlow with CytoDiff enhances the traditional data produced from a manual differential. For example, HematoFlow provides additional information, regarding T lymphocytes and B lymphocytes subsets and also greatly increased accuracy and precision for blast cell counts. In fact, according to the Ruempke table, when counting manually a 1% blast, values obtained can vary from 0.6 to 8.5 %, while HematoFlow gives a result with 12.9% CV (0.87 to 1.13%). This additional information helps in the interpretation of results by the laboratory in a very efficient workflow. “Not for In Vitro Diagnostic use in the United States

EW048 ADVANTAGES IN THE HEMATOLOGY LABORATORY USING THE HEMATOFLOW SOLUTION

o. Pradier

Laboratory Hematology Manager, Hopital Erasme, Brussels, Belgium

HematoFlow is a new solution for the routine hematology laboratory, which consists of DxH 800 analyzers, a sample preparer FP-1000 and a dedicated FC500 flow cytometer. This chain is controlled by the middleware Remisol and CytoDiff® is the mixture of antibodies which allows differentiation of the leukocyte populations and provides 9-part differential WBC count.
EUROMEDLAB 2013 - SCIENTIFIC SESSIONS

RETROSPECTIVE 18 MONTHS STUDY OF THE USE OF HEMATOFLOW IN ROUTINE DIAGNOSTIC IN THE HEMAOTLOGY LABORATORY OF AN ACADEMIC MIDDLE SIZE HOSPITAL. IN MARCH 2011, THE AUTOTAGING SOFTWARE TO ANALYZE CYTODIFF HAS BEEN IMPLEMENTED. BETWEEN SEPTEMBER 2010 AND FEBRUARY 2012, WE RUN 14,336 CYTODIFF (MEAN=796 PATIENTS BY MONTH, MEDIAN=752 PATIENTS). IT CONCERNED 5,658 DIFFERENT PATIENTS. THE CONTROL OF THE CYTODIFF DIFFERENTIAL BY MICROSCOPY REPRESENTS 7 TO 10 SLIDES/DAY. THOSE SAMPLES ARE DIFFICULT AND REQUIRE ALL THE EXPERTISE OF THE BIOLIGIST. HEMATOFLOW ALLOWS A PRECISE DIFFERENTIAL COUNT FOR LEUCOPENIA AS LOW AS 0.3 G/L. EVEN IN THESE CONDITIONS THE ACCURACY OF THE BASTIS COUNT REMAINS CLOSE TO 0.8%. WE ANALYZE THE SENSITIVITY OF THE FLAG VARIATION LYMPHOCYTES. THE ANALYSIS OF THE BALANCE KAPPA/LAMBDAY BY FLOW CYTOMETRY WHEN B CELLS ARE GREATER THAN 0.52 G/L LYMPHOCYTES AND NKT <3.2 G/L, REVEALED MONOCOLONALITY IN 44 PATIENTS, WHILE THE LYMPHOCYTE COUNT WAS WITHIN THE NORMAL RANGE AND THERE WAS NO MORPHOLOGICAL ABNORMALITY MICROSCOPICALLY DETECTABLE. IN 9 CASES, THE CONTINUITY OF CARE HAS CHANGED, FOUR WITH A CHANGE IN TREATMENT. HEMATOFLOW AND CYTODIFF EFFECTIVELY AUTOMATE THE ROUTINE HEMAOTLOGY REDUCING TAT WITH THE HELP OF AUTOTAGING STRATEGY AND REMISOL WATERFALL RULES. THE ADDITIONAL COST REAGENT IS OFFSET BY THE BENEFITS OF THE INCREASED PRECISION (20,000 CELLS COUNTED INSTEAD OF 100 CELLS), THE BLAST COUNT PRECISION, THE NEW INFORMATION; T/NK AND B CELLS COUNT, RESULTING IN BETTER CARE FOR PATIENTS AND THE DRASTIC REDUCTION OF THE USE OF MICROSCOPY ALLOWING THE TRANSFER OF TECHNOLOGIES TO NEW POSTING. BECKMAN COULTER, THE STYLIZED LOGO, DXH 800, CYTODIFF AND HEMATOFLOW ARE TRADEMARKS OF BECKMAN COULTER, INC. BECKMAN COULTER, THE STYLIZED LOGO, IS REGISTERED WITH THE USPTO. ALL OTHER TRADEMARKS ARE THE PROPERTY OF THEIR RESPECTIVE OWNERS. *NOT FOR IN VITRO DIAGNOSTIC USE IN THE USA.

EW049 DETECTION OF CELL CHANGES IN NEONATAL SEPSIS BY AUTOMATED WHITE CELL MORPHOLOGY

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Background: Larger, immature neutrophils pour in the bloodstream during neonatal sepsis. Current technology allows to assess the neutrophil volume and distribution, an index that has previously shown been significantly different in patients with bacterial infections in the adult. We have studied automated neutrophil volume and its distribution in late onset sepsis from very low birth weight neonates. Patients and methods: Consecutive very low birth weight symptomatic neonates were screened for sepsis using complete blood count (CBC), absolute neutrophil count (ANC), immature to total (I/T) ratio, C-reactive protein (CRP). Mean Neutrophil volume (MNeV) and neutrophil volume distribution width (NDW) were determined both in infants with suspected sepsis and in a group of controls matched for gender, birth weight and gestational age who were not symptomatic for sepsis. Blood culture was used as the gold standard for infection. Receiver operator curves, area under the curve, sensitivity, specificity, positive and negative predictive values were calculated for each test. Results: We enrolled 120 neonates with suspected sepsis and 60 controls. MNeV analysed in cases with sepsis on a single determination have shown a sensitivity =95% and a specificity=88%; cut off = 148 arbitrary units) performing statistically better than CRP (sensitivity=65%; specificity=96%; cut off=0.9 mg/dL), white blood cells count, ANC and I/T ratio. NDW was of poor value (sensitivity=80%; specificity=52%; cut off=27.5). When CRP and MNeV were considered together, sensitivity was unchanged while specificity rose to 97%. MNeV was positive in 1 out 60 controls. Conclusion: MNeV is a reliable and inexpensive adjunct and performs statistically better than the current screening tests evaluated in patients with neonatal late-onset sepsis.

EW050 EVALUATION OF EMERGING BIOMARKERS IN RENAL DISEASE

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University of Miami, Miller School of Medicine

The discovery that mutations in fibroblast growth factor 23 (FGF23) cause autosomal dominant hypophosphatemic rickets transformed our understanding of phosphate homeostasis in health and disease (White et al. Nat Genet 2000). Since then, the critical role of FGF23 in mineral metabolism in chronic kidney disease has been established (Larsson et al. Kidney Int 2003; Gutiérrez et al. J Am Soc Nephrol 2005). Elevated FGF23 has emerged as the earliest alteration of disordered mineral metabolism in chronic kidney disease (Ishakova et al. Kidney Int 2011). Several studies now demonstrate that elevated FGF23 is also a novel biomarker of risk for CKD progression, adverse cardiovascular outcomes and death among patients with chronic kidney disease (Fliser et al. J Am Soc Nephrol 2007; Gutiérrez et al. N Engl J Med 2008; Ishakova et al. JAMA 2011; Kendrick et al. J Am Soc Nephrol 2011; Jean et al. Nephrol Dial Transplant 2009). Most recently, FGF23 was demonstrated to be a contributing mechanism of left ventricular hypertrophy, which may underlie an important component of its association with high rates of adverse cardiovascular outcomes (Faul et al. J Clin Invest 2011). These studies elevated FGF23 and disordered phosphate homeostasis from powerful biomarker to novel mechanism of cardiovascular disease and chronic kidney disease. Other novel biomarkers of bone and disordered mineral metabolism, for example, sclerostin, are under intense investigation. In addition, controversy continues to swirl as to the best approach to measure parathyroid hormone in patients with chronic kidney disease. This session will describe the latest data on assessing disordered mineral metabolism in chronic kidney disease and how these biomarkers may potentially impact the care of the millions of individuals worldwide who suffer from chronic kidney disease.

EW051 MEASUREMENT OF VITAMIN D METABOLITES IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Until recently, nephrologists neglected the use of native vitamin D in patients with chronic kidney disease (CKD), especially in those undergoing haemodialysis, and active vitamin D analogs were almost exclusively used. Indeed, native vitamin D was considered ineffective as it would not be converted to its active metabolite by the damaged kidneys. Over the last few years, a large body of data on vitamin D in CKD has accumulated, and practices have changed substantially. In several observation studies, a negative association between serum 25OHD levels and all-cause mortality, but also between serum 25OHD levels and the progression of renal failure has been reported in CKD patients. Furthermore, treatment with native vitamin D has been shown.
to reduce modestly but significantly serum PTH levels in non-dialysis, and in dialysis CKD patients, as well as in kidney transplant recipients. The recent KDIGO guidelines suggest measuring serum 25-hydroxyvitamin D (25OHD) levels, the biochemical index of vitamin D store, in patients with CKD stages 3-5, including those undergoing dialysis, and repeating this measurement depending on the baseline value and therapeutic interventions. They also suggest to correct vitamin D deficiency/insufficiency using treatment strategies recommended for the general population. These guidelines do not recommend to measure calcitriol, the active vitamin D metabolite, with the exception of a few special cases. However, the fact that calcitriol levels increase in some dialysis patients when they are given native vitamin D is puzzling and deserves further studies. Recent data which need to be confirmed suggested that bioavailable 25OHD levels could be of better clinical value than total 25OHD level. However, this index is calculated with the aid of the vitamin D binding protein serum level, and a careful, evidence-based, cost-benefit analysis is needed. Finally, as FGF23, which is increased precociously during the course of CKD, stimulates the 24-hydroxylase pathway (inactivation pathway) of vitamin D metabolism, research on the clinical/biological value of measuring serum levels of 24-hydroxylated vitamin D metabolism is currently underway.

EW052
BIOMARKERS IN STROKE DIAGNOSIS, CLASSIFICATION AND PROGNOSIS
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KAT General Hospital, Athens, Greece

Stroke is a devastating condition that encompasses a wide range of pathophysiological entities that include thrombosis, hemorrhage, and embolism. Current diagnosis of stroke relies on physician clinical examination and is further supplemented with various neuroimaging techniques. The current diagnosis of stroke remains hampered and delayed due to lack of a suitable mechanism for rapid (ideally point-of-care), accurate, and analytically sensitive biomarker-based testing. A single blood biomarker or better multiple sets of biomarkers that could be used in an acute setting to diagnose stroke, differentiate between stroke types, or even predict an initial/reoccurring stroke or predict the severity and the outcome of an acute stroke would be extremely valuable. Also prediction of the severity and the outcome after an acute stroke is important for clinicians, patients, and researchers. The best validated clinical prognostic models are probably not accurate enough to predict outcome in individual patients with stroke. The performance of clinical models might be improved by blood markers of any of the pathological processes in acute stroke such as inflammation, hemostasis, neuronal or glial injury, and cardiac dysfunction. We discuss here the diagnosis and classification of acute stroke focusing on use of novel biomarkers (either solitary markers or multiple markers within a panel) that have been studied in a prospective study. For this purpose we have used the analytical platform of Evidence Investigator from Randox and the technique employed here is the biochip array technology (BAT) that allows the quantitative determination of multiple markers simultaneously. The reason for a multi-marker approach is that • No single biomarker has ever been demonstrated to be clinically useful as a standalone diagnostic test for stroke. • One way to address this difficulty is by simultaneously evaluating multiple biomarkers that contribute complementary information. • Preliminary studies suggest that such a biomarker panel may add time-sensitive diagnostic information in the early evaluation of stroke

EW053
TOWARDS DEVELOPMENT OF A NOVEL MULTIPLEX TEST FOR ACCURATE STROKE DIAGNOSIS EMPLOYING BIOCHIP ARRAY TECHNOLOGY
C. Richardson
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Stroke is the third leading cause of death worldwide and can be defined as the rapidly developing loss of brain function due to interruption in the blood supply to the brain. In order to minimise neurological damage following stroke, it is crucial that stroke patients are rapidly and accurately diagnosed so that appropriate treatment can be administered. At present, the diagnosis of ischaemic stroke is particularly challenging due to the poor sensitivity of computerised tomography for ischaemic stroke identification. Magnetic resonance imaging (MRI) does have improved sensitivity for ischaemic stroke detection but the employment of MRI can be limited by restricted accessibility and the unsuitability of this approach in certain acutely ill patients. Consequently, there remains a reliance on an experienced stroke clinician to make an ischaemic stroke diagnosis by supplementing brain imaging results with a physical examination of the patient. There is an urgent need for a rapid blood test for ischaemic stroke diagnosis to be developed to confirm clinical and imaging observations. This test would ideally be low-cost and applied to near patient technology. It is anticipated that a panel of biomarkers will be required for the development of a robust diagnostic test for acute stroke diagnosis. Randox aims to develop such a test by employing their proprietary biochip array technology (BAT) to multiplex a unique collection of stroke biomarkers.

In this talk, we will detail on-going efforts to develop a stroke multiplex test and discuss the clinical promise exhibited by candidate biomarkers for this multiplex array. Candidate biomarkers include glutathione S-transferase pi (GSTP1), nucleoside diphosphate kinase A (NDKA) and Parkinson disease protein 7 (PARK7 also called DJ-1). A recent publication concluded that these 3 biomarkers performed best from a total of 29 biomarkers evaluated with respect to differentiating stroke patients from controls and discriminating early time-point from late time-point stroke patients. This study has suggested that GSTP1 monitoring has the potential to significantly increase the number of patients having access to life-saving thrombolytic therapy due to the ability of this biomarker to accurately predict the time of stroke onset.

