**AACC Sympoisum Selected topics in laboratory medicine: quid novi, quo vadis**

## CARDIAC TROPONIN I STANDARDIZATION: CURRENT STATUS, FUTURE PROMISE

D.M. Bunk[1]
[1]*National Institute of Standards and Technology, Gaithersburg MD, USA*

The IFCC Working Group for Troponin I Standardization (WG-TNI) is developing a reference measurement system to support clinical measurement of cardiac troponin I.
The IFCC WG-TNI performed a pilot study, in collaboration with industry, to investigate the feasibility of preparing a stable, commutable, pooled serum cTnI certified reference material (CRM). cTnI-positive serum samples from 90 patients, presenting to the emergency department with suspected acute myocardial infarction, were used to prepare seven pools with cTnI concentrations in the range, 200-10,000 ng/L. All pools were assessed for commutability through measurement by 16 commercial cTnI assays according to predefined testing protocols. The data from the pilot study was also used to evaluate the potential for standardization of the 16 assays.
Through pair-wise comparisons of the commercial assay measurement results of both the candidate reference materials (RMs) and 90 individual patient samples, it was observed that all candidate RMs behaved equivalent to patient samples for all assays. Specifically, in pair-wise linear regression analysis of assay results, the measurement data from the candidate reference materials all fell within the 95% prediction interval of the Passing-Bablok regression line derived from the individual patient samples. Each assay was assessed against median cTnI concentrations measured by 16 systems using Passing-Bablok regression analysis of 79 patient samples with cTnI values above each assay's declared detection limit. An 8- to 9-fold difference in cTnI concentrations was observed among assays. After correction by a mathematical recalculation using slope and y-intercept values, between-assay variation was re-assessed. Overall, the 16 assays demonstrated negligible bias after realignment.
Although the effort to achieve equivalence of clinical cTnI measurement through standardization has been a long process, the WG-TNI has made significant progress in the development of a serum-based CRM. The WG-TNI anticipated this CRM will be available in 2015.

**AACC Sympoisum Selected topics in laboratory medicine: quid novi, quo vadis**

## THE LABORATORY MEDICINE BEST PRACTICES PROGRAM: AN EVIDENCE-BASED APPROACH TO LABORATORY MEDICINE

R. Christenson[1]

[1]*University of Maryland School of Medicine, University of Maryland Medical Center, Baltimore, MD 21201 USA*

The Laboratory Medicine Best Practice (LMBP) initiative is sponsored by the US Centers for Disease Control and prevention. The purpose of this program is to systematically identify, develop, and pilot test evidence-based quality/performance recommendations aimed at improving public health with measureable laboratory practices which are safe, timely, efficient, effective, equitable and patient-centered.

Development of an LMBP is based on a validated six-step model, termed "A-6", that is comprised of Asking the question, Acquiring the evidence, Assessing the evidence, Analyzing the data, Applying the findings into practice, and Assessing (or Auditing) the results. The LMBP methodology was recently published (Clin Chem 2011;57:816-25). The LMBP program utilizes systematic review methods to evaluate evidence of laboratory practice effectiveness, with a particular focus in the pre-and post-analytic phases. There is a fundamental paucity of evidence in most healthcare disciplines, including laboratory medicine. For this reason the LMBP program specifically seeks unpublished quality assurance studies from the field. These studies are held to the same standards as published studies and have contributed to the ability to promulgate best practice recommendations.

Examples of questions that have been addressed include: Does the use of rapid laboratory identification techniques to quickly identify microbes in positive blood cultures result in decreased time to targeted therapy? When drawing blood samples for laboratory testing from ED patients, what practices are effective in reducing hemolysis rates among these samples? What practices are effective for timely communication of laboratory critical value results in an inpatient healthcare setting to the licensed caregiver who can act on them?

The LMBP program is designed to answer key questions and develop recommendations using evidence based methodologies and processes. Systematic reviews have been disseminated that particularly target pre- and post-analytical testing phases, because they are most susceptible to error. Effectiveness of the program is evaluated by post implementation audits.

**AACC Sympoisum Selected topics in laboratory medicine: quid novi, quo vadis**

## TOWARD PATIENT-CENTERED HEALTHCARE: IMPROVING PUBLIC KNOWLEDGE OF LABORATORY MEDICINE

D.R. Dufour[1]
[1]*George Washington University Medical Center*

Patient-centered care has become a preferred model for health care, and has been shown to have increased effectiveness and lower costs compared to provider-centered care models. Laboratory tests provide up to 70% of the objective data found in medical records, and are heavily used in diagnosis and management of disease. Although the focus of a 2009 report by the US Centers for Disease Control and Prevention, there is little concrete data on patient-centered laboratory medicine. A key component of patient-centered care involves explanation of information in terms understandable by the patient. Laboratory test results are usually presented in numeric format; numeracy skills are among the weakest health literacy skills found in typical patient populations studied. The US Agency for Healthcare Research and Quality has identified 9 tips for patients to ask before medical tests are performed, but have not provided similar guidance regarding the significance of test results. Because laboratories will be increasingly called to provide test results directly to patients, approaches for increasing patient understanding of test results will be increasingly important. Efforts such as the global Lab Tests Online program will become even more essential in providing patient-centered laboratory medicine. This session will review approaches to education of the public, including patients and their supporting family and friends, on laboratory test importance and the meaning of laboratory test results, such as patient-friendly laboratory reports, internet-based resources, and direct interaction with patients to explain their results simply. Examples of each of these approaches will be presented.

**AACC Sympoisum Selected topics in laboratory medicine: quid novi, quo vadis**

**INTERNATIONAL HARMONIZATION CONSORTIUM: CURRENT STATUS, FUTURE PROMISE**

G. Miller[1]
[1]*Virginia Commonwealth University - Richmond, USA*

between different clinical laboratory measurement procedures should be comparable, within clinically meaningful limits, to enable optimal use of clinical guidelines for diagnosis and patient management. The ISO standard 17511:2003 "In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials" describes a hierarchy of calibration traceability schemes to accomplish harmonization of results. The most desirable and best developed approaches utilize primary (pure substance) reference materials to prepare calibrators for high level reference measurement procedures. The ISO standard provides for the situation when there is no reference measurement procedure and traceability is to a secondary (matrix) reference material. However, inadequate attention to the commutability of secondary reference materials has led to the situation when clinical laboratory procedures are traceable to a reference material, yet results for patient samples are not equivalent when measured with different procedures. In addition, inadequate definition of the measurand and inadequate analytical specificity for the measurand contribute to lack of harmonized results. For many measurands, secondary reference materials are not available, and alternative processes based on panels of patient samples are needed to achieve harmonization. Despite many organizations in many countries addressing harmonization, there is no systematic approach to prioritize measurands based on medical importance and to coordinate the efforts of different groups. An International Consortium for Harmonization of Clinical Laboratory has been formed to provide a global infrastructure to enable a systematic approach for identification and prioritization of measurands to be harmonized, an information portal to foster collaboration among all organizations contributing to harmonization of measurands, and a technical focus on measurands that do not have reference measurement procedures. The Consortium is managed by a Council and a Harmonization Oversight Group. Interested stakeholders may join the Strategic Partners Group and submit measurands for consideration at www.harmonization.net.

**Emerging vascular markers**

## ANTIOXIDATIVE DEFENSE MECHANISMS IN DIABETES MELLITUS

S. Awadallah[1]
[1]*University of Sharjah, United Arab Emirates*

Oxidative stress and overproduction of reactive oxygen species (ROS) is a significant player in the development of diabetic complications. Moreover, oxidative stress is exacerbated by concomitant impairment of antioxidative defense mechanisms in the form of decreased or altered enzymatic and non-enzymatic mechanisms. Clinical and experimental studies have shown that significant variations in the extent of susceptibility to oxidative stress exist among diabetic patients. Genetic polymorphism has been suggested as a factor that could precipitate such differences in susceptibility to oxidative stress. Although several genetic factors have been implicated, haptoglobin (Hp) is emerging as a strong candidate in this regard.

Haptoglobin is a polymorphic antioxidative plasma protein existing in three phenotypes (Hp1-1, Hp2-1, and Hp2-2). It binds with free hemoglobin (Hb) in circulation forming stable Hb-Hp complexes thus preventing heme-iron mediated damage. This antioxidative function of Hp is phenotype-dependent; in that, Hp2-2 is an inferior antioxidant compared to Hp1-1 or Hp2-1. Specifically, heme iron of the Hb-Hp2-2 complex is more redox active and clears less efficiently than that of other Hp phenotype complexes. Furthermore, the antioxidative potential of Hp2-2 precipitously decreases with increased levels of Hb glycoyslation. Several studies have demonstrated that diabetics with Hp2-2 are under increased oxidative stress and at higher risk of vascular complications. Furthermore, the activity of antioxidative enzymes like superoxide dismutase (SOD), glutathione peroxidase (Gpx), catalase, and ferroxidase was recently shown to be strongly related to Hp phenotype in diabetics. In this presentation, we hope to give an updated overview of recent work on the role of haptoglobin polymorphism in modulating the antioxidative defense mechanisms in diabetes mellitus.

**Emerging vascular markers**

## PHARMACOGENOMIC APPLICATION OF THE HAPTOGLOBIN GENOTYPE IN THE PREVENTION AND TREATMENT OF DIABETIC CARDIOVASCULAR DISEASE.

A. Levy[1]
[1]*Technion*

The overwhelming consensus from multiple prospective clinical trials performed over the past 10 years is that vitamin E supplementation does not provide any cardiovascular benefit. To the contrary, meta-analysis of these studies suggests that high dose vitamin E supplementation may increase mortality. A possible explanation for why these studies failed in spite of solid preclinical and observational data is the inadequate nature of patient selection in these studies. The Hp gene is polymorphic with two common classes of alleles denoted 1 and 2 and three corresponding Hp genotypes (Hp 1-1, 2-1 and 2-2) with genotype prevalence's of 16%, 48% and 36% respectively in most western populations. In this presentation we will provide clinical data supported by compelling mechanistic studies showing the Hp genotype may predict which individuals with Diabetes Mellitus are at highest risk of cardiovascular disease and who may benefit from vitamin E supplementation.

**New horizons of laboratory medicine for autoimmunity**

## CD22 POLYMORPHISM AND SUSCEPTIBILITY TO MURINE LUPUS-LIKE DISEASE

L. Reininger[1]
[1]*USR3151-CNRS, Station Biologique, Roscoff, France*

CD22 (Siglec-2) is a transmembrane protein expressed on B cells which regulates B cell receptor (BCR) signaling, cell survival, proliferation and antibody production. CD22 contains an extracellular N-terminal V-set Ig domain, which mediates binding to -2-6 linked sialic acids, and a cytoplasmic tail with immunoreceptor tyrosine based inhibitory motifs (ITIMs) mediating interaction with SHP1 tyrosine phosphatase. In view of the proximity of the Cd22 gene locus to susceptibility loci to lupus-like glomerulonephritis and autoimmune hemolytic anemia in mice, we analyzed the polymorphism of Cd22 in several mouse strains prone to develop autoimmunity.
Characterization of CD22 alleles was performed by sequencing of cDNAs amplified from inbred strain mouse spleen. The expression of aberrant forms of CD22 on B cells was performed by antibody staining, flow cytometry and western blot. The presence of serum autoantibodies was assessed by ELISA.
Polymorphism in the Cd22 gene of inbred mouse strains is based on three different alleles. The Cd22b allele is found in non-autoimmune mouse strains such as BALB/c and C57/BL6. The Cd22a allele, present in NZB and NZW mice, as well as the Cd22c allele, present in BXSB mice, are found in lupus-prone strains. The Cd22a and related Cd22c alleles lead to the expression of aberrant CD22 molecules with deletions in the ligand-binding domain of the protein. B cells from B6.Cd22a congenic mice show an activated phenotype similar to CD22 knockin mice with a mutated ligand-binding domain, confirming that the ligand-binding domain of CD22a is functionally defective.
These studies have identified a polymorphism of CD22 in mice and indicate that the Cd22a allele promotes B cell activation and spontaneous development of autoimmunity. Although genome-wide association studies have not detected human CD22 as susceptibility loci to SLE, other studies have linked CD22 deficiency/dysregulation to the development of B cell hyperplasia or leukemia. This work should prove useful as a basis for assessment of the functional capacities of CD22 in autoimmune diseases and other diseases in which B cell function is dysregulated.

**New horizons of laboratory medicine for autoimmunity**

**THE CD19 SIGNALLING MOLECULE IS ELEVATED IN NON-OBESE DIABETIC (NOD) MICE AND DRIVES AUTOIMMUNITY LEADING TO TYPE 1 DIABETES (T1D).**

R.M. Slattery[2], A. Ziegler[2], M. Le Page[2], M. Maxwell[2], J. Stolp[1], H. Guo[2], A. Jayasimhan[2], M. Hibbs[2], P. Santamaria[3], J. Miller[4], M. Plebanski[2], P. Silveira[1]
[1]*Centenary Institute, Sydney*
[2]*Monash University, Department of Immunology*
[3]*University of Calgary*
[4]*Walter & Eliza Hall Institute*

Type 1 diabetes is an autoimmune disease that results from mononuclear infiltration of pancreatic islets and targeted destruction of the insulin producing beta cells. The inflammatory infiltrate consists of many cell types including B lymphocytes capable of processing and presenting antigen to the T cells that kill beta cells. A crucial role for B lymphocytes in type 1 diabetes (T1D) pathogenesis has been established since non-obese diabetic (NOD) mice lacking B cells are protected from developing T1D. We show that CD19 expression on B cells, important for the internalization of membrane-bound antigens, is elevated in NOD mice, and results in increased signaling. Furthermore, while NOD B cells deficient in CD19 can adequately present peptide antigen and promote the expansion of T cells with specificity for the soluble beta cell protein pro-insulin, they possess significantly diminished capacity to expand T cells with specificity for the membrane-bound autoantigen, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). We propose that elevated CD19 on NOD B cells leads to increased uptake of membrane-bound antigen, mediating the expansion of autoreactive T cells specific for membrane-bound autoantigens which are critical for invasive insulitis, beta cell destruction and type 1 diabetes.

**Laboratory medicine practice guidelines: a multidisciplinary approach**

**EVIDENCE AND COST EFFECTIVENESS REQUIREMENTS FOR RECOMMENDING NEW BIOMARKERS.**

P. Collinson[1]
[1]*St George's Hospital and Medical School*

When assessing the potential application of a new biomarker two criteria must be met. The first is evidence to support the use of the biomarker. This evidence will be Analytical, Plausibility and Treatment, is this test APT. Analytical factors include pre-analytical factors, analytical performance and the ability of the biomarker measurement to be incorporated into existing workflow. Plausibility is both pathophysiological (does the test have an underlying accepted or verified role in the pathophysiology for which it is being advocated) and clinical. Clinical evidence should be based on test performance in a large enough sample of the population of interest and reproducible across different locations. Treatment impact is the single most important feature that defines biomarker acceptance into routine practice. The test must pass the "so what" question – so what will I (as a clinician) do differently with this result, that I would not have done before with the previous biomarkers I have available.
The second criterion is the impact on healthcare resource utilisation. There are three strategies for evaluation of the impact on healthcare costs, cost minimisation analysis, cost effectiveness analysis and cost utility analysis. Cost minimisation analysis has the objective of delivering the same health outcome at lower cost. At its simplest, if the new biomarker replaces an existing test at the same price (or ideally cheaper) then direct substitution can occur. This is not often the case but a more sophisticated analysis is possible examining process costs with or without the biomarker. Cost effectiveness analysis compares the relative costs and outcomes (effects) of two or more courses of action and is expressed as a gain in health in one main parameter such as life years gained. Where there is more than one dimension to measure change, cost utility analysis measures change in health status valued one against the other to produce an overall index of health gain. Typically this uses the quality adjusted life year (QALY, years of health gained). For diagnostic testing, estimating both costs and QALY's can be difficult and frequently involves modelling. This is often because of a lack of good cost effectiveness studies.

**Laboratory medicine practice guidelines: a multidisciplinary approach**

**GRADING EVIDENCE FOR LABORATORY TESTS BEYOND DIAGNOSTIC ACCURACY.**

A.C. Don-Wauchope[2], P.L. Santaguida[1]
[1]*Department of Clinical Epidemiology and Biostatistics, McMaster University*
[2]*Department of Pathology and Molecular Medicine, McMaster University*

GRADE has been proposed and utilised as a means of evaluating evidence for clinical practice guidelines. The initial GRADE papers discussed many aspects of clinical care including diagnostic accuracy. However, the use of laboratory testing covers a much wider scope than diagnosis and includes screening, monitoring, prognosis, risk stratification and prediction of therapeutic effect. The core principals of GRADE are risk of bias, consistency, directness and precision. These are combined to form a single score. The Agency for Healthcare Research and Quality has proposed a system that uses similar principals for evaluating evidence. Laboratory medicine practice guidelines should also consider evaluating evidence and the principals described in the GRADE system may be appropriate.
For a systematic review that included prognostic questions we considered the GRADE (quality of evidence) principals (risk of bias, consistency, directness, precision, dose-response association, plausible confounders, strength of association and publication bias) along with the system for evaluating strength of evidence as proposed by the The Agency for Healthcare Research and Quality. We applied these principals to the synthesis of evidence. To facilitate this we modified tools to evaluate the quality and risk of bias of individual papers.
The tool we used to evaluate prognostic papers facilitated the evaluation of risk of bias. We were able to consider consistency, directness, precision. strength of association, plausible confounders and publication bias to variable degrees. The dose-response association was not applicable to our questions. From these we could suggest a quality of evidence ranking, or the strength of evidence rank as well as consider applicability.
The GRADE approach may be useful in the context of prognostic laboratory tests but requires the use of appropriate tools to assist in the evaluation of primary papers.

**Laboratory medicine practice guidelines: a multidisciplinary approach**

**ARE GUIDELINES GUIDING US ON HOW TO UTILIZE LABORATORY TESTS?**

A.R. Horvath[1]

[1]*SEALS Department of Clinical Chemistry, Prince of Wales Hospital; Screening and Test Evaluation Program, School of Public Health, University of Sydney and School of Medical Sciences, University of New South Wales, Sydney, Australia*

Increasing health costs and risks due to market-driven, uncontrolled use of novel biomarkers make evidence-based guideline recommendations increasingly important. The translation of basic scientific discoveries into clinically meaningful studies and then to evidence-based guidelines for best practice or health policy is, however, not straightforward. Numerous CPGs are released on similar topics worldwide, but their quality and content validity are highly variable and their recommendations may differ even when using the same sources of evidence. This can be due to the limitations of the evidence base or that value-based judgments on the balance between benefits, harms, risks, patients' preferences and the organizational and financial aspects of care differ among countries and regions. Addressing these issues requires careful discussions between relevant multidisciplinary stakeholders involved in the management of conditions.

Although CPGs potentially influence clinical decisions and patient outcomes, current approaches to CPG development often fail scientific publication standards. Critical appraisal of CPGs has revealed that: many do not involve laboratory professionals in formulating recommendations on the use of tests; the composition of the panel could influence the scope of guidelines and over-represent certain stakeholders' views; numerous CPGs do not have rigorous evidence-based methodology and miss essential information important for the correct interpretation and application of test results. Variation in the assessment of the underlying evidence is partly due to the lack of agreed test evaluation methods and easy-to-use evidence rating schemes that can be universally adapted to diagnostic recommendations.

Clinical practice guidelines are developed to close the gap between research and practice, but the appearance of so many guidelines created a new gap between their development and utility in practice. Poor quality and lack of explicitness of recommendations on laboratory testing call for methodological and reporting standards for guidelines. The profession also needs an evidence-grading scheme and international collaboration of guideline development activities to increase the validity and practicality of recommendations for good laboratory practice.

**Epigenetics and laboratory medicine**

**PROBING THE CANCER METHYLOME**

S. Beck[1]
[1]*UCL Cancer Institute, London, UK*

What determines a phenotype is one of the fundamental questions in biology and medicine. In addition to genetic changes, epigenetic changes such as altered DNA methylation have been shown to play important roles. In the context of cancer, epigenetic changes often outnumber genetic changes which in turn frequently occur in genes encoding chromatin modifiers, thus adding another level of complexity to the dynamics of cancer epigenomes. To understand the rules governing DNA methylation and their functional consequences requires genome-wide analysis of methylome dynamics. I will present our efforts using array- and sequencing-based platforms for methylome analysis and discuss some functional insights gained from introducing multi-dimensional perturbations.
For further details, please see: http://www.ucl.ac.uk/cancer/medical-genomics/

**Epigenetics and laboratory medicine**

**REPROGRAMMING THE CANCER EPIGENOME**

C. Plass[1]
[1]*German Cancer Research Center (DKFZ)*

Cancer genomes are characterized by massive alterations in the epigenome. This includes alterations of DNA methylation patterns, as well as on the histone level. Currently the mechanisms that lead to these changes are unknown. However there is evidence from cancer genome sequencing projects that mutations in genes that regulate the establishment of epigenetic patterns occur frequently. During my presentation I will focus on our current knowledge on how mutations in epigenetic enzymes might affect the epigenome. I a second example I will discuss the role of a lncRNA in alternating the epigenetic pattern of tumor suppressor TCF21.

**New technological developments in laboratory medicine**

## ADVANCED TECHNOLOGIES IN STUDYING HIV INFECTION

A. Cossarizza[1]
[1]*Univ. of Modena and Reggio Emilia*

In the last three decades, the immunological changes that occur during the infection with the human immunodeficiency virus (HIV) have been largely investigated by using flow cytometry (FCM). Since the earliest times (1982-3), in which even the use of two monoclonal antibodies (mAbs) conjugated with two different fluorophores excited by a single laser (for the simultaneous recognition of two antigens) was more than problematic, this technology has facilitated the understanding of the interactions between the virus and the host, and in particular the mechanisms that the immune system activates to fight HIV. Furthermore, FCM is crucial to monitor the efficacy of the potent antiretroviral therapy currently used. In the '90s, the use of a second laser allowed to add a third and then fourth fluorescence to the "cytometric armamentarium", and to investigate more and more immunological parameters. About a decade ago, other lasers and fluorescence channels were available, and now it is possible to recognize more than 20 molecules in a single cell. Sophisticated softwares have been developed to allow the precise identification and functional characterization of an incredible number of cell populations that typically circulate in the blood of human beings and patients. So, polychromatic flow cytometry has permitted to identify the strategies that the immune system uses to react to HIV infection, from the earliest phases to the latest stages, to understand the importance of polyfunctional cells, and to identify possible cellular targets for innovative therapies.
In the last years, a new technology defined "mass cytometry" has been developed that uses mAbs conjugated with rare transition element isotopes not normally found in biological systems, that are analyzed by atomic mass spectrometry. This system, defined cytometry by time-of-flight, or CyTOF, in 2011 has first allowed to measure 34 different parameters in human bone marrow cells, then to show that CD8+ T cells recognizing different viruses had a much greater complexity than previously appreciated. Currently, several studies on the specific response to HIV peptides are in course, and will soon untangle other aspects of the complex changes that the virus induces in the host immune system.