EW054
EARLY DIAGNOSIS OF MYOCARDIAL INFARCTION USING HIGHLY SENSITIVE TROponin I ASSays
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Cardiology, University Hospital Basel, Switzerland

Background: Patients with symptoms suggestive of acute myocardial infarction (AMI) account for about 10% of all emergency department (ED) consultations. Electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstone of clinical assessment. A limitation of former generation cTn assays is a delayed increase of circulating levels for mandating serial sampling for 6-12 h with delays in diagnosing disease (“rule-in”) and delays in excluding disease (“rule-out”). The recently developed sensitive and high-sensitivity cardiac troponin (hs-cTn) assays have enabled measurement of cTn concentrations not reliably detected before. The new tests have been shown to improve the diagnostic accuracy for AMI
at presentation, and rule-in and rule-out of AMI might be feasible more rapidly. On the other hand the number of positive hs-cTn results in various acute and chronic conditions with cardiac involvement other than AMI has increased. As a consequence, the positive predictive value (PPV) of an elevated hs-cTn level has decreased, and many physicians treating patients with symptoms suggestive of AMI have been confused. It is currently unknown how to best take advantage of the novel hs-cTn tests in clinical practice. Accordingly, there is an on-going debate whether and to what extent a shortening of the time interval to the second sample is feasible and safe. The aim of this presentation will be to highlight an algorithm for rapid rule-in and rule-out of AMI using hs-cTnI levels

Methods: In a prospective, observational, multicenter study enrolling consecutive patients presenting with acute chest pain, we derived and validated algorithms on how to best apply hs-cTnI data either alone or in conjunction with other clinical information. Blood samples were collected at presentation and after 1,2,3 and 6 h in a blinded fashion. The final diagnosis was adjudicated by 2 independent cardiologists using all information including hs-cTnT values. All patients also received long-term follow up.

Results: The results will be presented and discussed.

Conclusions: Validated algorithms will help clinicians to best use the advantages provided by novel hs-cTn assays in the early rule-in and rule out of AMI.

EW055

GALECTIN-3 AS A BIOMARKER IN HEART FAILURE MANAGEMENT

R. De Boer

University Medical Center Groningen, The Netherlands

Cardiac remodeling in response to myocardial stress and injury is the main precursor for heart failure (HF). Despite the availability of numerous pharmacological and device therapies, HF remains a major burden to global health care associated with substantial morbidity and mortality. Therefore, there is an ongoing need for better HF diagnostics, risk stratification, and improved therapeutics. Experimental and clinical research has suggested that galectin-3, a β-galactoside-binding lectin, acts a key player in the maladaptive response to myocardial injury. Galectin-3 exerts its role in inflammation and fibro genesis, which are key mediators of cardiac remodeling and HF. In experimental studies, it was shown that galectin-3 is secreted by activated macrophages, and turns quiescent fibroblasts into active matrix secreting myofibroblasts. Disruption and blockade of galectin-3 attenuates and reverses the HF progression. By hitherto unknown mechanisms, galectin-3 is secreted into the systemic circulation and can reliably be measured. Plasma galectin-3 levels independently predict outcome in human patients with acute and chronic HF. Furthermore, in the general population, plasma galectin-3 levels strongly associate with cardiovascular risk factors and independently predict mortality and new-onset HF. There a several issues that need further clarification. First, there are indications that galectin-3 may be particularly important in specific forms of HF, e.g. HF with preserved ejection fraction and the cardio renal syndrome. Second, clinical studies have been conducted to show that the use of galectin-3 as a biomarker changes our daily clinical routine. Such galectin-3-based clinical decision making would clearly strengthen the case for the use of galectin-3, and currently trials are ongoing that endeavor this concept. Finally, galectin-3 seems to be more than just a marker: it may be a pathophysiological layer in HF. If proven, this would open up possibilities for galectin-3-targeted therapy.

EW056

BIOMARKERS OF CARDIO-RENAL RISK AND CLINICAL OUTCOME

P. Murray

Mater Misericordiae University Hospital, Dublin, Ireland

Congestive heart failure is commonly (30%) associated with chronic kidney disease (CKD), which complicates management and worsens outcomes. Furthermore, acute kidney injury (AKI) is common (30% incidence) in patients hospitalized with acute decompensated heart failure (ADHF), and is associated with increased morbidity, mortality, management complexity, and cost. Efforts over many decades to prevent or treat AKI in such high-risk patients have been hampered by lack of time-sensitive and mechanism-specific markers for AKI to target early interventions. In AKI, serum creatinine (the generally accepted “standard of care” test for the clinical diagnosis of AKI) may not increase until days after renal tubular injury has begun. Furthermore, because serum creatinine is primarily a functional marker of glomerular filtration, it is not optimally suited to diagnose AKI, but rather serves to define severity of the resulting loss of renal function. Use of serum creatinine increments for AKI case definition has led to the conduct of numerous unsuccessful clinical trials of putative therapies for AKI in patients with established acute tubular injury and a significantly elevated creatinine. Creatinine changes that define AKI by validated classification systems (RIFLE, AKIN, WRF- worsening renal function), are currently the most useful biomarkers for AKI case identification and staging. Whichever functional criterion is used for AKI case definition, appropriate differential diagnosis and prognostic assessment can be further improved by the use of traditional and novel biomarkers of renal tubular damage. These markers can be used to differentiate cases of acute tubular necrosis (ATN) versus prerenal azotemia or chronic kidney disease, potentially leading to more accurate diagnosis, and improved triage and management. In patients with CHF, the use of cardiac biomarkers such as BNP can help to effectively reverse prerenal azotemia caused by ADHF, while monitoring of kidney damage markers can provide additional safety and prognostic information. In tandem with timely, sensitive, and specific markers of kidney damage, more time-sensitive markers of acute functional GFR change may further improve the monitoring and diagnostic assessment of AKI in the future.

EW057

LABORATORY PERSPECTIVES IN THE DIAGNOSIS AND MONITORING OF PLASMA CELL DYSCRASIA

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Background: Quantitative immunoassays for serum free light chain (FLC) have changed the approach to identifying and managing monoclonal gammapathies. The FLC assay has been incorporated into diagnostic screening panels, prognostic testing, and disease monitoring.

Methods: Summary of current published data on the impact of FLC assays on diagnosis, monitoring, and prognosis of proliferative plasma cell diseases. International guidelines and recommendations by expert groups will be reviewed.

Results: The diagnostic sensitivity of the FLC K/L ratio for detecting monoclonal free light chains has decreased the need for urine as part of the screening panel for monoclonal gammapathies. The recommended panel is serum PEL, IFE,
and FLC (urine studies are also suggested if AL is suspected). For the laboratory, this means that urine PEL will be predominantly performed for assessment of renal function and quantitation of urine M-spikes and that highly concentrated urine will rarely be required. The FLC K/L ratio is also useful for prognosis of MGUS and SMM progression to MM. MGUS progresses at 1%/yr. Patients with a normal FLC ratio (0.25–1.65), an M-spike <15 g/L, and a gamma heavy chain have a 0.1%/yr risk of progression (2% lifetime risk). Low-risk MGUS patients don’t need monitoring unless clinical symptoms occur. Identification of low-risk MGUS will decrease medical costs and patient anxiety. For the laboratory, this means we need to change our recommendations for long-term follow-up. FLC quantitation also has a role in disease monitoring. To date, that role has been restricted to patients with no measurable serum or urine M-spike. The long-term variability of serum FLC and urine M-spike measurements, however, are comparable, and either should suffice for monitoring. For the laboratory, this means additional requirements to reduce inter-assay variability and eliminate artifacts due to dilution and antigen excess. Conclusions: Quantitative FLC immunoassays must be integrated into the electrophoretic and nephelometric processes used for evaluating plasma cell dyscrasias. Their use increases diagnostic sensitivity, reduces the need for highly concentrated urine, and increases the need for consistent laboratory test protocols for disease monitoring.

**EW059**

**MULTIPLE MYELOMA IN CLINICAL PRACTICE: FROM DIAGNOSIS TO TREATMENT AND FOLLOW-UP.**

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Background: Measurement of serum free light chains (FLC) is a valuable laboratory test for monitoring of patients with light chain or oligosecretory multiple myeloma and AL amyloidosis. A single test available since 2001 has been used to establish value thresholds for response criteria. A new test for serum FLC* was developed recently. These tests differ in the characteristics of the antibody employed (monoclonal vs. polyclonal). Absolute concentrations of FLC observed with these tests are different without the possibility of applying a conversion factor. The objective of our study is to evaluate if the tests are comparable for follow-up of patients, in particular for the evaluation of response to treatment.

Methods: This study aims to compare these two tests in the follow-up of patients with multiple myeloma or AL amyloidosis. The secondary objective is to estimate the percentage of discrepancy between the tests in a large cohort of patients with various plasma cell proliferative disorders. All the patients in our institution for whom the serum FLC assay is prescribed will be included, regardless of the reason for prescription. The planned period of inclusion runs from July 1 to December 31, 2012. During this period, the Biochemistry laboratory will perform measurements with both tests for all prescriptions of the serum FLC assay. Patients treated for multiple myeloma or AL amyloidosis are followed every 3 or 6 weeks (according to the plan of chemotherapy) and will thus have samples collected at least four times during the 6-month period of the study.

Results: As of December 31, 2012, we have collected 1380 samples corresponding to 771 patients. 203 patients have been tested at least twice. Diagnosis is known for 249 patients. The main diagnoses are multiple myeloma (142 patients), MGUS (103 patients), and AL amyloidosis (15 patients). We observed good agreement between the two tests for kappa (concordance 88% and Cohen’s k 0.789), lambda (82%, 0.690) and kappa/lambda ratio (85%, 0.759). We compared evolution of FLC concentrations during follow-up of 127 multiple myeloma. Evolution was concordant for 121 patients (95%). We observed discordant evolution in 6 patients (5%). Conclusion: Our results confirm good agreement between the two tests for diagnosis but also for evaluation of response. However we observed few discordant results which emphasize the need to correlate the results of FLC tests with results of traditional tests and with clinical outcome.

*not available for sale in the U.S.
Background: The conflict between what societies are able to pay for health care and the population’s need for health care is still increasing. Health economics aims to find the best possible way to spend the available financial means. In order to apply economic thinking to healthcare, one should view the health sector as a productive sector whose aim is to produce health, by ensuring that people live longer and/or more healthily. Priority must be given to those health investments which result in the greatest amount of health for the money that is invested. Interventions with a good ratio between the invested money and the resulting health outcome are called cost-effective. Over the past years several health economic evaluations have been performed and published in the field of diagnostics.

Methods: A review of the literature was performed to obtain insight in the differences in methods and in applied decision criteria. A common tool was proposed and presented to payers and to experts in the field.

Results: Many different health economic approaches towards diagnostics are observed, applying different perspectives, and different outcome parameters. The level of required evidence varies a lot among advisory bodies and decision makers. HTA bodies such as NICE in the UK or the KCE in Belgium require high methodological standards and expect results in the format of cost per QALY (quality adjusted life year) gained. The QALY combines quality and quantity of life in one parameter and is theoretically the best approach to express health gains. Other decision makers criticize the practical implementation of the QALY and yet other only consider costs and budget impacts thereby missing an important part of the picture. We proposed a set of common criteria (a ‘tool’) for the evaluation of health investments for diagnostics. A key observation is that without added clinical utility of a diagnostic test there can be no additional economic value.

Conclusions: Current decision making on paying for diagnostics with public means is characterized by high diversity in methods and applied criteria. Diagnostic companies should strive to show the clinical utility of their diagnostic tests as a key requirement to demonstrate value for money.

In 2010, urine NGAL as early was included into the decision-making algorithm as the only change as compared to 2006-2009. Incidences of HF need and length of therapy (LOT) were recorded and compared. Costs associated with routine NGAL testing, need for HF and ICU stay were analyzed, and the economic impact of the new algorithm was determined.

Results: In 2010, 528 patients were operated with no statistical differences in numbers, case mix, risk scores and outcomes to previous years. With the inclusion of NGAL into the algorithm for HF-initiation (2010) HF was initiated in 26% less patients (14 patients) but also start of HF was on average 1.1 days earlier compared to 2006-2009. Length of therapy decreased by 25%. Comparison of direct costs for HF therapy based on expected versus actual seen HF cases in 2010 resulted in calculated overall savings of more than € 120 000, which corresponds to more than € 200 per cardiac surgery patient. This does not yet include savings associated with reduced or avoided ICU stay. In the current study this would result in more than 200 ICU days saved.

Conclusion: Routine NGAL testing in patients after cardiac surgery and consecutive changes in management reduced the number of HF therapies, the length of therapy and the overall ICU stay in our institution resulting in substantial net savings maintaining high quality of patient care.
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EW063
THE PROBLEMATIC OF NOSOCOMIAL INFECTIONS BY CLOSTRIDIUM DIFFICILE
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Since its discovery in 1978, Clostridium difficile has emerged as a major nosocomial pathogen. It is the leading cause of antibiotic-associated diarrhoea, with a clinical spectrum of the disease ranging from mild diarrhoea to life-threatening pseudomembranous colitis. The main virulence factors are two high molecular weight exotoxins, namely toxins A and B that both exhibit cytoxic and enterotoxic activities.

Ten years ago, the epidemiology of C. difficile infections (CDI) dramatically changed in North America and Europe. A significant increase of incidence as well as of severity of CDI were reported on both sides of the Atlantic ocean. The rapid emergence and spread of a specific clone of C. difficile was rapidly demonstrated. This clone constitutes a specific type that belongs to PCR-ribotype « 027 » or pulsotype « NAP1 ». The increased virulence of this clone is associated with the overproduction of toxins A and B and the production of binary toxin. Primarily detected in North America, C. difficile « 027 » was rapidly identified in outbreaks that occurred in several european countries (UK, The Netherlands, Belgium and France). In elderly patients, the mortality linked to CDI caused by the 027 ribotype reached sometimes percentages higher than 10%. Hospital outbreaks of CDI are frequent. They are mainly due to the rapid contamination of the environment by spores disseminated by the diarrhoeal stools. Hence, an early and accurate diagnosis of CDI is a keystone in the optimal management of this nosocomial disease. Firstly it allows to initiate an adequate therapy but, moreover, it also allows to implement hygiene and infection control measures in the patient’s room. All strategies should aim at a same-day diagnosis in case of suspicion of CDI. In case of a positive result, the immediate treatment of the patient will improve his condition and limit the risk of room contamination. And the rapid implementation of hygiene measures will prevent further spread of the disease. With such a goal and such implications however, the accuracy of the laboratory diagnosis is of crucial importance. False positive results may induce inadequate treatment and increase cost due to isolation procedures and false negative results may lead to outbreaks.