**New technological developments in laboratory medicine**

**RECENT ADVANCES IN CYTOMICS FOR IMMUNOLOGY**

J. O'Connor[1], S. Balaguer[1], L. Díaz[1], A. Gomes[1], G. Herrera[2], A. Martínez-Romero[3], F. Sala-de-Oyanguren[1]
[1]*Center for Cytometry and Cytomics, Dpt. Biochemistry and Molecular Biology, The University of Valencia, Valencia, Spain*
[2]*Cytometry Service, UCIM, Incliva-Faculty of Medicine, University of Valencia, Valencia, Spain*
[3]*Cytomics Technological Service, Principe Felipe Research Center, Valencia, Spain*

Cytometry is one of the choice methodologies for the study of the Immune System in normal and pathological conditions. The technical evolution of cytometry, as a single-cell based technology has led sequentially from Cytology to Cytomics. In analogy with other -omics, the targets of Cytomics are the heterogeneous cellular systems known as the cytomes. The cytomes can be understood as the heterogeneous cellular systems and functional components of pluricellular organisms. Of special importance is the cell-by-cell basis of cytomic analysis, an approach that allows to resolve heterogeneous systems and avoids the loss of information that characterizes bulk technologies, in which average values are obtained from large number of cells or from tissue homogenates.Since the functional heterogeneity of the cytomes results from both the genome and extracellular environment, Cytomics can be considered also as the discipline that links Genomics and Proteomics to cell and tissue function, as modulated by external influences. Based upon these premises, the Immune System has been always considered one of the most challenging cytomes for Cytomics. In the most recent years, the application of Cytomics to immunological studies has been greatly improved by novel technological approaches that enhanced either the velocity of sample analysis (High-Troughput Flow Cytometry) and data acquisition (Acoustic-Focusing Flow Cytometry) or the content of biological information generated per each single cell analyzed, by means of fluorescence automated imaging of single cells in suspension (Multispectral Imaging Flow Cytometry) or in solid matrices (High-Content Analysis by Bioimaging). A particularly promising development in this context is Time-of Flight Mass-Spectrometry Cytometry, a single-cell multiparametric flow technology based on inductively coupled plasma mass spectrometry. In this approach, antibodies are labelled with non-radioactve isotopes of rare earth atoms, rather than with fluorophores, which currently allows the simultaneous quantification of about 50 membrane- or intracellular proteins, while avoiding the problem of spectral overlap, a complex issue in modern polychromatic Flow Cytometry.

**New technological developments in laboratory medicine**

**BIOMARKERS, BIOSIGNATURES AND BIOSENSORS OF CHRONIC INFLAMMATION**

A. Radbruch[1]
[1]*Deutsches Rheuma-Forschungszentrum Berlin, a Leibniz Institute*

The early diagnosis and stratification of patients suffering from rheumatic diseases remains a major challenge. While clinical manifestation, radiography and laboratory tests, such as determination of auto-antibody profiles and inflammatory parameters are indispensable for diagnosis they often lack sensitivity and specificity and fail to predict treatment response.
Cells of the peripheral blood are easily accessible. We could show that monocytes circulating in the blood are sensitive "biosensors" to indicate and type chronic inflammatory diseases. Isolated monocytes from patients show disease-specific gene expression signatures in their global transcriptomes, allowing to discriminate different types of rheumatic diseases. These "biosignatures" reflected the action of cytokines and other signals driving the diseases.
Since transcriptomes are not easy to obtain routinely, do not inform on the single cell level and do not allow to monitor parameters in conjunction, we developed a cytometric single cell proteomic approach, with a multicolor staining panel of 50 different monoclonal, fluorochromated antibodies. This panel identifies all major leukocyte populations and their corresponding subtypes, the expression of surface proteins involved in complement and antibody binding, cell migration, and activation. This cytometric profiling platform identifies cytometric biosignatures, but also can be adapted to translate the transcriptome signatures. It classifies rheumatic diseases and allows to develop signatures that predict response of individual patients to therapy.

**WASPaLM Symposium Biomarkers and personalized pharmacotherapy**

## PHARMACOGENOMICS OF CARDIOVASCULAR DRUGS – THE EXAMPLE OF THIENOPYRIDINES

G. Siest[1]
[1]*UMR INSERM U1122 - IGE-PCV - University of Lorraine - Nancy - FRANCE*

Between 14 classes of cardiovascular drugs, the antithrombotic and the anticoagulants ones are the most sensitive to genetic variability. Thienopyridines are a therapeutic class of anti platelet drugs largely used and Clopidogrel, combined with aspirin is routinely used to treat patients with a variety of thrombotic disorders (prevention of ischemic events, acute coronary syndrome, prevention of thrombosis after stent...)
Clopidogrel inhibits platelet aggregation induced by adenosine diphosphate (ADP). Clopidogrel is a prodrug requiring several biotransformation steps, mediated mainly by cytochrome P450 (CYP), in order to generate an active metabolite that binds irreversibly to the platelet ADP receptor P2Y12. But the same CYP are metabolizing also arachidonic acid producing anti inflammatory derivatives. Inflammation reduction is a second target for thienopyridines.
The pharmacodynamic response to Clopidogrel varies widely from subject to subject, and about 30% of patients treated with standard dose of Clopidogrel, display low ex vivo inhibition of ADP-induced platelet aggregation. This poor response is partially linked to several functional polymorphisms found in genes encoding CYP isoforms involved in Clopidogrel metabolism.
Many clinical trials are in development to study, more extensively, the pharmacogenetics of Clopidogrel and the interaction between Clopidogrel and other widely-used medications, the pathologies influences, the ethnic differences...
During treatments with thienopyridines, we have also studied the inflammation response by measuring CRP and other plasma inflammation markers and discovered an interaction with smoking.
Point-of care testing (POCT) has been put on the market for giving quickly platelet reactivity or genetic information to clinicians before giving the right drug to the patient.
In summary, thienopyridines are good examples of Pharmacogenomics clinical implementation difficulties.

**WASPaLM Symposium Biomarkers and personalized pharmacotherapy**

## PHARMACOGENOMICS AN INDIVIDUALIZED DRUG THERAPY

M. Schwab[1]
[1]*Dr Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany*

Variation in drug disposition and response among patients is a major concern associated with many therapeutic agents used in all disciplines of medicine, particularly in oncology. The clinical relevance of interindividual variability is most evident with drugs that have a narrow therapeutic window (i.e., the dose used is close to the dose probably resulting in drug-related toxicity in most individuals). With increasingly information available from the Human Genome Project pharmacogenomics (PGx) aims to elucidate the genomic determinants of drug efficacy and toxicity. Variation of drug response however is caused by a combination of genetic and environmental factors as well as patient characteristics which can affect the pharmacokinetics and/or pharmacodynamics of drugs. PGx research has led to fundamental discoveries, and a large resource of PGx traits has been generated in which variation in the gene sequence and/or variation in gene expression of ADME targets such as drug metabolizing enzymes, drug transporters and nuclear receptors are associated with alterations in drug response. For instance clinically important cancer drugs related to PGx are thiopurines, tamoxifen, and irinotecan. However, a more comprehensive approach is required to consider PGx in entire biological and pharmacological pathways (Schwab M, Schaeffeler E. Pharmacogenomics: a key component of personalized therapy. Genome Med 2012).The combination of recently developed -omics approaches like epigenomics (e.g., DNA methylation, miRNA) and metabonomics together with integrative and holistic system pharmacology strategies are highly promising for the identification of novel putative ADME targets for better prediction of drug response (Meyer UA, Zanger UM, Schwab M.Omics and drug response. Annu Rev Pharmacol Toxicol 2013).

**WASPaLM Symposium Biomarkers and personalized pharmacotherapy**

**BIOMARKERS TO ACHIEVE PERSONALIZED IMMUNOSUPPRESSION IN TRANSPLANTATION**

M. Oellerich[1]
[1]*Institute for Clinical Chemistry, University Medical Center Göttingen, Germany*

Transplantation biomarkers attract much attention because there are still unresolved problems. Irreversible chronic rejection and side effects of standard immunosuppression limit long-term outcome. There are limitations to how immunosuppressive drugs are currently monitored. Therapeutic drug monitoring is more useful to prevent toxicity than to predict efficacy. Biomarkers are needed that can accurately diagnose or predict complications at their earlier stages. Various strategies are currently being evaluated, including biomarkers of immune response, drug target enzymes, and markers of graft injury. A particularly promising new approach for the early detection of graft injury is based on the determination of graft-derived circulating cell-free DNA (GcfDNA) using droplet digital PCR. This assay takes advantage of a single nucleotide polymorphism panel that can be used for any donor/recipient combination for the exact quantification of GcfDNA percentage. GcfDNA has the advantage that it directly interrogates the health of the donor organ ("liquid biopsy").
In a recent study, subtherapeutic tacrolimus levels < 8 μg/L, HCV+ and rejection episodes, but not cholestasis, were associated with significantly elevated GcfDNA. The significant increase of GcfDNA was already observed 4 to 6 days before full-blown acute rejection.
Optimal combinations of practical and cost-effective biomarker/TDM assays are needed. Before transplantation and during the early phase, the percentage of IL-2 producing CD8+ T cells may be helpful to identify patients who require more potent immunosuppression. In the early phase, better markers of graft injury like GcfDNA would be useful. In the maintenance phase, both markers of tolerance and GcfDNA would help to identify recipients who would benefit from immunosuppression minimization. In the future, personalized immunosuppression will shift emphasis from reaction to prevention which could make immunosuppressive drugs safer, more effective, and reduce the cost of health care.

**WASPaLM Symposium Biomarkers and personalized pharmacotherapy**

**BIOMARKERS IN TARGETED CANCER DRUG THERAPY**

P.D. Walson[1]
[1]*Institute for Clinical Chemistry, University Medical Center, Goettingen, Germany*

Biomarker targeted cancer drugs (CDs) are being increasingly used instead of or combined with traditional, non-specific, cytotoxic and radiation therapies. Biomarkers are also used to select therapy, diagnose tumors, individualize dosing, and monitor responses.
Examples from both the literature and our group will be used to illustrate the current use and future promise of both tumor and patient biomarkers as well as some challenges and unanswered questions concerning their successful clinical applications.
FDA approved CD labeling includes a number of pharmacogenomic tumor biomarkers (e.g. EGFR, KRAS, ABL, C-Kit, ERBB2, estrogen receptors, and BRAF V600E), germline toxicity biomarkers (e.g. TPMT & UGT1A1), and compendium resistance biomarkers (e.g. BRC-ABL). Blood and urine proteins are also used for both cancer detection (e.g. KLK3, PSA, MUC16, AFP, CGB, TP53) and response. Drug concentrations are used to assess compliance and to avoid excessive or insufficient doses of CDs (e.g. imatinib), antibiotics (e.g. meropenem), anti-viral, and anti-fungal drugs. New biomarkers continue to be described including gene expression arrays (e.g. FoxP3), mass spectrometric proteomics, and molecular targeted PET imaging. Particularly promising are next generation molecular sequencing "liquid biopsies" such as circulating cell-free DNA which provides a practical, sensitive, non-invasive direct measure of tumor mass using only blood or urine and can be used for diagnosis, response monitoring, and identification of minimal residual disease. Tumor heterogeneity and temporal changes, assay sensitivity/specificity, regulatory requirements, clinical testing and acceptance all remain challenges, but biomarkers have the potential to both replace more expensive and invasive screening tests and allow more effective, less toxic, individualized drug selection and dosing.
While additional controlled trials are still needed to identify tests and their combinations that have optimal sensitivity and specificity, biomarker targeted cancer therapy has already improved patient survival, quality of life, and the cost/benefits of cancer therapy. Biomarkers promise to continue to revolutionize how cancer is diagnosed and treated.

**IFCC Symposium The impact of laboratory medicine on clinical outcomes**

## DEMONSTRATING THE IMPACT OF LABORATORY MEDICINE ON CLINICAL OUTCOMES

M. Hallworth[1]
[1]*Royal Shrewsbury Hospital, Shrewsbury SY3 8XQ, UK*

Clinical laboratory workers believe that the work they perform in providing laboratory tests is valuable. However, data to validate this has been limited, and evidence of the contribution of laboratory medicine to the overall process of diagnosis and management is not easy to obtain. This session will describe the work of the IFCC Task Force on the Impact of Laboratory Medicine on Clinical Management and Outcomes (TF-ICO). It will examine existing evidence, review the gaps in our understanding and deficiencies in the way laboratory medicine is used, and indicate how these can be remedied.

Many articles and presentations seeking to promote the value of laboratory medicine have made use of what has become known as the "70% claim". This is presented in various forms, most commonly that "Laboratory Medicine influences 70% of clinical decisions", or minor variations around this figure. However, the data on which this estimate was based represents unpublished studies and anecdotal observations, and cannot now be objectively verified. The IFCC TF-ICO was established in 2012 to evaluate the available evidence supporting the impact of laboratory medicine in healthcare, and to develop the study design for new studies to generate evidence of the contribution made by laboratory medicine. This presentation will examine existing evidence, review the gaps in our understanding and deficiencies in the way laboratory medicine is currently used, indicate how these might be remedied and offer a vision of a future state in which laboratory medicine is used effectively to support patient care and enhance patient safety. An approach to measuring value will be proposed in which the net value of a testing process is defined as delivered benefits minus delivered harm (undesirable effects of testing). Value is maximized by increasing the benefits and reducing harm.

Much of the evidence relating to the value of laboratory medicine is poorly structured and does not relate to clinical outcomes. A more rigorous approach is required. Laboratory medicine has much to offer, but can cause adverse outcomes if not properly used. Laboratorians need to refocus their attention onto improving outcomes.

**IFCC Symposium The impact of laboratory medicine on clinical outcomes**

## COMMUNICATING THE IMPACT OF LABORATORY MEDICINE: THE "LABS ARE VITAL" GLOBAL INITIATIVE

E. Jacobs[1]
[1]*NYU School of Medicine / Henry J Carter Specialty Hospital & Nursing Facility*

Promotion of the role and value of laboratory medicine is critical for the future of our profession. Laboratory medicine is truly the hidden treasure in health care. The role of the laboratory in the provision of medical care is often underappreciated, if not appreciated at all by the public; patients, physicians and other healthcare workers, hospital administrators, and government & health policy makers. Laboratory services are often seen as a commodity rather than a professional activity. Thus it is important to communicate the essential contribution that lab medicine makes to the healthcare system.

In 2008 the Labs are Vital (LRV) international campaign to promote the field of laboratory medicine as a career choice was started by Abbott Diagnostic. In 2012 the transition from an Abbott Diagnostics initiative to one driven and managed by a consortium of global professional bodies was started. The four initial members of the consortium are: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), World Association of Societies of Pathology & Laboratory Medicine (WASPaLM), American Society for Clinical Pathology (ASCP), and International Federation of Biomedical Laboratory Scientists (IFBLS.)

The top-line objective for LRV is "To communicate the essential contribution lab medicine makes to our healthcare system – emphasizing lab professionals as being central to safe, effective patient care, and raising the profile of lab medicine as an attractive career choice. " While recognizing regional and local differences, the member board confirmed a common promotional message: Pathology and laboratory medicine – the essential partner in patient care

• Central to every patient pathway
• Evidence-based service delivery
• Driving change for better clinical outcomes

In order to bridge from the objective to the agreed messages below, the LRV goals include:

1) Plug the information gap that exists – collect data, as well as quantitative and qualitative information that evidences the clinical value of lab medicine
2) Uncover case studies that show how lab medicine affects individual patient experience
3) Act as a focus for discussion of IVD developments – POCT, genomics, etc. – to boost value of profession

**IFCC Symposium The impact of laboratory medicine on clinical outcomes**

**COLLABORATING WITH INTERNATIONAL CLINICAL ORGANISATIONS**

H.A. Morris[1]
[1]*University of South Australia and Chemical Pathology, SA Pathology, Adelaide South Australia 5000 Australia*

The provision of quality laboratory services for patient care and to improve healthcare outcomes is at the centre of the work of the IFCC. Included in its Aims are statements highlighting this relationship including working "to enhance the scientific level and the quality of diagnosis and therapy for patients throughout the world" as well as to "build on the professionalism of our members to provide quality services to patients." In the day to day work of laboratory medicine we often don't have direct contact with patients. How can the IFCC activities be improved to enhance patient outcomes?
At the beginning of each triennium, the IFCC Executive Board adopts a strategic plan. For 2012 to 2014 expanding our relationship with clinical organisations was a focus of a number of activities including specifically Item 14 under 'Broadening our Horizons' – "Develop a plan to increase collaboration between IFCC and international clinical organisations" and "Establish at least one new collaboration each year with an international clinical organisation".
A review of IFCC activities has indicated current collaborations with international health and clinical organisations at all levels. The IFCC Executive Board leads collaborations with international peak bodies including World Health Organisation and World Association of Societies of Pathology and Laboratory Medicine (WASPaLM). The current work of the Scientific Division involves collaborations with 16 clinical organisations at the level of the Executive Committee as well as with specific Committees and Working Groups. Furthermore in recent years the Executive Board has established a number of Task Forces with strong interaction with clinical organisations and clinicians.
IFCC is currently working to expand its relations with international clinical organisations to enhance the translation of developments in laboratory medicine to improve patient care and clinical outcomes as well as their adoption into clinical practice such as inclusion in clinical guidelines. The standardisation of the assay for HbA1c is just one example of technological improvement to not only improve the performance of the test for monitoring disease but increase its utility for diagnosis.

**IFCC Symposium The impact of laboratory medicine on clinical outcomes**

## THE ARCHITECTURE OF MEDICAL TEST EVALUATIONS: FROM ANALYTICAL PERFORMANCE TO CLINICAL EFFECTIVENESS

P.M. Bossuyt[1]
[1]*Academic Medical Center - University of Amsterdam*

Like all interventions in medicine, medical tests should be thoroughly evaluated before they are introduced into clinical practice, and laboratory tests are no exceptions. Patients, payers and other societal parties expect innovations in laboratory tests to improve or maintain health, or to contribute to health care efficiency.
Traditionally, the emphasis in laboratory medicine has been on analytical performance. Clinical evaluations have far and foremost focused on estimating diagnostic accuracy, by comparing test results against the clinical reference standard.
In this presentation we will introduce the clinical effectiveness of medical tests as a key concept. We will describe how evaluations of analytical performance and of clinical performance can be better scoped, so they can serve as an assessment of necessary conditions for clinical performance. In this process, the intended use of the medical test is quintessential.
In the end, medical test evaluations should answer the question whether the test is “fit for purpose”: whether we can say with confidence that it contributes to health outcomes and health care efficiency.

**EFLM Symposium Patient focused laboratory medicine**

## THE TRIALOGUE BETWEEN PATIENT, PHYSICIAN AND LABORATORY: DELIVERING PATIENT FOCUSED LABORATORY MEDICINE

J.X. Corberand[1]
[1]*University Hospital of Toulouse - France*

Medical biology is a medical specialty in its own right. This brings the patient on to the scene, passing from the status of a tube sample to that of a person. The relationship of the laboratory with the patient therefore comes into play.
Tests are ordered by a clinician. The clinician has all the information on the patient: complaints, history, and psychology, which should not be neglected (depression). The clinician is not competent in all fields of medical biology. The test results therefore need personalized interpretation, giving diagnostic hypotheses and suggesting further investigations.
The patient can personally give the laboratory medicine specialist (LMS) all personal information, but most often a patient's statements need to be filtered through clinical analysis, giving all the items of data their due weight. The LMS cannot be a listener to the patient who in general is not in contact with a professional competent to listen. Communication between the clinician and the LMS is often inadequate. The clinician's prescription contains little clinical data. Contact with the prescriber is difficult and often the LMS delivers the results without any personalized interpretation. Analytical data may give an immediate diagnosis. Diagnoses of extremely serious diseases can be established from a blood film. They cannot be announced without psychological preparation. This announcement falls to the clinician, as part of the "singular dialogue" between physician and patient. This does not exclude the LMS from giving the patient a full explanation of the test results, bearing in mind that the patient is primarily interested in their meaning and their seriousness.
Here we have three persons who are communicating about a single subject, the patient. The patient may be directly relating with each of the two others. But there is no direct relationship between the clinician and the LMS. To improve this, a fourth element comes into play: the availability of relevant information that can be consulted at any time. This is the digitalized patient's medical record (DPMR). For the LMS to assume a full role in the relationship with the patient, access to the DPMR is a necessity. All necessary steps need to be taken for DPMR to be accessible on a European scale.

**EFLM Symposium Patient focused laboratory medicine**

## IF I WAS A PATIENT, WHAT WOULD I EXPECT FROM POCT?

I. Watson[1]
[1]*University of Liverpool*

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!

**EFLM Symposium Patient focused laboratory medicine**

**GIVING LABORATORY RESULTS DIRECTLY TO PATIENTS: PRINCIPLES AND PRACTICE**

W. Oosterhuis[1]
[1]*Atrium Medical Center, Heerlen, The Netherlands*

Patients are regarded more and more as partners. Patient empowerment refers to informing patients and preparing them to discuss treatment options with the doctor. The WHO and the WONCA (the world organization of general practitioners) are two organizations that have included patient empowerment into their policy. This relates to shared decision making: in contrast to the paternalistic model where the doctor decides what is best for the patient, in this model choosing between treatment options has become a shared activity. Research has shown that this leads to better motivated patients with better treatment outcomes.
How can the laboratory take a role in these new developments? The first possibility is sharing the laboratory results with the patients. A recent survey performed across European countries by the EFLM working group of patient focused laboratory medicine has shown a very diverse picture. In many countries – as in my country The Netherlands – it is not a routine to give laboratory results to patients. In other countries this is very common, and the objections against sharing the results are not recognized. With the development of web-based patient records that can be accessed by the patients, it will inevitably become a reality that laboratory results can be obtained freely by patients. A survey in our hospital showed that 85% of the patients did want to receive their results from the laboratory. This underlines the great need by patients for information that also includes information about laboratory results. Most patients however will not be able to understand their laboratory results. The question is, whether the laboratory can play a role in informing the patient about the interpretation. Patients are often not very well informed by their doctors: not about the tests that were requested or about the meaning of the results. The laboratory could play a role in informing the patient about guidelines that might be applicable in their case. Patients can easily be informed about additional (verified) information available on the Internet. The concept of patient empowerment places the patient at the center, receiving information from different sides and participating in treatment decisions. The laboratory could very find a place in this new concept.

**Pandemia of obesity, metabolic syndrome and diabetes. Role of laboratory medicine**

## ADIPOKINE AP2 IS A NOVEL METABOLIC HORMONE CONTRIBUTING TO CARDIOMETABOLIC DISEASE

E.S. Calay[1]
[1]*Harvard School of Public Health*

Adipocyte lipid chaperone adipocyte protein 2 (aP2) has been previously identified as an important protein for pathogenesis of cardiovascular diseases, obesity and type 2 diabetes in disease models and body of literature exists supporting aP2 as a serum marker for some of these pathologies in humans. Dysregulated hepatic glucose production is one of the contributing factors to etiology of obesity and type 2 diabetes. Despite ample evidence hinting towards an adipose tissue contribution to hepatic glucose production, a direct link between adipose tissue and liver glucose production has been missing. We have recently identified aP2, previously thought to be intracellular protein, to be secreted adipokine in a regulated fashion, which signals liver for de-novo glucose production. Neutralization of this circulating factor corrects the diabetic phenotype of the obese mice, furthermore close association of this circulating factor with obesity in humans, may open up new therapeutic avenues for treatment of obesity and type 2 diabetes as well as provide a new clinical marker for diagnosis and prognosis.