EW064
LABORATORY MANAGEMENT OF DIABETIC PATIENTS CARRYING HEMOGLOBIN DISORDERS
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The prevalence of diabetes is increasing rapidly. There are estimated to be 366 million people in the world with diabetes and by 2030 this number is expected to reach 552 million. The clinical laboratory has a fundamental role in the management of patients with diabetes. The analyzes most commonly measured are glucose and hemoglobin A1c (HbA1c). Glucose is measured in the clinical laboratory for diagnosis and by the patients themselves for monitoring. The HbA1c concentration indicates the average glucose over the preceding 8-12 weeks and provides an additional criterion for assessing glycaemia. Large prospective randomized clinical trials, most notably the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS), documented that HbA1c predicts the risk for developing micro vascular complications. Several influential clinical organizations have recently advocated HbA1c for diagnosis of diabetes, augmenting the contribution of HbA1c to diabetes. HB abnormalities may be cause by defects in the HB molecule (HB variants) or change in the production of one of the subunits (thalassemia). Over 1150 HB variants have been identified, the most common of which are HBs, HBs, HBc and HBd. HbA1c can be measured accurately in individuals heterozygous for the common HB variants, provided an appropriate method is used (see www.ngsp.org). In subjects with homozygous variant HB or altered erythrocyte survival, HbA1c does not reflect glycaemia. Extracellular markers of long-term glycaemia (10-14 days), most commonly fructosamine or glycated albumin, are independent of both erythrocyte lifespan and HB modifications. However, they are altered by changes in albumin turnover and clinical studies are limited. Moreover, there are neither outcome data nor agreed target values for optimum glycemic control. While useful in conditions where HbA1c cannot be used, until more data are available their clinical value is limited. Measurement of HbA1c provides valuable information for the overwhelming majority of diabetic patients. A knowledge of the conditions that alter HbA1c enables appropriate use of HbA1c in patient management.

EW065
CARBAMYLATED HEMOGLOBIN IN DIABETES AND RENAL DISEASES
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HbA1c, the major glycated hemoglobin fraction, is characterized by the binding of glucose to the N-terminal valine residues of globin beta chains. It is considered the gold standard of diabetic survey and has been proposed for diabetes diagnosis. Besides glycation, other nonenzymatic modifications can affect hemoglobin and other proteins. During chronic renal failure (CRF), HB is also modified by carbamylation, due to the nonenzymatic binding of isocyanic acid, mainly formed in vivo by spontaneous dissociation of urea, to N-terminal extremities of globin b chains, generating carbamylated hemoglobin (cHb). Whereas cHb has been described as a classical interference in HbA1c assays, since cHb could co-migrate with HbA1c, most of currently available methods are no more prone to this interference in the majority of patient samples. However, the threshold of interference varies according to the method used. Besides, the formation of other adducts due to the accumulation of various mid-sized molecules modifies the chromatographic pattern of hemoglobin. Thus, the interpretation of HbA1c results in patients with renal disease must be cautious.

cHb assay has been proposed during CRF as marker of "uremic memory", cHb rate being correlated with uremia and duration of exposure to urea. Besides, cHb could be used to differentiate acute renal failure (ARF) from CRF, and could constitute a time-integrated urea index in hemodialysis. However, like in the case of HbA1c, many factors may interfere with red blood cell metabolism in patients with CRF (e.g.: shortened RBC lifespan, erythropoietin therapy) and alter the seminalogical value of the assay. The assessment of markers of plasma protein carbamylation (e.g.: e-carbamyl lysine, also called homocitrulline) may constitute a valuable alternative. Finally, it must be noticed that carbamylation and glycation, which both alter structural and functional properties of proteins, compete for the same NH2 sites, especially at the N-terminal valine of globin beta chains. It may be hypothesized that HbA1c formation may depend on the actual Hb carbamylation rate.
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**EW066**

**ACHIEVEMENT OF DESIRABLE ANALYTICAL GOALS AND WORKFLOW IMPROVEMENT FOR HBA1C TESTING: AN INNOVATIVE PROPOSAL**

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The use of HbA1c for diagnosis increases the need for improved assay precision and accuracy, as well as reduced interferences. In addition, variant detection is critical in the aid of diagnosis since the clinical cut-off of HbA1c in patients carrying a variant is unknown. The growing epidemic of Diabetes is also increasing HbA1c testing volumes and, consequently, this drives the need for workflow efficiencies. Automated testing systems should deliver faster time to result, easy to use technology and reduced time for interpreting results. Laboratories are experiencing the tightening of criteria for accuracy and precision from the National Glycohemoglobin Standardization Program (NGSP) for certification. Effective September of 2012, NGSP requires that 37/40 HbA1c results must be within ±7% of target value for an HbA1c method to pass NGSP certification. The FDA is also expected to have more stringent requirements for HbA1c testing such as improved precision of <2% CV, reduced bias to <±2%, and little to no interferences with any hemoglobinopathies for tests used in the diagnosis of Diabetes. In Europe, laboratories are expected to maintain IFCC criteria and, for most of the countries, to report in mmol/mol units. To this regard, recent evidences based on biological variation indicate that the desirable goals for imprecision, bias and total error be ≤1.3%, ≤±1.9% and ≤±3.9%, respectively. This presentation will introduce the D-100 Hemoglobin Testing System from Bio-Rad Laboratories and show how this system offers a solution to the new guidelines and requirements for HbA1c testing. The D-100 Hemoglobin Testing System offers the security of High Performance Liquid Chromatography (HPLC) HbA1c results and focuses on innovative solutions for workflow efficiency. Partnered with an accurate and precise HbA1c assay, workflow efficiencies are demonstrated with a quicker time to result, reduced hands on time with reagents, simple calibration, automatic test parameter updates, sat capability, intuitive user interface and automated result review.

**EW068**

**MANAGING DEMAND FOR LABORATORY TESTING IN A TIME OF ECONOMIC CRISIS.**

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With a constant increase in laboratory testing and a consistent decrease in health care resources, clinical laboratories need more active management in laboratory testing. It is well known, especially within hospitals, that some laboratory tests are ordered too often, time intervals being too short between consecutive tests, with no real clinical or patient care justification. Moreover, the use of request protocols, in which a number of tests are carried out simultaneously, and the ever-increasing general use of electronic ordering for laboratory tests mainly through the Hospital Information System (HIS), has also decisively contributed to increased activity, sometimes unnecessary or redundant. Whether laboratory testing is genuinely needed or not is an ongoing point of discussion. Different studies have suggested that up to 40% of the testing is questionable and 30% is attributed to unnecessary test repetitions. Managing the demand for laboratory tests can be useful for improving laboratory efficiency and the reduction of unnecessary activity. Due to the complexity of work in the laboratory area, development of managing tools are limited, based mainly on automated test rejection, avoid repeat testing, and redefinition of clinical and request protocols. At this moment, we would like to offer our experience in the development and implementation of a tool for managing the demand for laboratory testing. Via HIS, in the moment of an electronic laboratory order, the following information appears: minimum time suggested between two consecutive measurements of the same parameter, and the date and result of the previous order. When the order for a particular test is requested before the end of this suggested interval, an alarm is displayed on the HIS screen. With this information, the physician, taking into account specific patient care needs, can decide whether or not to order routine tests, while more specialized tests can be automatically rejected.

The implementation of this tool, together with regular updates and redefinitions of request protocols, including the
agreements between clinical departments regarding laboratory activity, has led to a remarkable reduction in both activity and budget.

**EW069**  
**INTRODUCTION TO RENIN AND ALDOSTERONE TESTING**  
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Derangements in the activity of the renin-angiotensin-aldosterone system (RAAS) play a pivotal role in causing a number of frequent and serious cardiovascular diseases. On the other hand a large body of evidence was accumulated showing that pharmacological blockade of RAAS may prevent or relent the unfavourable outcome of these diseases. Thus, it would be highly desirable having accurate and reproducible methods for measuring the components of RAAS. Unfortunately this is not the case. Indeed renin, the driving force of RAAS, is traditionally measured as plasma renin activity (PRA, ng/mL/h) i.e. as a function of the action of renin on angiotensinogen to generate Angiotensin I which is subsequently quantified by radioimmunoassay. This time-honoured enzymatic assay is admittedly very accurate but time-consuming and scarcely reproducible among laboratories. As an alternative to PRA, new assays, which measure plasma renin concentration (PRC, pg or μU/ml), were developed. These direct assays exploiting the high specificity of monoclonal antibodies directed against a specific epitope of the renin molecule, have the advantage of being fast and reproducible but were criticized because of the lack of sensitivity that is required for measuring the very low levels of renin which are found in primary hyperaldosteronism (PHA). However recent refinements of these direct assays have further improved their sensitivity and PRC is progressively replacing PRA. The appropriate assessment of the aldosterone/renin ratio, that is, at present, the recommended screening test for PHA, brings about the reliability of aldosterone assays. Aldosterone is usually quantified by radioimmuno assay after an extraction from plasma, an essential step that is often overlooked in many laboratories. This limitation, in combination with the use of an array of detection antibodies with different sensitivities, make the measurement of aldosterone among laboratories quite erratic, at best. However also in this area the availability of monoclonal antibodies and of new chemiluminescent tracers promise to provide new, accurate and fast methods very convenient for assessing aldosterone in the every day clinical setting.

**EW070**  
**PLASMA ALDOSTERONE TO RENIN RATIO ON AN AUTOMATED ANALYSER USING TWO NOVEL CHEMILUMINESCENCE IMMUNOASSAYS: ONE-YEAR EXPERIENCE**  
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Inappropriately high levels of aldosterone as in Primary aldosteronism (PA) can lead to cardiovascular damage and other complications. According to recent guidelines, plasma aldosterone to renin ratio (ARR) is recommended for screening in groups with an increased prevalence, and aldosterone after salt load testing can be used to confirm the diagnosis. Due to the lack of assay harmonization, method specific cut-off values are mandatory. We present data on our experience with two novel immunoassays for aldosterone and renin on the IDS-ISYS automated analyser. Aldosterone and renin levels were measured in 130 healthy, 166 essential hypertensive (EH) and 117 PA patients to determine the ARR using the IDS-ISYS analyser (Boldon, UK). Furthermore, the iSYS Aldosterone assay was also used to examine levels during the saline-infusion confirmatory test in PA (n=27), EH (n=49) and confirmed non-PA (n=65). Based on our experience with our previous routine assay, PA was defined by lack of aldosterone salt-load suppression to <5 ng/dL using the Siemens RIA. Mineralocorticoid receptor antagonists and b-blockers were excluded. Hypokalemia was controlled in all subjects. Our traditional cut-off during screening for PA is 1.2 [ng/dL]/[mU/L]. Good agreement was seen between the ISYS and Siemens aldosterone assays (y=1.1x ± 0.02, R2=0.942, n=275) and ISYS and Diasorin Liaison renin assays (y=1.05x ± 0.13, R2=0.921, n=137). ROC curve analysis of PA vs EH ARR values showed that a slightly lower cut-off at 1.1 [ng/dL]/[mU/L] would provide a 96.6% sensitivity with 87.4% specificity using the iSYS assays, equivalent to the currently used methods. Sodium-load confirmatory testing revealed 3 of 27 PA cases with false negative suppression and 1 confirmed non-PA patient case with a borderline non-suppressible aldosterone using both the Siemens and ISYS assays. In conclusion, the data suggest a similar cut-off to the one currently used for the ARR at >1.1 [ng/dL]/[mU/L] could be used to diagnose PA as well as a similar confirmatory post-saline cut-off at >5ng/dL. The availability of a completely automated, single-sample method would provide a simpler and higher throughput alternative to facilitate the screening and diagnosis of PA in larger population studies.

**EW071**  
**ANALYTICAL CONSIDERATIONS ON THE IDS-ISYS ASSAYS FOR HYPERTENSION**  
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Introduction: Aldosterone, the founder of mineralocorticoid hormones, is produced in the adrenal cortex by a distinct biosynthetic pathways from glucocorticoids. Aldosterone plays an important role in electrolyte balance and blood volume-pressure regulation, its secretion is the result of the action of several factors among which the most important is the increase of the plasmatic potassium concentration and the rennin-angiotensin-aldosterone system activation when sodium concentration decrease. Measurement of Aldosterone and Renin levels is very useful in hypertension as part of the diagnostic protocol to highlight hyper or hypo secretion. Measurement of Aldosterone in urine is needed as a 24 hour collection gives the whole estimation of its secretion over the entire day. In this study we evaluated the analytical performance of a new automated chemiluminescence immunoassay. Methods: Aldosterone was measured in 88 urine remnant samples and 95 plasma EDTA samples from subjects referred to our laboratory (48.4 ± 18.3 years of age, 93 females, 90 males). Determinations were run with the ALDOCTX Diasorin RIA assay (Stillwater, US) and the IDS-ISYS Assay (Boldon, UK) on the automated iSYS platform. Aldosterone concentrations ranged from 1.3 to 40.5 ng/dL and from 12.6 to 94.1 ng/dL for urine and plasma respectively. Reproducibility was evaluated according to CLSI EP5-A2 in urine samples with Aldosterone concentrations ranging from 14.1 to 97.7 ng/dL. Results: the Passing-Bablok regression of ISYS against RIA shows a slope of 1.20 (95%CI 1.11-1.28) and intercept 2.1
demonstrated to be accurate in the detection of newborns with IEM, robust, and without the risk of the exposure to toxic detected. The mean SUAC level in non-affected newborns was 74 patients with IEMs including three with HT1 were extracted separately for SUAC, converted into hydrazone, after extraction from DBS punches. The residual blood spots were analyzed by a novel protocol and compared to a in-house method. All samples were derivatized with butanol-HCl buffer to adjust required concentrations of the individual drugs used. Methods: A commercial kit was evaluated to analyze amino acids, acylcarnitines and SUAC with a significantly less harmful hydrazine derivative in a NBS laboratory. DBS specimens from pregnant women. The modular MassTox® TDM System consists of three components: The BASIC Kit A, the Analytical Column A, and 14 PARAMETER Sets which specify the measurement of up to 150 analytes. The specific parameter set for AEDs encompasses 26 drugs. Preparation of samples including AEDs is same for all parameters, based on a simple, effective protein precipitation process. Briefly, precipitation is achieved by addition of 25 µL of extraction buffer and 250 µL of precipitation reagent containing all labeled internal standards. When centrifugation supernatants are diluted with buffer to adjust required concentrations of the individual drugs according to MS/MS instrument sensitivity and parameter set. Results were generated on an ABI Sciex API 4000 equipped with an electro spray ionization source.

**EW073**

**THERAPEUTIC DRUG MONITORING OF ANTIEPILEPTIC DRUGS DURING PREGNANCY**

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Epilepsy is the most frequent neurological disorder worldwide with a prevalence of approximately 0.5% in western countries. Around one quarter of people with epilepsy are women of reproductive age and most of them use antiepileptic drugs (anticonvulsants, AEDs) for adequate control of their seizures. Additionally, anticonvulsants are also used for the treatment of a broad range of other medical conditions such as bipolar disorders, cancer or neuroepathic pain. Recent clinical studies revealed that physiologic changes during different stages of pregnancy may lead to altered pharmacokinetics for AEDs and broad individual variations resulting in difficulty to predict the appropriate drug dosage. It is well known that fetal drug exposure to some AEDs like valproic acid increases the risk of minor or major congenital malformations. Therefore, therapeutic drug monitoring for AEDs plays an important role in improvement of the effectiveness of treatment while minimizing fetal and maternal drug exposure. Here, we describe the evaluation of a commercially available mass spectrometry kit (MassTox® TDM Series A) from Chromsystems, Munich for the fast and efficient monitoring of AEDs in serum/plasma samples from pregnant women. The modular MassTox® TDM System consists of three components: The BASIC Kit A, the Analytical Column A, and 14 PARAMETER Sets which specify the measurement of up to 150 analytes. The specific parameter set for AEDs encompasses 26 drugs. Preparation of samples including AEDs is same for all parameters, based on a simple, effective protein precipitation process. Briefly, precipitation is achieved by addition of 25 µL of extraction buffer and 250 µL of precipitation reagent containing all labeled internal standards. When centrifugation supernatants are diluted with buffer to adjust required concentrations of the individual drugs according to MS/MS instrument sensitivity and parameter set. Results were generated on an ABI Sciex API 4000 equipped with an electro spray ionization source.

**EW074**

**POINT-OF-CARE TESTING OF CARDIAC MARKERS – COMING OF AGE?**

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The assessment of blood levels of biomarkers such as cardiac troponins and brain natriuretic peptides have become more or less indispensable in the management of patients with heart disease, be it in patients with acute coronary syndromes or in heart failure. The assay of cardiac troponins is included in the universal definitions of acute myocardial infarction and the assay of brain natriuretic peptides may help the doctor in the decision of the rapid rule-in or rule-out of acute heart failure when a patient presents as an emergency with symptoms reminiscent of heart failure, such as dyspnea. The introduction in recent years of more and more sensitive cardiac troponin assays has clearly shown their advantages in the care of patients with the acute coronary syndrome. One is the earlier recognition of myocardial injury and another is the recognition of significant changes in troponin levels around the diagnostic cut-off levels of the 99th percentile URL i.e. objective sign of myocardial injury, which may stay unrecognized by less sensitive assays. These advantages are important since they
allow lifesaving interventions more rapidly. However, in order to
take full advantage of these assay improvements the time
between blood sampling, “vein”, to the actual decision made
by the doctor, “brain”, should be kept at a minimum. In most
hospitals the vein-to-brain time is 1-2 h if the blood sample is
sent to the laboratory. The development of point-of-care (POC)
assays for cardiac troponins and brain natriuretic peptides to be
used in the emergency room has the potential to reduce the
vein-to-brain time, since these assays can provide results
within 15-20 min. One disadvantage of POC-assays for
troponins, however, is their relative insensitivity, since we
showed that the most popular and wide-spread POC-assays
would miss many patients with adverse outcomes that were
recognized by the sensitive laboratory assays.
Conclusion: There is still a gap between the analytical and
clinical performances of laboratory and POC assays for cardiac
troponins and it is a major challenge to develop POC assays
that match the high sensitive laboratory assays.

EW075
CLINICAL DECISION SUPPORT SYSTEMS, PROCESSES
AND INTEGRATION OF POINT-OF-CARE TESTING IN
ACUTE CARDIAC PATIENTS
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Clinical support systems are crucial in Emergency Medicine,
as decisions often need to be taken under time pressure and
in patients in critical situations. We have therefore developed a
support system (www.emergencystandards.com), open to
professionals, with the aim to improve decisions in Emergency
Medicine.
Our research focuses on the evaluation of fast and frugal
decision trees, a method that has been described to be equal
or even superior to more complicated tools aiming at decision
making in terms of diagnostic reasoning. The diagnostic
challenge in Emergency Medicine is often focusing on unclear
or non-specific presentation where decision trees might help
the speed, timing, and accuracy of establishing an early
working diagnosis in order to give treatment to patients with
serious disease, such as myocardial infarction, at the earliest
timepoint possible. The majority of elderly patients with
myocardial infarction in the previously described BANC cohort
does not present with typical complaints, such as chest pain.
Therefore, a strategy involving broad testing for frequently
occurring problems, such as myocardial infarction is warranted.
We have taken the subset of patients with myocardial infarction
that did not present with typical symptoms in order to
retrospectively analyze the timepoints at which the crucial steps
in diagnosing myocardial infarction took place. In order to
calculate the theoretical benefit of point-of-care testing for
troponins, we have chosen the real lag-time in these patients
as compared to an estimated lag-time, if point-of-care testing
would have been performed. This analysis showed a
hypothetical advantage of point-of-care testing over the
traditional laboratory analysis. We will discuss the advantage of
clinical support systems in Emergency Medicine in general, the
use of fast and frugal decision trees, and the possible advantages of point-of-care testing in situations where decision
support is available and point-of-care is most likely a benefit to
patients.

EW076
A REVOLUTIONARY OPTOMAGNETIC IMMUNOASSAY
TECHNOLOGY FOR POINT-OF-CARE TESTING
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Point-of-care (POC) diagnostics is very demanding in a number
of areas: performance, ease-of-use, and reliability. We are
developing the Minicare handheld instrument designed with a
focus to specifically address these demands. The performance
of a POC system should preferably be comparable with the
quality that can be obtained in the central lab to be a viable
alternative. Not only in terms of sensitivity, but precision is
equally important; in particular with respect to the latter there is
still a gap between the central lab and current POC systems
available. We present an optomagnetic immunoassay
technology based on nanoparticles that are magnetically
actuated and optically detected in a stationary sample fluid.
The dynamic control of nanoparticles by magnetic fields
impacts the key immunoassay process steps, giving
unprecedented speed, assay control and seamless integration
of the total test. The optical detection yields sensitive and
multiplexed assays in a cost-effective disposable cartridge.
Applications are foreseen in the emergency department and
the first application under development is a TnI assay with a
turn-around time of less than 10 min. The system is self-
monitoring and works with a single droplet (25-40 µl) of whole
blood. The results of the test are automatically transferred to
the laboratory information system to ensure a smooth
integration in the clinical workflow.

EW077
MEDICAL AND HEALTH-ECONOMIC CHALLENGES OF
SEPSIS
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Sepsis incidence: According to new data from the Center for
Sepsis Control and Care (CSCC) funded by the Federal
Ministry for Science and Research (BMBF) and located at
Jena University Hospital, a total of 180,311 patients developed
septic conditions in Germany in 2010 (1). Of these, 78,208
patients had sepsis, 81,073 severe sepsis, and 21,708 a septic
shock. Figures are based on the new ICD-10 encodings
introduced by the German Sepsis Society (DSG) in 2007 (1).
The peak age was between 70 and 75 years and 60,199
patients died in hospital. Hospital mortality rate for sepsis was
12 %, for severe sepsis 46.9 % and 60.5 % for septic shock
exceeding data on incidence and mortality reported by the
German Competence Network, SepNet prevalence study in
2004 (2). Given the increasing age of the population, figures
can be expected to continue rising and there is no reason to
believe that there are substantial differences between
European countries. Sepsis costs: A calculation of direct
hospital costs according to the actual costs for a large German
health insurance fund agency revealed that annually
5,822,098,251 US$ are spend for sepsis treatment in German
hospitals. The mean costs per case are 76,048 US$ for
survivors and 67,022 US$ for non-surviving patients.
Furthermore, the indirect costs by premature death are
calculated to an additionally 4,634,718,126 US$ per year.
Sepsis – a call to action: Both the currently being revised
guidelines from the German Septic Society on “Prevention,
diagnosis, treatment and long-term care of sepsis” and the

biochimica clinica, 2013, vol. 37, SS  S71
next congress of Weimar sepsis update 2013, will question issues relating to improving structure and process quality to counter the continuing high mortality. Questions to be answered are: • Where are the deficits in diagnosis and what new therapeutic approaches need to be taken? • Is there an iatrogenic post-therapy-related (adverse event) that we should have paid too little attention to? • Have we underestimated the potential for preventive measures, since about 70% of sepsis patients show a nosocomial infection? • And finally, is there an association of the shorter length of stay in hospitals and the quality of outcomes? What is the long-term survival rate and the health-related quality of life?

EW078 CURRENT CLINICAL PRACTICE IN SEPSIS TESTING
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Background: Sepsis and severe sepsis are increasing in recent years. Early recognition and diagnosis of severe sepsis is a crucial part to improve outcome.

Methods: The current literature has been reviewed with respect to laboratory testing in sepsis. Results: Several laboratory parameters are currently used in patients suspected to suffer from sepsis. Most of these are markers of inflammation and infection. In daily clinical practice clinicians use white blood count, C-reactive protein (CRP) and procalcitonin (PCT). Major advantage of PCT is its early and highly specific increase in response to bacterial infections. In sepsis increased PCT levels can be observed 3-6 hours after infection. Low PCT values (<0.25 µg/L) in patients with clinical signs of infection indicate a low probability for blood culture proof of bacterial infection, whereas elevated PCT values (>0.25 µg/L) seem to correlate with positive blood culture result. PCT levels in sepsis are generally >1-2 µg/L peaking to 10-100 µg/L. In healthy people, plasma PCT concentrations are below <0.05 µg/L. PCT levels are usually low in viral infections, chronic inflammatory disorders or autoimmune processes. PCT has been demonstrated to be the best marker for differentiating patients with sepsis from those with systemic inflammatory reaction not related to infectious cause. Moreover, PCT was shown to be the only laboratory parameter that made a significant contribution to the clinical diagnosis of sepsis. Information obtained from IL-6, IL-8 and CRP had no impact on the clinical diagnosis of sepsis on admission. As the septic infection resolves, PCT reliably returns to values below 0.5 µg/L with a half-life of 24 h. Consequently PCT can be used to monitor the course and prognosis of life-threatening systemic bacterial infections and to tailor the therapeutic interventions more efficiently.

Conclusions: Currently several inflammatory biomarkers are used for sepsis diagnosis. The only biomarker with sufficient specificity, positive and negative predictive value for sepsis diagnosis and prognosis is PCT. C-reactive protein and leukocyte count are too unspecific and cannot reliably differentiate patients with sepsis from those with systemic inflammatory reaction not related to infectious causes.

EW079 FUTURE OUTLOOK OF SEPSIS TESTING
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Nearly 200 markers have been reported in the literature to aid in the diagnosis and management of sepsis. The markers reflect the diversity of the causative agent and response mechanisms that are activated. However, only a small number have demonstrated sufficient potential to warrant further investigation. A brief review will be made of some of these markers and their potential application. A report will be given on a recent study of Procalcitonin, Pancreatic Stone Protein (PSP), solubleCD25 (alpha chain of the IL2 receptor) and interleukin 6 with comments on how they could aid in the detection of sepsis and severe sepsis/septic shock. Plasma levels of the biomarkers, PCT and selected inflammatory cytokines were measured in samples taken from 219 patients during the first 6 hours of admission to intensive or high dependency care. Patients with a systemic inflammatory response were categorised as having a non-infective aetiology or sepsis, with or without markers of severity, using standard diagnostic criteria. Both PSP and sCD25 performed well as biomarkers of sepsis irrespective of severity of illness. For both markers the Area Under the Receiver Operating Curve (AUC) was greater than 0.9; PSP 0.927 (0.887-0.968) and CD25 0.902 (0.854-0.949). Procalcitonin and IL6 also performed well as markers of sepsis; PCT 0.840 (0.778-0.901) and IL6 0.805 (0.739–0.870). Levels of both PSP and PCT reflected severity of illness and both markers performed well in differentiating patients with severe sepsis from severely ill patients with a non-infective systemic inflammatory response; AUCs 0.955 (0.909-1) and 0.837 (0.732-0.941) respectively. Although levels of sCD25 did not correlate with severity, addition of sCD25 to either PCT or PSP in a multivariate model improved the diagnostic accuracy of either marker alone. Studies in 3 hospital emergency room departments are ongoing to demonstrate if any of these markers are efficacious in the early detection of sepsis.

EW080 DIAGNOSTICS OF AUTOIMMUNE DISEASES USING INDIRECT IMMUNOFLUORESCENCE TESTS FOR DETECTION OF ANTINUCLEAR ANTIBODIES: 60 YEARS OLD AND IT DOES NOT SHOW
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The indirect immunofluorescence test certainly has withstood the test of time, still being used after the introduction in 1957. Though a number of modifications have been added since its first presentation the same simple principle is used. The cell substrate nowadays consists of thoroughly tested and reproducibly grown cell lines such as human laryngeal carcinoma HEP-2 cells) attached to glass slides, sometimes supplemented by cryosections of animal tissues. Substrate is fixed to ascertain access of patient serum immunoglobulins into the various compartments of the cell, and fluoresceininated anti-immunoglobulin is added to allow binding to and excitation of fluorescence pattern in a fluorescence microscope. The technique is somewhat labour intensive and demands visual expertise of the microscopist interpreting the results. International committees on standardization of ANA detection as well as European and American rheumatology organizations recommend that the IIF ANA test using HEP-2 cells be used as the primary screening method for ANA detection. The ANA test today includes detection of autoantibodies to cytoplasmic as well as nuclear structures, these antibodies being found at a clinically relevant level (titer). The test should focus only on IgG antibodies by use of an IgG (Fcγ chain) specific conjugate. Today presence of IgG ANA is used as support for or even classification criteria for certain systemic rheumatic diseases (SRD). A positive ANA result is used as an initial stepping stone for detecting the specific
autoantibodies underlying the IIF reaction using other methods e.g. enzyme immuno-assay, micro-array or addressable laser bead immuno-assays. Until recently no unified nomenclature for HEp-2 cell IIF reaction patterns existed, but collaboration between expert diagnostic laboratories in the CANTOR project supported by the European Commission has led to the proposal on a contemporary IIF HEp-2 cell nomenclature. Details of this proposal will be presented in the symposium.