**Pandemia of obesity, metabolic syndrome and diabetes. Role of laboratory medicine**

## THE ROLE OF THE LABORATORY IN THE DIAGNOSIS AND ASSESSMENT OF DIABETES

E. Kilpatrick[1]
[1]*Department of Clinical Biochemistry, Hull Royal Infirmary/Hull York Medical School*

For most of the last century, diabetes has been diagnosed on the basis of raised blood glucose values. While haemoglobin A1c (HbA1c) has been routinely used for the last 30 years to monitor patients with established diabetes, its use has recently been extended to the diagnosis of type 2 diabetes.
Using HbA1c for diabetes diagnosis has brought with it advantages and disadvantages. One of the disadvantages is that HbA1c may not be suitable for some patients with haemoglobinopathies, especially in countries where diabetes incidence is predicted to increase most markedly in the coming decades. Existing other markers of glycaemia, such as fructosamine and glycated albumin, have just acquired an evidence-base which may now allow them to be more confidently used as alternatives to HbA1c when assessing both the risk of developing diabetes as well as the likelihood of acquiring microvascular complications in patients already known to have the disease.

**Pandemia of obesity, metabolic syndrome and diabetes. Role of laboratory medicine**

**ADIPOKINES IN MORBID OBESITY**

G. Schernthaner[1], G. Schernthaner[2]
[1]*Department of Medicine, General Hospital Vienna, Medical University Vienna, Vienna, Austria*
[2]*Department of Medicine, Rudolfstiftung Hospital, Medical University Vienna, Vienna, Austria*

Adipose tissue had been considered for many decades as an non-secretory, inert, depot storage tissue, that only serves the purposes of insulating the body from cold and/or merely storing energy that is not needed for immediate use.
In the last decade, adipose tissue has been acknowledged as a major endocrine and paracrine organ that produces hundreds of proteins. These molecules are known under the term “adipokines” or „adipocytokines“ and contribute as enzymes, hormones or growth factors in the modulation of insulin resistance and metabolism of fats and glucose and thus have an indirect effect on atherosclerosis.
Inappropriate secretion of several adipokines by the excessive amount of white adipose tissue, in particular in patients presenting with morbid obesity, seems to participate in the pathogenesis of obesity-related pathologic processes including endothelial dysfunction, inflammation, atherosclerosis, diabetes mellitus, and chronic kidney disease. Most adipokines with pro-inflammatory properties (e.g. Interleukin-6 and -8, Monocyte Chemoattractant Protein-1, Leptin, Resistin, Adipsin) are overproduced with increasing adiposity, whereas some adipokines with anti-inflammatory or insulin-sensitizing properties, such as Adiponectin, are decreased.
This dysregulation of adipokines production may promote obesity-linked metabolic disorders and cardiovascular disease. World wide research has documented that adipokines are not only involved in the modulation of the immune system, but also in angiogenesis, energy homeostasis and metabolism, vasoconstriction/vasodilatation, lipid metabolism and energy storage as well as in coagulation and fibrinolysis.
In the last decade we have studied many of the recently identified adipokines (Interleukin 6, TNF-alpha, Monocyte chemoattractant protein-1, YKL-40, Retinol-binding protein 4, Resistin, and HMW Adiponectin) in patients with morbid obesity before and after dramatic weight loss induced by metabolic surgery. Remarkably, many of those adipokines were strongly associated with the insulin resistance state before surgery and changes of the serum levels of adipokines after surgery were closely linked with improvement of glucose metabolism and insulin sensitivity.

**Clinical laboratory's role in decision making: risk assessment and interpretation of laboratory test results**

**TROPONINS, ACCELERATED DIAGNOSTIC PATHWAYS AND CLINICAL DECISION MAKING.**

C. Florkowski[1]
[1]*Canterbury Health Laboratories, Christchurch, New Zealand*

In clinical decision making, the optimal use of troponins for diagnosis of acute myocardial infarction is best guided by clinical outcomes. Previously published that have addressed this include the 2-h diagnostic protocol to assess patients with chest pain symptoms in the Asia-Pacific region (ASPECT) and the 2-Hour Accelerated Diagnostic Protocol to Assess Patients With Chest Pain Symptoms Using Contemporary Troponins as the Only Biomarker (ADAPT) studies. In both studies, patients presenting to the Emergency Department (ED) with chest pain, accelerated diagnostic protocols (ADP) were defined using TIMI pre-test probability score, lack of new ECG changes and cardiac biomarkers including troponins; and identified subsets of patients at low-risk (9.8% and 20% for ASPECT and ADAPT respectively), with >99% negative predictive values for major adverse cardiovascular events (MACE) at 30 days.
In a randomized, controlled clinical trial (RCT), patients were randomized to usual clinical care versus an ADP modelled on the ADAPT study, including TIMI score, ECG changes, 0 and 2 hour troponin I (Abbott Architect; cut-off 0.03 ug/L). In the ADP, 19.3% versus 11.0% ($p<0.05$) were able to be discharged within 6-hours of presentation and with no MACE up to 30 days. The ADP has been advocated as the usual standard of care within our institution. Based on outcome data, a refined Australasian risk probability score has been derived and incorporated into a new on-going RCT of an updated ADP, also incorporating high sensitivity TNI. It is projected that earlier discharge from the ED will translate into considerable cost savings and especially when extrapolated to larger populations.
For clinical decsision making, optimal utilisation of troponins depends critically upon assessment of the full clinical context, namely pre-test probability coupled with short term evaluation of troponins. In accordance with the best principles of Evidence Based Laboratory Medicine, this should ideally be supported by RCTs based on clinical outcome data.

**Clinical laboratory's role in decision making: risk assessment and interpretation of laboratory test results**

## APPLICATIONS OF CARDIAC NATRIURETIC PEPTIDE MEASUREMENTS IN THE CLINICAL MANAGEMENT OF HEART FAILURE

A.M. Richards[1]

[1]*Director, Cardiovascular Research Institute, National University Heart Centre, Singapore, NUHS. Director, Christchurch Heart Institute, University of Otago, Christchurch, New Zealand*

Plasma cardiac natriuretic peptide concentrations reflect cardiac injury and overload and are powerful independent prognostic markers in cardiovascular disease. This background underpins their established applications in acute and chronic heart failure. The B type cardiac natriuretic peptides BNP and NTproBNP (with some evidence for mid-region pro-ANP also) are best proven to assist in the diagnosis of acute heart failure among patients presenting to the Emergency Department with recent onset breathlessness and selected "rule out" levels have been established for both markers. NTproBNP diagnostic performance is enhanced by use of age adjusted thresholds. Overall the B peptides have ~90% sensitivity and specificity together with 98% negative predictive values for the diagnosis of HF among breathless patients. Admission and discharge values of B peptides and the shift between are powerful prognostic markers for death or readmission in the months following discharge. In the setting of chronic HF titration of treatment according to serial measurement of plasma B peptide values in addition to customary clinical practices is able to improve clinical outcomes including overall mortality and readmissions with new acute HF at least in those with HF and reduced ejection fraction. The diagnostic, prognostic and titration performance of B plasma peptides has resulted in their introduction into authoritative guidelines for the diagnosis and management of HF.

**Clinical laboratory's role in decision making: risk assessment and interpretation of laboratory test results**

## GENOMIC PREDICTION AND RISK STRATIFICATION FOR COMMON DISEASES

J. Whitfield[1]
[1]*QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Existing predictors, often based on biochemical and physiological measurements, have had an important role in identifying high-risk individuals before they develop overt disease and in reducing disease incidence through lifestyle or pharmacological interventions. The best-developed examples are for cardiovascular disease and type 2 diabetes. Two important challenges are to improve the accuracy of prediction algorithms, and to extend the range of diseases which can be prevented in this way. These may be achievable through use of genotypes as additional markers of risk.
Most common diseases are thought to have a genetic component, and single-nucleotide polymorphisms (SNPs), identified through genome-wide association studies (GWAS), have been shown to associate with risk for hundreds of common diseases or quantitative traits. Such SNPs are present at comparatively high frequency in the population (usually > 5%) but tend to have small effects on disease risk. Genotyping of multiple SNPs is cheap and reliable, and can be used to calculate a genomic risk score. However, addition of genomic risk data to conventional algorithms for cardiovascular or diabetes risk has not yet led to significant improvement in prediction of disease. Larger GWAS, powered to discover additional genetic associations, may still lead to improved predictors.
Although most genetic risk for common disease is due to the cumulative effects of many common variants of small effect, common diseases can also have uncommon causes. In the cardiovascular field, familial hypercholesterolaemia is uncommon, has a large effect on risk, and is due to many different variants in several genes. Such rare variants may only make a small contribution to the population attributable risk but they are important to the affected individuals and their families. At present they are usually investigated by sequencing of selected genes in people known to be at high risk from their family history.
It is possible that the technologies for assessing common and rare genetic contributions to common disease will converge through the introduction of low-cost genome-wide sequencing. However, there are technical, clinical and ethical issues to be resolved and evidence of benefit must precede adoption of this approach.

**Data generation and ethical issue in laboratory medicine**

## TO KNOW OR NOT TO KNOW: THE IMPORTANCE OF ETHICS IN THE OMICS AGE

J.J. Jonsson[1]
[1]*Dept. of Biochemistry and Molecular Biology, University of Iceland and Dept. of Genetics and Molecular Medicine, Landspitali – National University Hospital, Reykjavik, Iceland*

How ought we live our lives? This is the central question in ethics. There is no final one answer, but decisions still have to be made. Clinical laboratorians are busy people faced with many issues and preciously little time to deal with them. Is ethics becoming more or less important in this situation and is training in ethics helpful?
To address these questions we take an example from the dominant new technology. Omics techniques are evolving from research applications into the clinical laboratory. By definition they involve comprehensive analysis generating results unrelated to the indications for testing. This has re-awakened the thorny problem of what to do with incidental findings. This question first came in focus with mechanized autoanalyzers offering panels of tests. Now it resurfaces on a much bigger scale. Under what circumstances should incidental results be included in laboratory reports and when should they be communicated to the patient? Analysis of these situations requires considering concepts in ethics foremost beneficence, non-maleficence, autonomy and justice. In particular patient autonomy is central in deciding when patients are entitled to know or not to know incidental results.
Clinical laboratorians need to learn and apply concepts and techniques from many disciplines. In addition to mastering the science, they need to learn statistics, quality management, business, human resources and communication. Although ethics is central in many issues in the clinical laboratory, ethics training has apparently not been a prominent part of training programs. To further examine this situation the Ethics Task Force of IFCC is conducting a survey on the teaching of ethics in training programs for clinical laboratory directors. The objective is to better define educational goals and to suggest optimal educational materials for ethics. Preliminary results show that only 25% of the responding programs provide a formal medical ethics course and 31% offer a formal research ethics course. These courses are generally 6 hours or less. Almost half the training programs, however, reported plans to enhance ethics training with most interest in using online resources and self-learning. Providing such materials is an important task.

**Data generation and ethical issue in laboratory medicine**

## ARE THERE CORE BIOETHICAL PRINCIPLES THAT SHOULD BE DEALT WITH IN PRIORITY IN THE CLINICAL LABORATORY?

J. Watine[1]
[1]*Hôpital de la Chartreuse, Villefranche-de-Rouergue, France*

A standard approach to biomedical ethics, developed by Beauchamp and Childress in their book "Principles of Biomedical Ethics", resolves ethical issues in terms of four ethical principles i.e. respect for the patient' autonomy, non-maleficence, beneficence, and justice, also called equity. Just like physicians, or nurses, specialists in Laboratory Medicine should permanently bear these values in mind when they perform their duty, including when they generate data. Non-maleficence imposes an obligation not to inflict harm on patients. Many medical interventions may be both harmful and beneficial, but the harms should be proportionate to the benefits. Beneficence means that health-care professionals have to contribute to the welfare of the patients in a way that makes sense to the individual patient. Respecting the autonomy of the patients means respecting the decision-making capacities of individuals. Justice, also called equity, means that patients in similar positions should be treated in a similar manner.
The presentation will be illustrated by a few examples showing that in many circumstances the generation of inappropriate, or too many, or too few, data by the laboratory, mostly conflict with these four values. Few key points or considerations will be highlighted:
- Health equity is one of the main objectives of public health policy across the world, as disadvantaged populations have poorer health, and poorer access to health care. The collective resources on earth are limited and must be fairly distributed not only between the sick and their health-care professionals, but also between these two categories of people and other citizens. It would be equally reasonable to provide disadvantaged people with a better access to hygiene and health-care, and to prevent many citizens from being over-tested, over-diagnosed, and ultimately over-treated.
- We probably have much to gain from education and training in bioethics. This can potentially help us to better understand important concepts such as evidence for, or against, testing. This can also protect specialists in Laboratory Medicine against their own conflicts of interest. Ultimately this can better guide practice, research and policy.