**EW081**

**THE USE OF DIFFERENT TECHNOLOGIES FOR AUTOMATED DETECTION AND CLASSIFICATION OF ANTI-NUCLEAR ANTIBODIES**

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Background: The Zenit G-Sight system (Menarini Diagnostics, Italy) is a motorized microscope for the screening of antinuclear antibodies (ANA) on HEp-2 cells. The system is capable to discriminate between positive/negative samples, to measure fluorescence intensity (probability index), and interpret and classify 5 fluorescence pattern (homogeneous, nucleolar, speckled, centromeric and mitochondrial).

Methods: We evaluated the Zenit G-Sight (I-Sight IFA) on 134 selected sera. 98 of these sera were positive and 36 were negative by the conventional immunofluorescence method (IIF) on HEp-2 cells (HEp-2 Zenit IMMCO). The 98 positive sera showed the following patterns: 17 homogeneous, 24 speckled, 22 cytoplasmic, 3 dense fine speckled, 3 centromeric, 1 CENP-F, 5 nuclear matrix, 5 nuclear membrane, 2 midbody, 1 NuMa, 5 multiple nuclear dots, 5 nucleolar, 4 PCNA. 14 had a titer of 1:80, 13 of 1:160, 20 of 1:320, 20 of 1:640 and 39 of ≥1:1280.

Results: Positive/negative concordance between conventional microscopic results and the Zenit G-Sight reading was 92.5%. 83 sera agreed for positivity; 27 for negativity; 14 IIF-positive and 7 IIF-negative were in the Zenit G-Sight grey zone; 1 IIF-positive serum was negative with the G-Sight and 2 IIF-negative sera were positive with the G-Sight. Agreement was 100% for the homogeneous pattern, 77.1% for the speckled pattern, 100% for nucleolar and centromeric patterns, and 75% for the mitochondrial pattern. In 36 IIF-positive sera, Zenit G-Sight did not assign any pattern, despite a positive probability index. Correlation between titer and G-Sight fluorescence intensity was excellent for sera with speckled, homogeneous, nuclear membrane, centromeric and nucleolar patterns, whereas a non-linear correlation was observed in positive sera with other patterns.

Conclusion: The Zenit G-Sight system is highly accurate in picking out ANA-positive sera; the use of this instrument in clinical laboratories is able to standardize the first step of ANA detection; Zenit G-Sight gives good performance in discriminating 5 patterns but is not yet able to replace the pathologist in this interpretative phase. The fluorescence index must be related to the type of pattern in order to express marker concentration correctly.

**EW082**

**THE CLINICAL RELEVANCE OF AUTOMATED MODERN METHODS FOR PLATELET COUNTING IN 2013**

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Impedance Platelet (PLT) counting methods still have limitations. Cell size analysis cannot discriminate PLT from other similarly sized particles. More recently fluorescence methods have been introduced for PLT counting on haematology analysers. International Council for Standardization in Haematology (ICSH) introduced a reference method for PLT counting which utilises monoclonal antibodies to PLT surface antigens. This enables the calibration of cell counters, to assign values to calibrators, and to obtain PLT counts in a variety of pathological samples. The Sysmex XN performs fluorescence analysis of PLT (PLT-F) using a novel marker. Algorithms are designed for thrombocytopenic samples for an accurate PLT count and immature PLT fraction (IPF) by prolonged counting sequence and elimination of interference from non-platelet particles or platelet abnormalities. The PLT-F channel can be selected for testing or only used as a reflex test if there are abnormalities or if the PLT count is below a pre-set limit. PLT counts and IPF were evaluated on 390 samples compared to the XE-2100. PLT counts were compared to the ICSH flow cytometric method on samples, range 1-1728 x 10^9/l (n=185), 67 samples were at, or below, the PLT transfusion threshold. Carryover, linearity, precision and stability for PLT and IPF were also investigated according to ICSH guidelines. Results for all samples were similar between instruments for the impedance method compared to the reference method, XE-2100 R^2 0.986, XN 0.972. The PLT-F method was superior to the XE-2100 optical method when compared to the reference method on samples with a PLT count of <20 x 10^9/l, XE-2100 R^2 0.500 and XN 0.875. Discrepancies on 8 patients were demonstrated for IPF between the XE-2100 and XN (low on the XN and high on the XE-2100). From the diagnosis of the 8 patients it may be results were more correct on the XN. 6 patients were undergoing chemotherapy and 2 had aplastic anaemia, all had low PLT counts. Results for carryover, linearity, precision and stability were good. The evaluation showed excellent correlation of PLT-F count with the flow cytometric reference method for all samples, and an improved accuracy in thrombocytopenic samples, especially at platelet transfusion thresholds of 10-30 x 10^9/l.

**EW083**

**MANAGING THROMBOCYTOPENIC SAMPLES IN THE ROUTINE WORKFLOW**

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When dealing with thrombocytopenic patients, the clinical need in daily routine workflow is to determine whether the patient is truly suffering from thrombocytopenia, and to monitor the recovery success of thrombopoiesis in patients undergoing therapy. The Sysmex XN-Series counts platelets using two different methods: impedance counting and dedicated fluorescence analysis. Impedance counting is suitable for routine platelet measurement using the principle of differentiation by cell volume. In contrast, fluorescent platelet counting using a prolonged counting sequence and fluorescent RNA labeling can solve possible interferences derived from non-platelet particles or platelet abnormalities in thrombocytopenic samples. Managing thrombocytopenic samples in routine workflows is challenging for several reasons: i) in the presence of interfering particles one may have to use multiple methodologies for platelet counting. ii) One has to report an accurate “delta value” for platelets – the difference compared to previous results. This is only feasible when iii) the platelet results derive from the same methodology. These issues should not negatively influence iv) turnaround time and v) costs. To make this possible, one needs an intelligent set of algorithms embedded in a work area manager. This optimizes the workflow of thrombocytopenic...
samples in routine settings. We tested the Sysmex evaluation software, which includes an algorithm set for managing the platelet workflow. The study consisted of two parts: the first monitored the recovery success of thrombopoiesis. One hundred thrombocytopenic patients from the surgical intensive care unit (ICU) were examined over a period of five days. In the second part, current best practice was compared with the new algorithm by including 10,000 routine samples from around 1,500 patients to monitor turnaround time and costs.

EW084
HBA1C STANDARDIZATION IS ESSENTIAL FOR DIAGNOSIS AND MONITORING OF DIABETES
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If we are to limit the global effect of diabetes and its financial burden then a globally accepted standardization system for HbA1c measurement is vital. This will allow an international approach to the formulation and implementation on guidelines for diagnosis and monitoring of diabetes. The lack of international standardization of HbA1c resulted in several countries developing national standardization programs. The most widely recognized being the National Glycohemoglobin Standardization Program (NGSP); utilizing a High Performance Liquid Chromatography (HPLC) system which Secondary Reference Laboratories these in turn standardize Clinical methods. There are two main concerns with the NGSP solution: results reported are not true HbA1c concentrations, but the best estimates that analyzer technology from the 1980s could deliver. Secondly there is no primary reference material to allow true calibration of the HPLC system. Implementation of this program resulted in laboratories reporting similar values with the same sample; but, the similar values were not the true HbA1c value. This was also the case with the Swedish and Japanese standardization programs; these were also based on optimized HPLC methods (referred to as Designated Comparison Methods [DCMs]). These national initiatives were important steps toward improvement of the comparability of HbA1c test results; but national standardization programs based on different DCMs cannot replace uniform worldwide standardization anchored on a metrological sound international reference measurement system. To address the global issue the IFCC WG on HbA1c standardization has developed a complete reference measurement system. The system fulfils the concept of a Metrological Reference Measurement Procedure; thus providing clinical laboratories with calibration traceable to primary reference material with known uncertainty, and that uncertainty kept to a minimum due to the processes involved. But there remains a divide on the reporting of results; although much of Europe has standardized on mmol/mol as the reporting unit, this SI units has not yet been universally accepted. If global comparability is to be achieved, then the recommendations of the consensus statement on HbA1c reporting must be adopted.

EW085
QUALITY OF HBA1C TESTING: EXPERIENCE OF AN IFCC REFERENCE LABORATORY
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Hemoglobin A1c (HbA1c) point-of-care (POC) instruments are widely used to provide rapid turnaround results in diabetic care centers. Most of the POC instruments are NGSP certified but the analytical performance in daily use is not really known due to the fact that most of the users of HbA1c POC instruments are not members of external quality schemes. The results shown in the article: “Six of Eight Hemoglobin A1c Point-of-Care Instruments Do Not Meet the General Accepted Analytical Performance Criteria” (Clin Chem 2010;56:44-52), clearly showed that manufacturers need to improve their methods. Most of the manufacturers took this message seriously and, indeed, re-evaluation of these methods showed improved analytical performance. Also new HbA1c POC instruments entered the market and some of these methods are even better than most of the laboratory based methods. Given these results, the question arises if it is correct to exclude all HbA1c POC instruments for the diagnosis of diabetes as recommended by the American Diabetes Association (ADA). In our lab (European Reference Laboratory for HbA1c at the Isala klinieken in Zwolle, the Netherlands) the analytical performance of different HbA1c methods (POC and laboratory based methods) was investigated, using certified CLSI protocols and results of external quality schemes. Furthermore, the interpretation of HbA1c values among different health care professionals was investigated which produced, in combination with the outcome of the evaluation studies, remarkable results. In this presentation I will focus on the results of these studies.

EW086
BETWEEN METHOD VARIABILITY IN LABORATORY MEDICINE: "THE GOOD, THE BAD, AND THE UGLY"
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Background: The primary responsibility of a laboratory medicine specialist is the delivery of a high quality analytical and interpretive service. Patients are increasingly mobile and so liable to be investigated at centres and laboratories that use different methods for the same measurand. Between method differences in results can impact on patient safety, reduce the effectiveness of clinical practice guidelines and undermine the reputation of the clinical laboratory in the eyes of the user and the patient. Therefore, the laboratory medicine specialist has a secondary responsibility to facilitate a reduction in between method variability.

Methods: A survey was conducted of External Quality Assessment (EQA) schemes in the UK. This was used to estimate the number of measurands commonly available across the spectrum of laboratory medicine. This outcome was compared with the database of the Joint Committee for Traceability in Laboratory Medicine (JCTLM), which contains listings of reference materials, reference measurement procedures and reference laboratory networks. Method performance characteristics were assessed for selected measurands from clinical chemistry, haematology and microbiology and these were compared with the recommended use of these measurands in clinical practice guidelines.

Results: Only a small percentage of the measurands commonly available in laboratory medicine have been standardised or have the current potential to be standardised. Cholesterol and haemoglobin are examples of measurands that have been standardised and the between method variability is correspondingly small giving credibility to the recommendations in clinical practice guidelines. Parathyroid hormone and haemoglobin A2 are examples of measurands where there is no current method standardisation and the high between method variability compromises the validity of clinical practice guidelines.

Conclusions: It is not currently possible to standardise many
of the measurands currently available in laboratory medicine. High between method variability compromises patient safety. Therefore, a more pragmatic approach is required that will facilitate method harmonisation. This must be a collaborative effort between the laboratory medicine specialist and the diagnostics industry.

EW087
FROM CHAOS TO ORDER: THE ROLE OF HARMONIZATION
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Background: Results between different clinical laboratory measurement procedures should be equivalent, within clinically meaningful limits, to enable optimal use of clinical guidelines for disease diagnosis and patient management. When laboratory test results are neither standardized nor harmonized, a different numeric result may be obtained for the same clinical sample. Unfortunately, some guidelines base decisions on test results from a specific laboratory measurement procedure without considering the possibility or likelihood of differences between various procedures. When this happens, aggregation of data from different clinical research investigations and development of appropriate clinical practice guidelines will be flawed. A lack of recognition that results may not be equivalent when measured with different procedures can lead to erroneous clinical, financial, regulatory, or technical decisions.

Methods: Standardization of clinical laboratory procedures has been accomplished for a number of measurands for which primary (pure substance) reference materials exist and/or reference measurement procedures have been developed. However, the harmonization of clinical laboratory procedures for measurands that do not have reference measurement procedures has been problematic owing to inadequate definition of the measurand, inadequate analytical specificity for the measurand, inadequate attention to the commutability of reference materials, and lack of a systematic approach for harmonization.

Conclusions: To address these problems, an International Consortium for Harmonization of Clinical Laboratory Results will enable a systematic approach for identification and prioritization of measurands to be harmonized based on clinical importance and technical feasibility, and for management of the technical implementation of a harmonization process for a specific measurand.

EW088
IMPORTANCE OF SERUM FREE LIGHT CHAIN MEASUREMENTS IN AL AMYLOIDOSIS
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AL amyloidosis is caused by aberrant folding of monoclonal immunoglobulin free light chains (FLC) which leads to the aggregation and deposition of these light chains in organs and tissues. Historically, the presence of monoclonal light chains was identified using serum or urine protein electrophoresis (SPE, UPE), however <70% of patients detectable monoclonal proteins and less still are quantifiable by these methods. The introduction of the Freelite® immunoassay (for the quantification of serum FLC) changed the diagnostic and monitoring paradigm for AL amyloidosis patients. It is the single most sensitive test for detecting abnormal FLC’s and whilst not diagnostic (which relies upon tissue biopsy) of AL amyloidosis, patients frequently present with abnormal FLC kappa/lambda ratios.

In a retrospective analysis of 262 patients 98% had abnormal FLC ratios (by contrast only 3% had serum FLC concentrations sufficiently high for SPE quantification). The prognosis of patients with AL amyloidosis is often determined by cardiac involvement – patients with cardiac involvement having significantly poorer outcomes than patients with other end organ damage. A prognostic model utilising cardiac biomarkers troponin-T (cTnT) and N-terminal pro-B type peptide (NT-ProBNP) is routinely used. However, this model fails to account for the underlying light chain burden and a recent update utilises elevated FLC, cTnT and NT-ProBNP to allow for accurate patient stratification. Such prognostic models help risk stratify patients for appropriate therapy including stem cell transplantation. The importance of FLC measurements in this disease is now well established and has been recently validated in a large international collaborative effort to define response criteria in using reductions in the serum FLC components. Achieving a dFLC (difference in the involved and uninvolved serum FLC) of <40 mg/L is now the minimum goal of therapy in AL amyloidosis.