**Data generation and ethical issue in laboratory medicine**

## ETHICAL CHALLENGES IN THE COLLECTION, GENERATION, INTERPRETATION AND APPLICATION OF CLINICAL RESEARCH DATA

M. McQueen[1]

[1]*Mc Master University and Hamilton Health Sciences, Ontario, Canada*

The Nuremberg Code 1947, the Declaration of Helsinki 1964 (with 5 subsequent amendments) and the Belmont report 1979, were landmark clinical research guidelines responding to specific abuses. Clinical laboratory workers may ask why they should care about clinical research ethics? You may be involved as a research subject, your regular prescription and your over-the-counter medicine may become part of a database for post-marketing research. Your laboratory may test as a formal part of a study, or informally for safety monitoring. Laboratory physicians may be clinical investigators, or may provide care for their patients receiving experimental medication. Many countries have structures to protect the rights of subjects, requiring clinical studies to be formally approved. Even with guidelines for good clinical practice, ethical breaches occur e.g. in a gene therapy trial at the University of Pennsylvania, the subject was not told of a change in his liver function tests and died when re-challenged with the gene therapy vector. The diabetes drug Avandia had bad effects on lipid profiles but the data were not made public. Many studies results are not published. Almost 1 in 3 large clinical trials in the U.S. are still unpublished five years after study completion, thus withholding results from the public domain (~250,000 people have been exposed to the research risks involved in those trials without the benefits to society that they were told would result). Autonomy has been a key principle, with recent questions relating to honesty and openness in research. Published data may be more positive than that submitted to regulatory agencies. The presentation and interpretation of study results may be distorted. Clinical, epidemiological, diagnostic, prognostic and pre-clinical research cannot be interpreted and applied properly if it is biased or the results distorted, producing less precise estimates of the effectiveness and safety. The STARD statement was first published in 2003 to facilitate the complete and transparent reporting of studies of the accuracy of medical tests. With greater understanding of the sources of biases in study design and the deficiencies listed earlier, there is now an initiative to update STARD.

**Biologic variation and its effect on the reference values**

**BIOLOGICAL VARIABILITY AND ITS EFFECT ON REFERENCE VALUES: AN OVERVIEW.**

W.A. Bartlett[1]
[1]*Blood Sciences, Ninewells Hospital & Medical School, Dundee, UK. DD1 9SY*

Biological variability (BV) confounds the derivation and application of reference values (RVs) in clinical practice. The utility of populaton based reference intervals is affected by the biological variation of test results.
Literature relating to biological variation has been considered in the context of impact of BV on the utility of reference values.
Endogenous rhythms that underly natural BV can be well characterised, understood and accounted for in appropriately stratified reference intervals (e.g. hormone concentrations during the stages of the menstrual cycle). BV within populations means that RIs need to be stratified on the basis of age, gender and stage of development. Variability around homeostatic set points, diurnal, circadian, circannual and seasonal variability's also impact on the application of reference intervals (RIs), definition and utility. There is an increasing evidence base indicating that BV is not always identical in diseased and non diseased subjects. The reasons for this may be complex. Generational change in method specificity for example may be a cause of observed variability (e.g. cross reactivity with parathyroid hormone fragments in renal disease). Genetic polymorphisms (e.g. angiotensin converting enzyme insertion deletions) may impact on reference intervals depending on the proportionality of the phenotypes in the populations studied.
The utility of population based RIs is defined by the ratio of within to between subject BV (index of individuality (II)). If II is low (<0.6) conventional population based RIs have a low utility. Here, knowledge of the individuals own data is the useful point of reference with reference change values (RCV) providing an alternative means to identification of a clinically significant result. Knowledge of BV allows stratification of RVs to deliver valid RIs
Biological variation affects the clinical utility of reference intervals and must be taken into account when defining them. It is accepted that reference intervals should be accompanied by a description of the population from which they were derived. The question arises as to whether reference intervals should be qualified also by transmission of estimates of indices of biological variation.

**Biologic variation and its effect on the reference values**

**BIOLOGICAL VARIATION – IMPLICATIONS FOR RENAL MEDICINE**

E. Lamb[1]

[1]*Clinical Biochemistry, East Kent Hospitals University NHS Foundation Trust, Kent and Canterbury Hospital, Canterbury, Kent, CT1 3NG, United Kingdom*

Perhaps more than any other clinical speciality, renal medicine relies on quantitative laboratory data for diagnosis, classification and management of patients. Until recently scant regard has been paid to the impact of biological variation on data interpretation in this area. Critical evaluation of the significance of changes in results obtained on analysis of serial specimens can occur only by consideration of biological and analytical variation. Diagnosis and classification of chronic kidney disease (CKD) relies heavily on measurement of serum creatinine/estimated GFR and urinary albumin. Prevalence estimates can differ markedly when multiple as opposed to single measurements are taken into consideration. A critical issue is the ability of biomarkers to detect CKD progression. GFR itself has intrinsic variability, estimated at between 5% to 10%. Taking both analytical and biological variation into account data suggests that a true change in kidney function in an individual (i.e. that exceeding the reference change value, RCV) can only be inferred to have occurred when the change in serum creatinine (or eGFR) exceeds 13%. It is debatable whether use of cystatin C could reduce the RCV. GFR changes of this order exceed the limit that most nephrologists would consider a clinically insignificant change. In the setting of acute kidney injury there is a need to assemble biological variation data on biomarkers (e.g. urinary NGAL, KIM-1) to define disease detection, compare markers against each other and assess whether correction for urinary concentration is desirable. Biological variation may differ in chronic disease states compared to health, although much available evidence relates to variation in health. Biomarkers used to monitor treatment effect in dialysis patients (e.g. haemoglobin, parathyroid hormone) have higher variance in stable dialysis patients than in healthy controls. In many of the above examples the RCVs exceed what most clinicians would consider a clinically significant change: it is likely management is being adjusted in response to changes that reflect biological variation. It is crucial that this message is clearly conveyed to our clinical colleagues. This talk will provide examples of application of biological variation data in nephrology.

**Biologic variation and its effect on the reference values**

## BIOLOGICAL VARIATION AND ITS EFFECT ON THE REFERENCE VALUES: A STUDY IN TURKEY

Y. Ozarda[1]
[1]*Uludag University*

Awareness of the effect of the components of biological variation (BV) on reference intervals (RIs) is essential to establish the reference data used in laboratory testing to notify clinicians of change of patient status. A nationwide multicenter study was conducted to establish RIs in the Turkish population for 26 commonly tested biochemical analytes and to explore sources of variation in reference values, including regionality. Parallel to the nationwide study, the BV of the same parameters were investigated for the region of Bursa, the location of the central laboratory.
In the RIs study, blood samples were collected in 28 laboratories (≥400 samples/region, total 3066) and sera were analysed in Uludag University, Bursa using Abbott reagents and analyzer. RIs were derived by parametric and non-parametric methods. Three-level nested ANOVA was used to evaluate variations among sexes, ages and regions. For BV data, same condition examinations were made of blood samples were taken at 0,1,2,7,14,21 and 28 days from 21 healthy, fasting, reference group volunteers (F=10, M=11). The Fraser and Harris methods were used to calculate the components of BV and reference change value (RCV).
No significant regional differences were determined in any of the 26 analytes by ANOVA. Significant gender differences were observed for 11 analytes, respectively. The index individuality (II) of total BV data was determined as <0.6 in 15 analytes meaning that individual-specific RIs were appropriate and 0.6-1.4 for 11 analytes, which indicated borderline acceptability. In the separate gender evaluation, IIs were calculated as 0.6-1.4 for 20 analytes and in 5 analytes the II was >1.4 indicating that population-based RIs were appropriate.
BV impacts on clinical utility and must be considered in both the generation and application of reference values. Greater focus on RCV and BV will improve the effectiveness of clinical outcomes. The results of this study showed the benefits of reference value stratification with separate calculations for males and female increasing the utility of the population-based RIs. Thus, the RIs derived from the nationwide study are relevant for the entire Turkish population as no regional differences were determined.

**Preanalytical, analytical and postanalytical aspects in molecular diagnostic**

## MASSIVE PARALLEL SEQUENCING IN MOLECULAR ONCOLOGY

M. Neumaier[1]
[1]*Inst. for Clinical Chemistry, Medical Faculty Mannheim, University of Heidelberg, Germany*

Neoplastic transformation of tissues is based on molecular defects in the genome and leads to the development of malignant tumour diseases including cancer. In most cases, there is no single route in the cellular and metabolic pathways through which tumours arise or progress. Particularly, the solid tumours are characterized by extensive genetic and epigenetic heterogeneity. Current breakthroughs in sequencing technologies put cancer genomes at the fingertips of researchers. There are multiple targets for predictive or diagnostic molecular analyses including predisposition and susceptibility genes, somatic driver and passenger mutations/defects for tumour initiation or genes for progression and metastasis. Also, tumour profiling can provide information on druggable targets to be used for therapeutic strategies. Finally, the genetic make-up of a patient´s germline can be important to avoid unwanted drug side effects or to assure effectiveness of a chosen therapy regimen.
Much of the critical information for patient management may be condensed and investigated in so-called cancer gene panels, for which there exist commercial kits on the different NGS platforms. Considering that there are still many cancer entities and individual cancers, in which no pathobiochemical leads have been found, more research is needed to unravel important targets not yet identified. Massive parallel sequencing will provide the raw data, which need to be evaluated by appropriate bioinformatics to make them meaningful for our understanding of the underlying principles of malignant transformation and provide means for future therapies.

**Preanalytical, analytical and postanalytical aspects in molecular diagnostic**

**STANDARDIZATION OF THE PRE-ANALYTICAL PHASE FOR MOLECULAR METHODS IN BLOOD**

M. Pazzagli[4], R. Wyrich[2], K. Gunther[2], P. Verderio[1], S. Pizzamiglio[1], C.M. Ciniselli[1], M. Kubista [3], S. Gelmini[4], F. Malentacchi[4]

[1]*IRCCS Istituto Nazionale Tumori, Milan, Italy*
[2]*Qiagen GmbH, Hilden, Germany*
[3]*TATAA Biocenter Gothenburg, Sweden*
[4]*University of Florence, Italy*

Molecular Diagnostics have allowed a great progress in medicine but their use can be limited by the lack of guidelines for collection, handling, stabilization and storage of biosamples. The development of evidence-based quality guidelines for blood samples requires the identification of critical steps in the pre-analytical procedure that need further development.

To reach this goal, within the FP7 EU granted project "Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics" (SPIDIA; www.SPIDIA.eu) it was planned the implementation of a panel of Pan- European external quality assurance schemes (EQAs) specifically designated for monitoring the performance of the pre-analytical phase of DNA, cell-free DNA and blood RNA testing in blood samples.

With the support of the European Federation for Clinical Chemistry and Laboratory Medicine (www.efcclm.org) more than 320 applications have been collected from about 220 laboratories of 30 different European countries. The participants to the SPIDIA EQAs received the same sample/s (whole blood, plasma) and performed, in two separate runs, sample extractions using their own protocol and reagents or following detailed procedures. Participants then sent back the extracted DNA/RNA to SPIDIA facilities for further analysis, plus details about reagents and protocols used for the extraction phase.