To date all guidelines and utilities for FLC measurements have utilised the Freelite assay. More recently a monoclonal antibody based assay has been introduced, preliminary assessments of this assay suggest international response criteria cannot be applied and further work is required to evaluate its clinical utility.

EW089
THE IMPORTANCE OF SERUM FREE LIGHT CHAIN MEASUREMENT IN KIDNEY DISEASE
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Kidney disease is a serious complication of multiple myeloma and other monoclonal diseases. Serum free light chain (sFLC) measurements have a central role: (i) in diagnostic algorithms for patient with newly diagnosed acute kidney injury (AKI) or chronic kidney disease (CKD); (ii) as early determinants of clinical outcomes, particularly in patients with dialysis dependent AKI. It is now clear that rapid and accurate identification of the nature and burden of the monoclonal disease and confirmation of the role of the disease in kidney injury is a crucial determinant of patient outcome. This allows the prompt commencement of disease specific treatment and protects the kidneys against the profound toxicity of some light chain clones. This field is rapidly developing: recent insights include; the recognition of light chain monoclonal gammopathy of uncertain significance (LC-MGUS) as an entity; the developing concept of monoclonal gammopathy of renal significance (MGORS), clinical studies evaluating the direct removal of sFLC in MM and AKI, the impact of kidney impairment on the ‘normal’ range of sFLC and the capacity of polyclonal sFLC to risk stratify for mortality and other . Serum FLC measurements had and have a crucial role in these and other recent advances. The assay is now established as a standard of care of laboratory assessment in the diagnosis, monitoring and management of kidney involvement in the setting of monoclonal disease.
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EW090
ROLE OF HEVYLITE FROM A LABORATORY PERSPECTIVE
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Background: Identification and quantification of monoclonal immunoglobulins by serum protein electrophoresis (SPEP) and immunofixation (IFE) are fundamental to diagnosis and monitoring of multiple myeloma (MM) and other plasma cell disorders. The biochemical nature of some intact immunoglobulins can present significant challenges to the laboratory in the accurate quantification by SPEP either because of their co-migration with other serum proteins (seen in up to 60% of IgA monoclonal protein) or concentrations <10 g/L which leads to substantial imprecision. Methods: In 2009, 3 paired immunoassays (Hevylite-HLC) identifying the Igk/Igλ variants for IgG, IgA and IgM isotypes became available. These assays are targeted against conserved, junctional epitopes between the light and heavy chain. Calculation of an Igk/Igλ ratio (as with the serum free light chain (FLC) Igk/Igλ ratio) can be used to detect clonality. In a series of MM monitoring patient samples we compared commonly utilized methods for detection of monoclonal protein, including HLC chain determination. Results: While concordant results were achieved between HLC and SPEP monoclonal protein determination in the abundance of clonal protein, IgA HLC ratios to have a greater sensitivity than SPE at low concentrations. In IgG patients the two assays were broadly concordant, thus Hevylite may alleviate issues associated with accurate quantification. Additionally, substantial, yet incomplete, concordance was observed between IFE, SPEP, FLC and HLC monoclonal protein detection. Conclusions: The clinical impact of the HLC assay is still incompletely understood. Further studies delving into the utility of HLC in diagnosis, monitoring, and prognostic application continue across the globe. Published studies looking at MM patients indicate HLC ratios identified all patients. However in a large MGUS study the HLC ratio sensitivity did not match that of IFE (97%, IgA, 80% IgM and only 56% of IgG patients). Intriguingly, the suppression of the uninvolved Hevylite pair had a strong impact on outcome. In MM this suppression may precede relapse, prior to measurable increases in monoclonal immunoglobin production; therefore HLC determinations may represent beguiling tools for patient monitoring.

EW091
THE IMPACT OF PROCALCITONIN ON CLINICAL DECISION MAKING: WHY THE CLINICAL LABORATORY SHOULD OFFER PROCALCITONIN TESTING
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The limitations of clinical signs and microbial techniques for the diagnosis of bacterial infections are eminent. Procalcitonin, a novel biomarker for infection, is released in multiple body tissues in response to bacterial infections via direct stimulation of bacterial cytokines. The use of Procalcitonin provides a promising novel approach to better diagnose infection and for antibiotic stewardship. Today, the concept of guided stewardship has been evaluated in fifteen randomized controlled trials including >4000 patients for efficacy and safety. PCT guidance lowered antibiotic prescription rates by 65% in primary care patients, 35% in the emergency department setting, and almost 30% in the critical care setting. A recent meta-analysis found no difference in mortality in procalcitonin group patients compared to control patients. However, a lower risk for treatment failure in procalcitonin-guided patients compared to control patients (19.1% vs 21.9%, adjusted Odds ratio 0.82, 95%CI, 0.71 to 0.97) was found. Today, there is strong evidence supporting the use of procalcitonin in respiratory infections and for sepsis in the intensive care unit. For other infections, disease and setting specific cut-off ranges must be validated and proposed and intervention studies conducted to tackle the existing vicious cycle of diagnostic uncertainty, antibiotic overuse and emerging multi-resistance.

EW092
CT-proAVP (COPEPTIN) IN THE DIFFERENTIAL DIAGNOSIS OFT HE POLYURIA-POLYDIPSIA SYNDROME
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Arginine vasopressin (AVP) is a key hormone for fluid and electrolyte regulation in the human body. Despite its clinical relevance in maintaining fluid balance and vascular tone, measurement of the bioactive mature AVP is difficult and subject to preanalytical errors. Copeptin, a 39-amino acid glycopeptide that comprises the C-terminal part of the AVP precursor (CT-proAVP), was found to be a stable and sensitive surrogate marker for AVP release that can be quickly and easily measured. The predictive and diagnostic relevance of copeptin has been demonstrated for various clinical indications, including the diagnosis of hyponatremia and the monitoring of sepsis and cardiovascular diseases. Recent data strongly suggest that copeptin may be an extremely useful and specific diagnostic marker also for the differential diagnosis of diabetes insipidus. The water deprivation test is currently the diagnostic routine procedure for differentiation of hypotonic polyuria, with either direct or indirect measurement of the AVP activity. But the test protocol is exhausting and its results offer only an extremely low diagnostic accuracy, especially in the identification of patients with central and nephrogenic forms of diabetes insipidus. Additional direct copeptin measurement during the dehydration test offers a much more accurate diagnostic differentiation. More importantly, even baseline copeptin measurement, without a functional test design, is able to reliably identify patients with a nephrogenic and a severe central diabetes insipidus; whereas the copeptin increase in response to an appropriate osmotic stimulation is able to even reliably differentiate between the two most difficult to diagnose forms of hypotonic polyuria, which is the primary polydipsia and a mild form of central diabetes insipidus. The pros and cons of the current diagnostic test standard in polyuria polydipsia syndrome will be discussed, and a concept of possible future diagnostic simplification and improvement in diagnostic accuracy will be illustrated based on the current state of research.

EW093
FROM THEORY TO PRACTICE: SAVING COSTS BY ADDING BIOMARKERS TO INFECTION MANAGEMENT
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Severe infections cause medical and economic problems in hospitals. While the importance of treating infectious patients
with the right antibiotic, as early as possible is well known and excellently investigated, the economical side of story is yet to be told. Especially the right moment to finish antibiotic therapy is an economical factor that cannot be underestimated. Not only does the termination of treatment save costs for antibiotics, it also contributes to a shorter length of stay in hospital and last but not least is an important part in the big puzzle of limiting antibiotic resistance. Using procalcitonin (PCT) as a biomarker to detect and to monitor bacterial infections is widely accepted in the area of sepsis and severe airway infections such as pneumonia. It was shown recently in randomized controlled trials, that an optimized monitoring algorithm using PCT to manage antibiotic therapy, can realize savings in antibiotic use by reducing the initiation as well as the duration of antibiotic treatment and intensive care unit days (ICU) without affecting clinical outcome. Applying these savings to medical data from 16 German hospitals resulted in savings of 886 € per ICU patient with sepsis. However the savings were calculated via a medical economic model and derived from a meta-analysis of several trials. Also cost economic benefits of PCT-algorithms were proven in clinical trials, we started the implementation of the new algorithm from mid 2012 in ICUs in three German hospitals to test its benefit in real life clinical settings. ICU staff was trained for the PCT algorithm, cases were peer reviewed and treatment data collected. Additionally ICUD were collected as well as the adherence to the PCT-algorithm. Analysis will be done after 12 months with an interim analysis after 6 months. Results will be statistically analyzed via a comparison with historical control groups before introduction of the new PCT-based treatment algorithm. It will be investigated, whether the introduction and adherence to the PCT-algorithm in real-life ICU settings will confirm the results being computed in the medical-economic model.

EW094
CONDITIONS FOR AN EFFECTIVE COLORECTAL CANCER SCREENING PROGRAMME: THE FRENCH EXPERIENCE
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Colorectal cancer (CRC) fulfills the conditions defined for mass screening. Currently the simplest screening method for CRC is periodic stool testing for occult blood. An organized screening program is being carried out in France (covering the whole country since 2009). Subjects aged 50 to 74 are invited to perform a guaiac fecal occult blood test every two years. The practical organization of CRC screening is decentralized. At the level of the department a coordinating center is in charge of organizing breast and CRC screening. Each screening round begin by sending an information letter to each invited subject. During the first 6 months of the screening round GPs offer the test free of charge to eligible patients seen at their office. For subjects who did not consult their GP during the medical phase, the coordination center subsequently mailed the test. However, because of the limits of guaiac-based tests, there are no longer the preferred tests for population-based screening programs. Studies conducted in France and in other European countries indicated that immunochromatochemical fecal occult blood tests outperform the guaiac tests and are associated to greater participation, a key element in the effectiveness of CRC screening. They allow the detection rate of CRC to be doubled and a 3-4 fold increase in the detection of advanced adenoma. The Ministry of Health has announced in March 2012 the shift from guaiac to immunochromatochemical tests, without deciding when it will occur. It will be based on a biennial one-stool immunochromatochemical test. The choice of the test will be based on test performances, the ease of sample collection and test interpretation and cost-effectiveness analyses.

EW095
SCIENTIFIC AND TECHNICAL ATTRIBUTES SUPPORTING WIDE ADOPTION OF FECAL IMMUNOCHEMICAL TESTS
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Colorectal cancer is the third most common cause of cancer deaths worldwide and the second most common cause in Europe. Whilst biochemical tumour markers largely play second fiddle in frontline cancer diagnosis, the clinical utility of faecal occult blood testing (FOBt) has finally been recognised after decades of making an uncertain contribution to the process of diagnosing colorectal cancer. During the 1990s, four randomised controlled trials demonstrated the clinical efficacy of FOBt as a screening tool to reduce mortality from colorectal cancer in an average risk population. England was one of several countries that adopted guaiac-based FOBt for population screening and between 2006 and October 2012, following 15.5 million invitations, had identified and treated over 14,000 cancers and removed 42,600 advanced adenomas. The diagnostic attributes of guaiac were recognised in 1862 but detection of haem using its peroxidase properties exposes it to interference and limits analytical specificity. The introduction of faecal immunochemical tests for haemoglobin (FIT) to detect human globin offers major analytical and clinical advantages and FIT was adopted two years ago by the European Union in revised guidelines on population screening for colorectal cancer. FIT is simpler to use and it enables automated, instrument-based analysis, which is essential for large throughput population-based screening. The numeric haemoglobin (Hb) concentration allows selection of a positive/negative cut-off level tailored to available endoscopic resources. FIT technology brings both opportunities and challenges. Globin is more susceptible than haem to accelerated denaturation from extended periods at high temperature, the reliability of quantitative measurement is dependent upon reproducibility of sampling across an expanding range of devices and the faecal matrix presents challenges for internal and external quality monitoring. Can the international biochemistry community guarantee consistent, high quality, professionally-led services for this major public health initiative? As this ‘Cinderella’ analyte moves ‘centre stage’ we are challenged to develop safe systems of analysis, adequate sample preservatives, traceable calibration and internationally agreed units of reporting.

EW096
HEMOGLOBIN STABILITY IN FECAL IMMUNOCHEMICAL TEST DEVICES: BETWEEN EXPECTATIONS AND REALITY
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Background: The assessment of faecal occult blood (FOB) is a fundamental tool for the early diagnosis in colon-rectal cancer even if, the stability of Faecal Haemoglobin (HbF) represents a major criticism because different storage temperatures as well as different time-delays between sampling and analysis may significantly affect the accuracy of the test. Some different devices have been commercially proposed with the aim to guarantee a better stability of faecal
samples as well as an improved analytical performance, such as FOB Gold Tube Screen and FOB Gold Tube NG with storage buffer H (BH) and N (BN) (Sentinel CH, Milan, Italy) recently evaluated in our laboratory.

Methods: Stability tests were performed on three real positive stool samples (HbF= 96.5, 227.8 and 649.5 ng/mL) collected with FOB Gold Tube Screen and on other three different samples (HbF=131.9, 277.2 e 632.3 ng/mL) collected with FOB Gold NG, both stored for 15 days at 4, 23, 30 e 37°C. One aliquot of each pool and of each temperature has been tested in triplicate at 0, 1, 3, 5, 8, 11, 14 and 15 days. All determinations have been performed using Cobas c6000 analyzers (Roche Diagnostics, MI).

Results: The HbF shows, as expected, a different degradation percentage according to time and storage temperature being after 5 days about 4% at 4 °C , about 10% at 23 °C and more than 30% at 30 °C. Similar results have been obtained after 8 days of storage in the same conditions. The analytical performance evaluated for BH storage buffer, show satisfactory results in terms of LOQ=18, ng/mL; total imprecision=6.5-9.41%; linearity (slope=0.99 intercept=1.26, range: 74.8-800.8 ng/mL) also in samples with haemoglobin variants (Hbs, Hbc e Hba1c).

Conclusions: The results of our study evidence the criticism related to the storage condition for the accuracy of HbF test. The comparison of the described storage buffers shows that BN assures an improved and adequate stability of HbF, but any of buffers evaluated assure the preservation at temperatures >23 °C. These preanalytical aspects should be carefully considered in the organization of screening program for the assessment of fecal occult blood.

EW097 NEW APPROACHES IN BODY FLUID MANAGEMENT

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Accurate and rapid analysis of white blood cells (WBC) and red blood cells (RBC) in body fluids can provide clinicians with useful information related to diagnostics and treatment effect. As matters stand, manual microscopy is considered the “gold standard” for counting WBC and RBC in body fluids, but it is time-consuming, with significant imprecision and inter-observer variability. As a consequence, many laboratories have replaced conventional manual differential counting with automated haematology analysers for initial screening and detection of cellular abnormalities so as to standardise their routine procedure. In this study, we evaluated the body fluid (BF) module of the XN-1000 automated haematology analyser for counting WBC and RBC and compared it with the manual reference methods. A total of 187 BF samples (73 cerebrospinal fluids (CSF), 48 continuous ambulatory peritoneal dialysis fluids (CAPD), 46 ascites fluids, and 20 pleural fluids) were collected randomly. All samples were analysed within 1 hour of entering the laboratory. Samples were first measured on the automated XN-1000, followed by manual microscopy. By setting manual microscopy as the reference method, total WBC and RBC were counted using the Fuchs-Rosenthal chamber followed by WBC differentiation into mononuclear cells (MN) and polymorphonuclear cells (PMN) by May-Grünwald Giemsa-stained cytospin slides. We also assessed precision, carry-over and linearity. Good agreement was found for counting WBC (y=1.06 x+0.09, n=67, R^2=0.96) and MN (y=1.04 x- 0.01, n=40, R^2=0.93) in CSF. However, the XN-1000 systematically counted more PMN (y=1.48 x+ 0.18, n=40, R^2=0.99) compared to manual microscopy. Excellent correlation for RBC > 1×10^9/L (y=0.99x + 116.56, n=26, R^2=0.99) in CSF was found. For other fluids (CAPD, ascites and pleural fluid) excellent agreement was found for counting WBC, MN, PMN and RBC. The lower limit of quantification for WBC was defined at 5×10^6/L. Linearity was excellent for both the WBC (R^2=0.99) and RBC (R^2=0.99) and carry-over never exceeded 0.05%. In total, we concluded that the BF module on the XN-1000 is a suitable tool for fast and accurate quantification of WBC (differential) and RBC counts in CSF and other body fluids in a diagnostic setting.

EW098 THE CLINICAL USEFULNESS OF AUTOMATED MEASUREMENT OF BODY FLUIDS

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Normal cerebrospinal fluid (CSF) contains no or very few white blood cells (WBC). An increase in WBC can be an indication of several diseases, including meningitis, encephalitis, malignancy or other neurological diseases. The total WBC count and the WBC differentiation into mononuclear cells (MN) and polymorphonuclear cells (PMN) can help clinicians with useful diagnostic and treatment effect information. CSF analysis is also useful for detecting or monitoring the response to intrathecal chemotherapy for detecting minimal residual disease (MRD) in patients with leukaemia. A high red blood cell (RBC) count indicates a cerebral haemorrhage or traumatic spinal tap. To answer whether it is fresh or older bleeding, finding certain macrophages such as erythropagocytes or haematoidin-containing macrophages can be helpful. Since erythropagocytes do not occur earlier than 12-18 hours after a bleeding, and haematoidin-containing macrophages are usually not found earlier than two weeks after the incidence, the appearance of those cells strongly points to a cerebral bleeding rather than a traumatic tap. Microscopic analysis of CSF is known as the gold standard for total WBC and RBC counting, and WBC differential. This procedure has several disadvantages, however: low precision, high cost, delayed results, the need for skilled personnel, and inter-operator variability. The obvious answer to these problems could be to introduce advanced automated analysis methods. They could reduce inter-operator variability and improve turnaround time and precision. However, in contrast to blood, the automated haemocytometric analysis of body fluids is a relatively unexplored area of research. This prospective clinical research study was designed to improve research software of the Sysmex XN-Series automated haematology analyser, which support the clinical questions: i) Is an infection or inflammation ongoing?; ii) Is the infection bacterial or viral?; iii) Did fresh bleeding or old bleeding occur? This evaluation analysis mode does not require pretreatment of the samples (dilution, enzyme digestion) and provides a full WBC count and WBC differential, an RBC count, and research parameters for detecting tumour cells and activated WBC.

EW099 QUANTIFICATION OF BTP FOR DIAGNOSIS OF CEREBROSPINAL FLUID LEAKAGE

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Background: Complications, morbidity, and mortality are high after trauma with injury to the meningeal space and leakage of...
FUNCTION IN DIALYSIS PATIENTS

SERUM BTP AS A MARKER OF RESIDUAL RENAL FUNCTION IN DIALYSIS PATIENTS

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Background: Survival on dialysis remains poor, with a 5-year mortality exceeding 60%. Kidney function in patients on dialysis, also known as residual kidney function (RKF), is associated with better survival, but there are no simple methods for assessing RKF. Beta-trace protein (BTP) is a novel endogenous filtration marker of kidney function. BTP is highly correlated with measured glomerular filtration rate (GFR). BTP, unlike urea and creatinine, is not removed during dialysis and may allow assessment of RKF, similar to serum creatinine in patients not on dialysis. The objective of this presentation is to discuss the role of serum BTP for assessment of RKF in dialysis patients.

Methods: We will present the results of two studies in this presentation. In the first study, we measured serum BTP in baseline samples from 503 participants of a U.S. national prospective cohort study of incident dialysis patients with enrolment during 1995–1998 and follow-up until 2004. Outcomes were all-cause and cardiovascular disease (CVD) mortality analysed using Cox regression adjusted for demographic, clinical, and treatment factors. In the second study, which is on-going, we are measuring GFR in dialysis patients and correlating it with serum BTP.

Results: In our first study, serum BTP levels were higher in individuals with no urine output at baseline compared with those with urine output (0.02±0.01 vs. 7.6±3.1 mg/L, P<0.001). There were 321 deaths (159 from CVD) during follow-up (median 3.3 years). Higher BTP levels were associated with higher risk of mortality. The adjusted hazard ratio (HR) and 95% confidence interval (95% CI) for all-cause mortality per doubling of serum BTP was 1.36 (1.09–1.69). Analysed as tertiles, the adjusted HR (95% CI) for all-cause mortality in the middle and highest tertiles compared with the lowest tertile was 0.95 (0.69–1.32) and 1.72 (1.25–2.37). Similar results were noted for CVD mortality. In our second study, preliminary results demonstrate that serum BTP is moderately correlated with measured GFR in dialysis patients; correlation coefficient for BTP is 0.609 compared with 0.407 for creatinine.

Conclusions: The serum level of BTP is associated with RKF and is an independent predictor of death in incident hemodialysis patients.

EW101 CHALLENGES IN HIV INFECTION DIAGNOSIS

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Diagnosis of HIV infection deals with several challenges: virus genetic variability, the epidemic level of the disease, the level of undiagnosed infection and resources involved in laboratory investigations.

HIV shows a high degree of genetic variability; 4 HIV-1 groups (M-P) were described. Group M is divided into 9 subtypes (A-K) and more than 50 circulating recombinant forms (CRFs), have been identified. HIV-2 includes 8 groups (A-H). The geographical distribution of HIV-1/M subtypes and CRFs is worldwide and linked to the epidemic level of the disease. HIV-2 and HIV-1 non-M are limited to West Africa and Western Central Africa, respectively and countries with links to these regions. This complex diversity has implications for diagnosis since false negative results attributed to viral diversity have been reported with serological assays and with some PCR-based commercial assays.

The diagnosis of HIV primary infection is crucial to prevent further transmission, to facilitate clinical management and early treatment, and to avoid viral transmission through blood transfusion. Traditionally, HIV infection is diagnosed on the basis of the detection of antibodies (Ab). Due to the Ab negative early phase, French health authorities mandated the use of HIV Ag/Ab assays able to detect at least 2 p24Ag IU/mL. These assays exhibit an overall high sensitivity for the detection of Ab from individuals infected by different HIV strains. However, even though their analytical sensitivity fulfils the legal requirements, many of them could fail to detect HIV primary infection due to HIV-1 non-B, non-M strains and HIV-2. Access to HIV testing in undiagnosed individuals who can unknowingly transmit infection is essential in resource-limited areas as well as in high-income countries. In these cases, even though globally less sensitive than EIAs, rapid tests which are affordable and easy to use, might improve medical care and reduce transmission of infection.

In conclusion, as the prevalence of HIV genotypes varies geographically and is continuously changing, there is a need to develop diagnostic tools able to detect a large spectrum of HIV polymorphism. Moreover, the high level of undiagnosed infections creates the need of highly sensitive rapid tests.
HIV SERODIAGNOSIS IN 2013
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Due to technical improvements, the reliability of serological laboratory diagnosis of HIV has improved considerably. The recommendation in the UK National Guidelines for HIV testing 2009 is the use of the fourth generation immunassays, called HIV Ag/Ab assay, for the first-line testing of blood in all healthcare settings in order to reduce the window period. In 2010, French health authorities mandated the use of a CE-marked HIV Ag/Ab assay able to detect at least 2 IU/mL of p24 Ag (based on the WHO HIV-1 Ag standard), which is the minimum threshold required by the current European legislation applicable to p24 Ag detection assays. HIV viruses have a high level of genetic diversity. Although HIV-2 is distributed into 8 genotypes, HIV-1 has been classified into 4 groups, M (major), O (outlier), and two non-major and non-outlier (N and P). This diversity can have an impact on the capacity of HIV Ag/Ab assays to equally detect all HIV genotypes. Ten HIV Ag/Ab CE-marked assays for their sensitivity in the detection of p24 Ag of diverse HIV-1 and HIV-2 isolates were evaluated. The Ag p24 limit of detection (LOD) ranged from 0.505 to 1.901 IU/mL with the WHO standard. But this analytical sensitivity obtained is not predictive for the performance of p24 Ag detection of different HIV-1 subtypes since the majority of investigated HIV Ag/Ab assays were not able to detect every HIV-1 strain with an optimal sensitivity. According to the mean LODs of different subtypes obtained, there are three categories of HIV Ag/Ab assays: the first category included those which failed to achieve the minimum required threshold of 2 IU/mL regardless of the genotype; the second gathered those which fulfil the requirements not for all genotypes and the third category comprised all the others assays which provided a mean threshold below 2 IU/mL independently on the genotypes. The variable sensitivity of HIV Ag/Ab assays observed in the detection of p24 Ag could compromise the diagnosis of early infection due to HIV-1 non-B genotypes, and HIV-2. As the prevalence of HIV-1 subtypes varies geographically and is continuously changing driven by such factors as immigration, travel and tourism there is a need to develop diagnosis tools able to detect a large spectrum of HIV polymorphism.

ENHANCED HIV SCREENING THROUGH MULTIPLEX ANALYSIS
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Objective: Create the next generation of HIV tests: an automated HIV assay with 4th generation sensitivity that can report antibody and antigen results separately, and distinguish HIV-1 from HIV-2 positives.
Methods: The BioPlex 2200 HIV Ag-Ab assay uses multiplex flow immunassay, a methodology that permits simultaneous detection and identification of many analytes in a single reaction vessel. The assay is performed by the Bio-Rad BioPlex 2200 automated analyzer. A mixture of four populations of dyed beads is used. Assay performance has been characterized in house using CLSI methods to determine limit of detection and precision (20 days, 2 runs/day) using weakly reactive specimens. Clinical sensitivity and specificity were evaluated using 24 commercial HIV seroconversion panels and 5239 routine specimens of unknown risk for HIV infection. HIV-2 detection was assessed using 177 known HIV-2 positives from West Africa and 90 known HIV-1 positives. Results: Total precision of index values (S/CO) were <10% for all analytes. Limit of detection of p24 is 0.63IU/mL based on the WHO Standard. p24 antigen was the first analyte detected in all 24 seroconversion panels. BioPlex 2200 detected HIV infection one donation sooner than Abbott Architect Combo HIV (4th generation) in three panels. BioPlex 2200 missed one donation positive by Architect. To assess specificity, 5239 samples of unknown risk were tested. Nineteen were reactive on BioPlex and confirmed by Abbott Architect Combo. Nine other specimens were reactive on BioPlex, but could not be confirmed, resulting in specificity of 99.83%. All known HIV-1 and HIV-2 specimens tested were reactive. All 90 HIV-1 samples were correctly identified as HIV-1, 153/177 HIV-2 samples were correctly identified as HIV-2; 24 were undifferentiated. Of BioPlex 2200 undifferentiated samples, 17 were undifferentiated on ImmunoComb.
Conclusion: The BioPlex 2200 HIV Ag-Ab assay, which is currently in development, is highly sensitive and specific, and can provide detailed screening results that will assist in identifying specimens from primary infection and HIV-2 positives. Its ability to report separate results for p24 antigen, HIV-1 antibody, and HIV-2 antibody qualify it as the 5th generation of HIV testing.

A BETTER PATIENT OUTCOME WITH A WIDE APPROACH ALGORITHM
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The evaluation of gastrointestinal symptoms has a major role in pediatric clinical practice. Careful patient’s history evaluation and physical examination are crucial for the formulation of a correct diagnosis and identification of useful tests for a definitive diagnosis. Cases in which the suspected diagnosis is poorly defined are frequent and the risk of a diagnostic delay or incorrect use of invasive or non-invasive tests is high. This is the case of many children with inflammatory bowel disease (IBD) in which the presence of non-specific intestinal symptoms or extra-intestinal manifestations can result in a significant diagnostic delay responsible for serious impact on body growth and development, unnecessary dietary regimens or therapies, and medical and familial costs. In these situations, as well as in the differential diagnosis of functional gastrointestinal disorders or food allergies, having effective laboratory tests can be of great help. Recently, several non-invasive diagnostic tools have been proposed to make a timely and accurate diagnosis of IBD. Among these fecal calprotectin, serological specific markers (i.e., anti-Saccharomyces cerevisiae and perinuclear staining anti-neutrophil cytoplasmic antibodies), together with the study of small intestinal permeability, and of bowel wall thickness by ultrasonography have a major impact on a modern approach to these subjects. The sequential incorporation of these new diagnostic tools into the work up facilitate clinical decision making when the diagnosis of IBD in children is uncertain. The diagnostic test should ideally be able to identify and quantify the risk of developing a disease, reflect the degree of severity and activity, be specific as well as easy to apply in daily clinical practice. These requirements seem more theoretical than real, but in recent years many advances have been made in this direction and several new tests are now available. In several cases a careful standardization is still advocated, but it is essential for the physician to know existence, potential and limits of these tests in order to meet the
needs of the patients and make diagnostic process as accurate and fast as possible.