At SPIDIA facilities, the extracted samples have been investigated for quality/quantity/integrity and stability and then the participants have received a report and a qualitative "score" which include the comparison of the performance of the single laboratory with that of the other participants.

From the analysis of the proposed SPIDIA-EQAs, the most critical steps of the pre-analytical procedure have been identified. The results of these studies are under evaluation by the European Committee for Standardization (CEN; www.CEN.eu) for the development of technical specifications as a basis for standardization activities.

**Preanalytical, analytical and postanalytical aspects in molecular diagnostic**

## COMPUTATIONAL METHODS AND INFRASTRUCTURES FOR POST-PROCESSING OF MASSIVE PARALLEL DATA SETS

T. Rattei[1]
[1]*University of Vienna*

Massive parallel molecular data have become fundamental in biological and medical research. The explosive growth of DNA sequence data impressively embodies the ongoing revolution in biology and medicine. However, most of these data are not published in a conventional sense, but deposited in databases and assigned a unique identifying number for quotation in publications. Sequence data from mega-sequencing projects may not even be linked to a conventional publication. This trend and the need for exhaustive computational management of the data makes Next-Generation-Sequencing (NGS) data the ideal showcase to discuss computational methods and infrastructures for post-processing of massive parallel data sets.

This lecture will give an overview in the current status and developments in NGS-oriented bioinformatics. Reduction of raw data, quality control and filtering techniques as well as de-replication methods are typical first-stage post-processing steps. Sophisticated mapping and de novo assembly algorithms reconstruct genomic and metagenomic, transcriptomic and metatranscriptomic from high-coverage short-read data. Statistical analysis of sequence coverage allows quantifying genotypic variants and transcript abundances. Peak identification and quantification methods are used in the analysis of e.g. ChIP-seq data and for the discovery of non-coding RNA genes. All these post-processing steps are computationally challenging, which has induced a broad, heterogeneous landscape of software solutions. Workflow tools allow combining individual programs to large, easy to handle processing workflows. The development of diagnostic tools based on massive NGS data, e.g. for oncology or microbiology, has just started.

Despite the impressive progress of computational NGS data processing within the least years, fundamental improvements will be needed already in the near future. These comprise e.g. storage, locality and availability of NGS data, distributed processing capacities as well as novel approaches for comparative analysis of de-centralized massive data sets.

**Expanding role of clinical laboratory in infectious diseases. Automation?**

**THE COMING OF AGE OF AUTOMATION IN THE CLINICAL MICROBIOLOGY LABORATORY**

P. Bourbeau[1]
[1]*BD Diagnostics*

While automation has steadily spread throughout other areas of diagnostic laboratories, the microbiology laboratory has largely been excluded from this trend. Microbiology specimen processing and culture workup remain largely manual tasks, and few changes to the methods used to perform these tasks have occurred for many years. In my opinion, the level and degree of automation in the microbiology laboratory is poised for a dramatic change, a change that will occur much more rapidly than most laboratorians may suspect. Important technical drivers of automation include MALDI-TOF and the adoption of liquid microbiology transport which have allowed microbiology laboratories to simplify collection and identification systems, creating a work-flow that can be optimized with automation. Other drivers include an aging workforce, increased testing demand, and cost constraints. There are 4 automated specimen processors that can standardize the plating of certain specimen types. While there are significant performance differences between these instruments, they all provide a reproducible streaking pattern, labeling of specimens, and varying media options. However, the real benefits of microbiology automation are realized with the adoption of total laboratory automation (TLA) solutions, of which there currently are 2: Copan WaspLab and BD Kiestra. TLAs offer incubator-imaging systems connected to the specimen processors with inoculated plates transferred into and out of the incubator by a moving track system. Camera systems are programmed to capture images from inoculated plates at predetermined intervals of time, permitting technologists to digitally examine culture plates, avoiding the inefficiencies of sorting plates and placement of plates at the workbench for hours while the technologists perform their work. TLA productivity increases of 2.5- 4 x have been achieved with the Kiestra system. Importantly, achieving productivity increases requires process change such as can be achieved by utilizing the metrics produced by the “Kiestra Dashboard”. Lastly, a recently introduced simulation software from Kiestra is now being used prior to system installation to model efficient specimen throughput, minimize culture turnaround time and appropriately size labor resources.

**Expanding role of clinical laboratory in infectious diseases. Automation?**

**APPLICATION OF AUTOMATION IN MOLECULAR DIAGNOSTICS**

D.H. Walker[1]
[1]*University of Texas Medical Branch-Galveston*

Molecular diagnostics (MD) for infectious diseases (ID) are well established and the "gold standard" for many diseases. MD of ID detect nucleic acids (NA) of infectious agents (IA) or host DNA mutations responsible for disease, including PCR, strand-displacement amplification, and loop-mediated isothermal amplification. If the target NA is present at high concentration, in vitro amplification is unnecessary. Direct hybridization of target DNA to probes is sufficiently sensitive. Automated MD instruments execute all operational steps. Hands-on steps are usually only loading reagents and "raw" sample into the instrument and reviewing and reporting results.
With fewer (now largely automatically executed) steps the test is classified "moderate complexity" by the FDA, meaning that personnel require less training than for "high complexity" tests.
Clinical laboratories adopts automated molecular diagnostic testing because of reduced labor costs, freed-up effort during automated periods, and decreased errors. Automated MD systems range from testing a single specimen on demand to testing batches of specimens. The labor advantage of batch systems is lost and materials cost per result is greatly increased if batch sizes are too small.
High throughput, batch-type automated MD systems include Abbott Real-time, BD Max, Cobas Amplicor, and Ampli-prep/Tagman, Panther, Tigris, and Viper. The IA detected vary among the instruments.
Viper tests urine, genital, throat and rectal swabs for Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, and herpes simplex types 1 and 2 in batches of up to 92 samples. The instrument extracts and purifies NA, amplifies DNA, and detects the product.
On-demand test systems include FilmArray, GeneXpert, IsoAmp, Simplexa Direct, and Verigene.
FilmArray has a 20 agent respiratory panel, a 27 target blood culture panel including three antibiotic resistance genes, and a GI panel of 14 bacteria, five viruses, and four protozoa. FilmArray uses a "pouch" that contains all reagents for sample processing, NA extraction, first round multiplexed PCR, and second round single-plex PCRs, each performed on an array spot. After PCR, DNA melting analysis performed automatically by the instrument identifies the pathogen.

**APFCB Symposium Environmental issues**

## ADOPTING ENVIRONMENTAL GUIDELINES AND COST SAVINGS

T. Badrick[1]
[1]*Faculty of Health Sciences and Medicine, Bond University, Robina, Australia*

Waste in the form of power, recyclables, chemicals or water represent cost and potential pollution. Drought, increased water costs, concerns about the viability of coal burning power plants, and the ever increasing cost of power are daily reminders of the need to conserve and recycle.
Clinical Laboratories are significant polluters and users of scarce resources, but generally are unaware of the impact they make. There is an ISO standard on Environmental Management Systems (EMS) - ISO 14001 which is available in order for any organisation to introduce a structured approach for identifying and managing legal obligations, its environmental impacts and to meet key stakeholder aspirations. The benefits of the established EMS to the organisation have been improved environmental awareness and performance, cost savings, business efficiencies, compliance with regulations, improved corporate image, marketing opportunities, reduced risk of disaster and improved relationships with the public and community. However organisations often believe there is a significant net cost in adopting an EMS. Implementing an EMS will lead to improved environmental awareness and performance, improved relationships with the local community, environmental and real cost savings, as well as a leaner and greener approach to business. The pay back cost in terms of the implementation cost for ISO 14000 has been estimated at between 18 and 24 months in other industries. This session will describe the process of implementing ISO 14000 in a large clinical network and the cost implications of that implementation.

**APFCB Symposium Environmental issues**

## ENVIRONMENTAL LABORATORY FACILITIES MANAGEMENT

D. Jackson[1]
[1]*ARUP Laboratories, Inc.*

The greening of the clinical laboratory is a phenomenon with far reaching implications. Laboratories face particular challenges and pressures to become more environmentally friendly. 24 hour operations, patient samples, regulatory requirements, and infection control play a role. Intensive requirements for energy, water, and chemicals make environmental consciousness a unique challenge. By implementing strategies of the US Environmental Protection Agency's symbol elements of Reduce, Reuse, and Recycle, any laboratory can "go green." ARUP Laboratories of Salt Lake City, Utah, has undertaken such a venture and has achieved some success in each of these areas. Owned by the University of Utah, ARUP provides esoteric testing for clients throughout the US. Ten years ago, ARUP determined to become more environmentally conscious. What started with a few simple conservation efforts steadily matured into an extensive program based on the reduce, reuse, and recycle philosophy.

ARUP followed critical steps to achieve its goals. First, a long range plan was developed entailing reducing, reusing, and recycling all aspects of our business. We committed to be determined, yet patient. The plan incorporated the Company's long term goals, as well as its financial capabilities. Second, we initiated an intensive education program for our employees. Helping them understand the value as well as the ease of recycling was critical to success. Third, over time we gradually built the infrastructure. Initiatives were added based on financial and space constraints.

ARUP has accomplished significant environmental improvements over the last decade. Some of the most significant accomplishments include reducing cleaning and floor stripping chemicals, landscaping watering, and dry extinguishing chemicals. We also reuse carpet and office and laboratory furniture. Finally, through employee efforts we recycle over 60% of our total waste annually, including, among others, styrofoam and glass.

ARUP started its environmental journey with blue recycling waste baskets placed in offices and laboratories. Environmental consciousness is now our culture. Any laboratory can become more environmentally aware regardless of the scale of its efforts.

**APFCB Symposium Environmental issues**

**ENVIRONMENTAL GUIDELINES FOR CLINICAL LABORATORIES**

J. Lopez[1]
[1]*Mahsa University*

Environmental Guidelines for Clinical Laboratories
Joseph B. Lopez
Department of Biomedical Sciences, MAHSA University, Kuala Lumpur, Malaysia
Abstract
All human activity results in carbon emission footprints which in turn contribute to climate change. While there has been an increasing societal awareness on the need to reduce carbon emission, clinical laboratories have yet to make a serious effort, if at all, to mitigate their impact on the environment. The effort needs to begin with a commitment by laboratories to reduce their environmental impact. Once this is done, laboratories should take the following steps to achieve good environmental practices: announce an environmental policy, secure the support of senior management, initiate documentation provide staff training, undertake environmental audits and appoint an environmental manager. Laboratories may work towards achieving accreditation to an environmental management standard such as the ISO 14000 series. Environmental management may be integrated with overall quality management as the two are closely linked. Although there will be some initial costs, good environmental practices eventually bring cost savings in the long run. They also contribute to corporate social responsibility which will improve public relations. Environmental improvement should be based on the universal 3R concept to reduce, reuse and recycle. Practices to reduce the consumption of power at a personal level may be implemented without cost. Policy initiatives should encompass ways to reduce energy and water usage and laboratory wastes. They may include a green purchasing policy for equipment, laboratory furniture and reagents as well as the management of packaging wastes. A reduction of test numbers and collection tubes should be attempted. Paper management involves all aspects of 3R. The recycling of solvents wastes could be practised with some initial investment on equipment. The construction of new laboratories or renovations to existing ones are opportunities to make them more environmentally-friendly. The imposition of environmentally-friendly conditions on contractors and advocacy of policies to associates are also integral parts of the programme.