EW105
A FRUITFUL AND EFFICIENT MODEL OF LABORATORY ORGANIZATION
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Background: Recent technological developments in laboratory medicine have led to a major challenge, maintaining a close connection between the search of efficiency through automation and consolidation and the assurance of effectiveness. The adoption of systems that automate most of the manual tasks characterizing routine activities has significantly improved the quality of laboratory performance; total laboratory automation being the paradigm of the idea that "human-less" robotic laboratories may allow for better operation and insuring less human errors.

Methods: After reviewing the number and types of tests requested in the field of allergy and autoimmune diseases, and the number of tubes and patients with tests requested in both diagnostic areas, we have integrated the diagnostic pathway by linking through a track the instruments used for allergy and autoimmune testing. Results: A significant reduction of the analytical turnaround time and the laboratory reporting within 24 h for tests requested for suspected allergies and/or autoimmune diseases was found. The possible addition of appropriate laboratory tests (reflective testing) on the basis of the initial data was also demonstrated. Conclusions: This experience confirms that automation allows clinical laboratories to improve analytical performances if trained staff operate in accordance with well-defined standard operative procedures, thus assuring continuous monitoring of the analytical quality. In addition, laboratory automation may improve the appropriateness of test requests through the use of algorithms and reflex testing. This should allow the adoption of clinical and biochemical guidelines. In conclusion, in laboratory medicine, technology represents a tool for improving clinical effectiveness and patient outcomes.

EW106
AUTOMATED, HIGH-THROUGHPUT WORKFLOW FOR THE ANALYSIS OF 25-HYDROXYVITAMIN D AND 3-EPI-25-HYDROXYVITAMIN D3 BY MULTIPLEXED TURBOFLOW LC-TANDEM MS
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Measurement of total serum 25-hydroxyvitamin D (25OHD) is considered a reliable marker of vitamin D status. In the past decade, there has been a move towards the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the analysis of 25OHD. However, MS/MS alone is an achiral technique. This can be problematic for some isobaric 25OHD metabolites, notably 3-epi-25-hydroxyvitamin D3 (3-epi-25OHD3). For accurate analysis of 25OHD3, LC-MS/MS methods require routine chromatographic resolution of 3-epi-25OHD3, but this requires extended chromatographic analysis times, which impacts on assay throughput. We have developed a method for the analysis of serum 25OHD2 and 25OHD3, which resolves interference from 3-epi-25OHD3, using automated sample preparation and multiplexed TurboFlow LC-MS/MS to maximise throughput without compromising assay specificity. All liquid handling was carried out using a VersetteTM automated system. Calibration, quality control standards and samples (100 µL) were transferred to a 96-well filter plate. Internal standard solution and precipitation reagent were separately added from reagent reservoirs. Filter plates were capped and vortex mixed (10 min). Supernatants were collected into a microtitre plate by centrifugation (200 g, 3 min). An Aria Transcend TLX-II system (Thermo Scientific) was used. Sample supernatants (100 µL) were injected onto a C18-XL TurboFlow column (50 x 0.5 mm i.d.). Retained analytes were back-flushed from the TurboFlow column onto an Accucore PFP analytical column (total 2.6 µm aps, 50 x 2.1 mm i.d.) at 40 °C. Mass spectrometry was carried out in positive ionisation mode using APCI. Retention times were 10.94 min, 11.47 min and 11.82 min for 25OHD3, 3-epi-25OHD3 and 25OHD2 respectively.

EW107
DETECTION OF DRUGS OF ABUSE IN EXHALED BREATH USING MASS SPECTROMETRY AND A SIMPLE COLLECTION DEVICE
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It has recently been demonstrated that amphetamine, methadone and tetrahydrocannabinol are detectable in exhaled breath following intake. Exhaled breath therefore constitutes a new possible matrix for drugs of abuse testing. The present work was aimed at exploring this possibility further by a study on patients treated for acute intoxication with abused drugs. Forty-seven patients were included in the study and breath, plasma and urine samples were collected following recovery together with interview data. Analyses of breath and plasma samples were done with liquid chromatography-mass spectrometry methods. Urine was screened using immunochromical reagents and positive findings confirmed with liquid chromatography-mass spectrometry methods. The following analyses were investigated: methadone, amphetamine, methamphetamine, 6-acetylmorphine, diazepam, oxazepam, alprazolam, morphine, benzoylecgonine, cocaine, buprenorphine and tetrahydrocannabinol. In most of the studied cases recent intake of an abused substance prior to admission was reported. In 43 of these (91%) the breath analysis gave a positive finding. Identifications were based on correct chromatographic retention time and product ion ratios obtained in selected reaction monitoring mode. Generally, data from breath, plasma, urine and self-report were in agreement. Detected substances in breath comprised amphetamine, methamphetamine, buprenorphine, 6-acetylmorphine, morphine, methadone, tetrahydrocannabinol, diazepam, oxazepam, alprazolam and cocaine. This study gives further support to the possibility to develop exhaled breath into a new matrix for drugs of abuse testing by extending the number of analyses that are documented to be detectable in breath.
EW108
THE INFLUENCE OF THE BLOOD DRAWING TECHNIQUE

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Blood drawing, a primary requisite of in vitro diagnostics, is the most vulnerable step of the testing process. An appropriate venipuncture is essential to obtain a quality specimen, wherein a mishandled or incorrect procedure can produce unsuitable specimens, compromise the quality of testing and ultimately jeopardize patient safety. The most frequent problems attributable to inappropriate sample collection, in order of frequency, entail spurious hemolysis, clotting, incomplete or inappropriate filling of primary blood collection tubes, undue clotting, inappropriate containers, contamination from infusion liquids, as well as misidentification and unsuitable conditions of temperature, time and humidity for transportation. The venipuncture procedure is typically complex, requiring both knowledge and skill. Although each phlebotomist usually defines a comfortable routine, a series of sequential steps must be fulfilled. These include correct patient identification, assessment of patient physical disposition, (i.e., diet, physical exercise, stress, basal state), check of requisition form, selection of a suitable site, preparation of equipment and puncture site. The tourniquet should applied to an area approximately 10 cm above the intended site of venipuncture, it should be tight enough to restrict venous flow but not arterial circulation, and – especially – should be ideally removed after 1 min but never left in site for more than 3 min. The blood samples must be labeled before venipuncture and collected in the appropriate container. The ideal needles are those with calibre comprised between 19-23 gauge, preferably straight. There is no analytical reason to limit the use of butterflies, but the incremental cost over the conventional straight needle should be adequately weighted as well as the stringent requisition of discarding the air volume with a discard tube. When mixing of the tube is required for the presence of various additives (anticoagulants, pro-coagulants, stabilizers), this should be done by 3-6 times gentle inversion. Primary blood tubes should never be mixed to prevent cross-contamination of additives. The specimens should hence be sent to the laboratory in a suitable time frame and under the best environmental conditions, with no injury.

EW109
PNEUMATIC TRANSPORT SYSTEMS: FACTS AND SPECULATIONS

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The pneumatic tube system (PTS) provides a rapid mode of sample transportation. Modern PTSs with soft start and variation of speed for different transports offer fast, reliable, and efficient transportation of blood samples to the laboratory. However, during transport in a PTS, blood samples are often subjected to high speeds and rapid acceleration and deceleration, which can lead to hemolysis. Usually, this problem can be revealed variously from sample to sample, and some recent reports have studied the different possible causes of this pre-analytical problem. The tube type can be correlated with the hemolysis caused by PTS. Serum samples are more susceptible than plasma samples, when sent through PTS, and serum tube with gel seems to confer some protection against the damage of erythrocytes and leukocytes. Hemolysis can be due to the specimen bumping on the internal sides of the shuttle, during the rapid accelerations or decelerations. Put the tube in a sponge-rubber or in a bubble wrap can reduce the chance of damage to red cells in whole blood specimen during the trip along the PTS. Speed of sample transportation by a PTS can influence the degree of hemolysis. Moreover also the distance is correlate with the level and percentage of hemolysed samples. Distance and speed can have a synergic effect, so that a higher speed can be accepted for short distance, while a slow movement can reduce the percentage of hemolysis in a longer route of PTS.

Last, some specific populations of patients (for instance, hematology and/or oncology patients) seems to have blood samples more susceptible to damage. Each installation of a PTS is uniquely characterized by architecture, technical specifications, and differences in speed and length, thus demanding individual evaluation of every single system. Each hospital should validate the PTS at the center before use to ensure that this pre-analytical step does not cause any spurious results. It is possible to avoid, or reduce, hemolysis by interventions like reducing speed, blocking samples inside the shuttle, changing tube type, centrifuging samples before the run and using plasma gel separator.

EW110
APPROACHES TO BE USED IN VERIFICATION OF IVD METROLOGICAL TRACEABILITY

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To be accurate and equivalent laboratory results should be traceable to higher-order references. Furthermore, their analytic quality should fulfill acceptable measurement uncertainty as defined to fit the intended clinical use. To this aim, IVD manufacturers should define a calibration hierarchy to assign traceable values to their system calibrators and to fulfill during this process uncertainty limits for calibrators, which should represent a proportion of the uncertainty budget allowed for clinical laboratory results. It is therefore important that, from one hand, laboratory profession clearly defines the clinically acceptable uncertainty for relevant tests and, from the other hand, end-users may know and verify how manufacturers have implemented the traceability of their calibrators and estimated the corresponding uncertainty, including, if any, the employed goal. Currently, the full information is usually not available as manufacturers only provide the name of higher-order reference material or procedure to which the assay calibration is traceable without any description of steps and their corresponding uncertainty of the implemented traceability chain. In general, it should be possible to establish if the current status of the measurement uncertainty budget associated with the proposed traceability chain is suitable or not for clinical application of the test. Important post-market tools for IVD traceability surveillance are related to the verification by clinical laboratories of the consistency of declared performance during routine operations performed in accordance with the manufacturer’s instructions and the organization of appropriately structured EQAS. The former activity should be accomplished by analyzing system control materials and confirming that current measurements are in control range, with no clinically significant changes in the assumed unbiased results. With regard to EQAS, it is mandatory that target values to materials (including their uncertainty) are assigned with reference procedures by an accredited reference laboratory, that materials are commutable and a clinically allowable
inaccuracy for participant’s results is defined in order to prove
the suitability of laboratory measurements in the clinical setting.

EW111
CURRENT PITFALLS OF CONTROL MATERIALS: THE
WAY FORWARD

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EU Directive on In Vitro Diagnostic (IVD) Medical Devices
obliges manufacturers to ensure traceability of their analytical
systems to recognised higher-order references. According to
IVD regulations, manufacturers should provide control
materials (CM) that shall give the user confidence that the
values obtained by use of the test system are traceable. Clinical
laboratories should verify the consistency of the performance
declared by the manufacturers through the organization of
appropriate analytical internal (IQC) and external (EQA) quality
controls. IQC used to monitor the analytical performance of the
methods should be reorganised to check the trueness of CE-
marked systems and to evaluate system imprecision. EQA
Assessment Programs provide an assessment of laboratory
performance. The CM used to check trueness and imprecision
must be provided by the manufacturer of the analytical systems
and by an independent source, respectively. The CM for
imprecision must be commutable and, when clinical decision
cut-points are employed, their concentrations should be around
those values. The CM for trueness and imprecision should
satisfy the analytical goals derived from biological variations of
each analyte. The EQA materials should suit the following
characteristics. First, the values must be assigned with
reference measurement procedures by an accredited
reference laboratory to check the measurement uncertainty of
participating laboratories against the established reference
measurement systems. Second, EQA materials should display
proved commutability to allow transferability of participating
laboratory performance to patient samples. Finally, the clinically
allowable uncertainty of measurements should be defined in
order to verify the suitability of laboratory measurements in a
clinical setting. A survey commissioned to the Working Group
for Analytical Quality by the Italian Society of Clinical
Biochemistry (SIBioC) demonstrated a gap from what reported
above and what at the moment is provided from manufacturers
and EQA organizers. For instance, the acceptable range of CM
for trueness respects manufacturer’s criteria rather than those
derived from biological variation and the CM for imprecision is
often the same used to check trueness.

EW112
HOW TO ESTIMATE THE MEASUREMENT
UNCERTAINTY IN CLINICAL LABORATORIES: IS THE
GUM APPROACH MANDATORY?

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It can be stated that the result of a measurement is only an
approximation (or estimate) of the true value of the measurand,
and thus it is complete only when accompanied by a statement
of the measurement uncertainty. The standard approach to
calculate the measurement uncertainty proposed by the Guide
to the expression of uncertainty in measurement (GUM) requires
the quantification of each and every source of variability to sum
them up for the final calculation (bottom up

Approach). To overcome the inherent difficulties in the
systematic application of this approach in a clinical laboratory
alternative ways were proposed. The so called “top-down”
approaches derive the calculation of measurement uncertainty
from already existing data provided by Internal Quality Control
(IQC) and External Quality Assessment (EQA).

In fact measurement uncertainty has a random component
that can be derived from IQC data and a systematic
component that should be minimized, but includes the
uncertainty of the value assigned to the calibrator. The amount
of bias to be considered for the calculation of the uncertainty
cannot be derived from different sources: measurements of a
value assigned reference material, peer group means of IQC
or EQA results.

We propose a practical way of calculating the uncertainty of
measurement of each measurand based on a spreadsheet that
uses all these parameters. Knowing the uncertainty of a measurement provides an
indication of the analytical quality of the result and allows
checking whether it is suitable for clinical purposes. Moreover
it allows a statistically sound comparison with previous results
of the same patient or with results from other laboratories. A
basic requirement for these comparisons is an easy,
homogeneous and standardized way of calculating the
measurement uncertainty and the tool we provide represents
an important step in this direction.