**IFCC Symposium Peer review and ethics in publications in the electronic age**

## PEER REVIEW IN SCIENTIFIC PUBLICATIONS

K. Adeli[1]
[1]*Chair, IFCC-Communications and Publications Division; Head and Professor of Clinical Biochemistry, Clinical Biochemistry, The Hospital for Sick Children, University of Toronto*

Peer review has been defined as a process of subjecting an author's scholarly work, research or ideas to the scrutiny of others who are experts in the same field. This process is used primarily by editors to select and review submitted manuscripts, and determine the acceptability (or validity) of the research findings or ideas. Peer review has been portrayed as a quasi-sacred process that helps to validate scientific findings. It functions to encourage authors to meet the accepted high standards of their discipline and to control the dissemination of research data to ensure that unwarranted claims, unacceptable interpretations or personal views are not published without prior expert review. Despite its wide-spread use by most journals, the peer review process has also been widely criticised due to the slowness of the process to publish new findings and due to perceived bias by the editors and/or reviewers. Often, authors of rejected manuscripts have concerns with the editors and reviewers making biased editorial decisions. It typically takes several months or even years in some fields for a submitted paper to go through the full peer review processed, be accepted, and published in print.

The major advantage of a peer review process is that peer-reviewed articles provide a trusted form of scientific communication. Even if you are unfamiliar with the topic or the scientists who authored a particular study, you can trust peer-reviewed work to meet certain standards of scientific quality. Since scientific knowledge is cumulative and builds on itself, this trust is particularly important. Unfortunately, the recent explosion in online only/electronic journals has led to mass publication of a large number of scientific articles with little or no peer review. This poses significant risk to advances in scientific knowledge and its future potential.

In this presentation, I will review the current peer review process models and discuss both the advantages and shortcomings of the current models. The audience will be familiarized with some practicals steps that can be taken by potential authors to ensure success with the peer review of submitted manuscripts.

**IFCC Symposium Peer review and ethics in publications in the electronic age**

## HOW TO WRITE A SCIENTIFIC PAPER: A PRACTICAL GUIDELINE

E. Delvin[1]
[1]*Department of Biochemistry, Faculty of Medicine, University of Montreal*

Writing a scientific paper is a major intellectual undertaking that requires time and energy, not only of the author, but also of the journal editor, reviewers and the publisher. When deciding to write a manuscript, the question that arises is why and for whom is the paper written. Is it for promotion, obtaining grants or amplifying curriculum vitae? Although these reasons are valid, they are not sufficient. The main reason should be to share, with the science community, data or hypotheses that advances knowledge and understanding in a specific field or domain.
In determining the suitability of submitted articles for publication, particular attention should be placed on: the degree of novelty and significance of the research and the extent to which it adds to existing knowledge in Laboratory Medicine.
The following serious issues are encountered on an occasional basis, but more often than liked. Multiple submissions of the same manuscript to two or more journals, submission of a paper already published in another language and plagiarism (especially of small parts of a paper).
Content is essential in any publication. The scientific message should be clear, useful, and exciting. Thus, the presentation is critical. It should convey the authors' thoughts in a logical manner such that the reader arrives at the same conclusions as the author. It should be constructed in the format that best showcases the authors' material, and written in a style that transmits the message clearly. The response to reviewers is equally important in the process of submission. These issues will be briefly covered.

**IFCC Symposium Peer review and ethics in publications in the electronic age**

## OPEN ACCESS PUBLISHING IN THE ELECTRONIC AGE

G.L. Kovacs[1]
[1]*Szentagothai Research Centre, University of Pecs, Hungary*

The principle of open-access publishing (OAP) is more and more prevalent also on the field of laboratory medicine. Open-access journals (OAJs) are available online to the reader usually without financial, legal, or technical barriers. Some are subsidized, and some require payment on behalf of the author. OAJs are one of the two general methods for providing OA. The other one is self-archiving in a repository. The electronic journal of the IFCC (eJIFCC) is a platinum OAJ - i.e. there is no charge to read, or to submit to this journal. Traditionally, the author was required to transfer the copyright to the journal publisher. Publishers claimed this was necessary in order to protect author's rights. However, many authors found this unsatisfactory, and have used their influence to effect a gradual move towards a license to publish instead. Under such a system, the publisher has permission to edit, print, and distribute the article commercially, but the author(s) retain the other rights themselves. An OA mandate is a policy adopted by a research institution, research funder, or government which requires researchers to make their published, peer-reviewed journal articles and conference papers OA by self-archiving their peer-reviewed drafts in a repository ("green OA") or by publishing them in an OAJ ("gold OA"). Creative Commons (CC) is a nonprofit organization that enables the sharing and use of creativity and knowledge through free legal tools. The free, easy-to-use copyright licenses provide a simple, standardized way to give the public permission to share and use creative work. CC licenses let you easily change your copyright terms from the default of "all rights reserved" to "some rights reserved." OA publishing also raises a number of new ethical problems (e.g. predatory publishers, fake papers).
Laboratory scientists are encouraged to publish their scientific results OA (especially in eJIFCC). They should, however, be aware of their rights, institutional mandate, the procedures of publishing and post-printing, and the potential risks of OAP. Recent research shows that OA articles are wider seen, and are just starting to be better cited than equivalent papers published in traditional subscription journals.

**IFCC Symposium Peer review and ethics in publications in the electronic age**

## ETHICS IN ONLINE PUBLICATIONS

P. Vervaart[1]
[1]*Principal Scientist Pathology Services, Royal Hobart Hospital, Australia*

Journals have been the publishing the results of scientific investigations since the founding of Philosophical Transactions in 1665. Since then we have witnessed a massive expansion in the number of journals to the point that there are now approximately 28,000 active, peer reviewed journals collectively publishing more than 1.8 million articles per year. Before the mid-1990s, these journals were only available on paper but by the end of the 20th century, most journals had moved to online platforms. Online publication has also served as the impetus for the move to 'open-access' to the information contained in journals. The fact that a publication is 'on-line' and 'open-access' does not negate the responsibility of the author and the publisher to publish in an ethical way.

The document produced by the IFCC Ethics Task Force (TF-E) on publication ethics states that 'Ethics in Science at its broadest level encompasses research ethics, medical ethics, publication ethics, conflicts of interest, ethical responsibilities as educator, plus many other areas.' Thus publication ethics is a continuum from the first step of research design through to the information being read by the reader. In general terms 'publication ethics' includes the ethical behaviour of the authors in writing and submitting a scientific manuscript to a publisher for the purpose of publication, thus any discussion of publication ethics must include the role of the authors, referees, publisher and reader and the issues of authorship (and the use of 'ghosts'), plagiarism, duplicate publication (including in different languages), image manipulation (particularly in the era of digitisation), and conflict of interest. To aid the authors, and others involved in the process of publication, a number of resources are now available particularly those from the Committee on Publication Ethics (COPE) and the World Association of Medical Editors (WAME).

More recently the issue of 'publisher ethics' has also been raised, particularly with the sudden increase of what could be termed 'predatory' publishers utilising the open access model to publish low quality articles, which often do not adhere to the guidelines mentioned above, utilising an author-pays model of open-access publishing for their own profit.

**COLABIOCLI Symposium Bleeding and thrombotic disorders: evaluation by haemostasis laboratories**

**INHERITED THROMBOPHILIA**

J. Annichino-Bizzacchi[1]
[1]*Hemocentro/Faculty of Medical Science - UNICAMP*

Heritable thrombophilia screen usually includes protein C (PC), protein S (PS), and antithrombin (AT) assays; tests for FV Leiden and prothrombin G20210A mutation. There is a debate regarding the benefit of thrombophilia screening in clinical practice: it is not cost-effective in unselected patients; there is little evidence to support the use to predict VTE recurrence. This diagnosis may be useful to family members, particularly women regarding contraceptive and pregnancy.

Plasma samples should preferentially be frozen at -40oC into multiple aliquots post venepuncture. Functional assays are affected by freeze-thawing of samples. Local reference ranges and internal quality should be done. Only functional assays of heparin cofactor activity, preferentially using bovine thrombin, will detect both type I and II AT deficiency. Chromogenic method for PC deficiency is the first line option, as clotting-based assays are subject to interference by a number of variables like FV Leiden, lupus inhibitor, elevated FVIII levels, and heparin. Diagnosis of PS deficiency is widely accepted as problematic. Free PS antigen estimation is the method of choice for PS deficiency. PS activity assays is influenced by many variables, and low results should always be further investigated with an immunoreactive assay of free PS. FV Leiden and G20210A prothrombin gene mutation detection relies on amplification of the mutation site from either genomic DNA or from mRNA, followed by enzyme digestion. Many DNA-based assays have been described including ELISA, melting curve analysis and fluorescence allele-specific discrimination method. Errors occur in genetic testing and repeats should be considered.

Testing should be done one month after stop anticoagulation. Pregnancy and estrogen reduce PS level. PC activity is related to age and sex. Measuring of other vitamin-K dependent factors is interesting to differentiate heritable from acquired PC or PS deficiency.

Thrombophilia testing is expensive,requiring especialized laboratory. Clinicians should be advised about the clear benefit to request these tests.

**COLABIOCLI Symposium Bleeding and thrombotic disorders: evaluation by haemostasis laboratories**

## OVERVIEW OF HAEMOSTASIS

M. Echenagucia[1]
[1]*Banco Municipal De Sangre-Escuela De Bioanalisis Universidad Central De Venezuela*

When a blood vessel is ruptured blood clots promptly at the site of vessel injury. Extensive research has been performed in an effort to understand the mechanisms of both the maintenance of blood fluidity within blood vessels and the prompt clotting upon injuries to blood vessels. At the 19th century, it was understood that prothrombin, could be activated in the presence of "thrombokinase" and calcium to produce thrombin. Before the second war the prothrombin time was described, heparin was discovered, and it was realized that people with haemophilia were deficient in a factor present in the plasma. After the war, Ratnoff and Davie (USA) and Macfarlane (UK) described the "waterfall" theories of blood coagulation, which helped to explain the increasingly complex function of the rapidly expanding numbers of discovered coagulation factors. This scheme did not take into account the participation of platelets nor other cellular components. The concept has been challenged by a new one in which cells are important participants in the coagulation process. In a modern view, the cell-based model of coagulation actually occurs in a overlapping step-wise process: initiation, amplification and propagation on the surface of activated cells, to produce the burst of thrombin that causes stabilization of the fibrin clot and stop bleeding. In more recent years there has been a growing interest in the causes of arterial and venous thrombosis and it has become necessary to revise the model propose. Nevertheless, the essential elements of haemostasis remain the vessel wall, the platelets, and the blood coagulation and fibrinolytic mechanisms. Remarkable advances have been made in our understanding of the mechanisms of blood coagulation, our knowledge has increased with the introduction of new technologies: from simple laboratory tests to protein chemistry, to DNA technology, and to gen targeting technology. Advances in basic research have been successfully translated into improved methods for the diagnosis of bleeding disorders as well as thrombosis, end the development of recombinant clotting factors for replacement therapy in patients with haemophilia. New promising anticoagulants have also been developed for the treatment of thrombotic disorders.

**COLABIOCLI Symposium Bleeding and thrombotic disorders: evaluation by haemostasis laboratories**

## THE ANTIPHOSPHOLIPID SYNDROME

R. Forastiero[1]
[1]*Favalaro University*

The Antiphospholipid Syndrome (APS) is an autoimmune disease that requires clinical manifestations (venous or arterial thrombosis, and/or recurrent pregnancy morbidity) and laboratory tests for diagnosis. Antibodies to a variety of phospholipids usually detected by coagulation and immunology assays are referred to as antiphospholipid antibodies (aPL). The laboratory criteria include lupus anticoagulant (LA), and high titers of IgG and/or IgM anticardiolipin (aCL) and anti-β2 glycoprotein I antibodies (aβ2GPI). The diagnosis of LA relies on a set of successive phospholipid-dependent clotting tests. In the aCL test, cardiolipin or a mixture of phospholipids is coated onto an ELISA plate, and in the aβ2GPI assay, β2GPI is directly coated onto a high-binding ELISA plate or other forms of solid phases. The diagnosis of aCL and aβ2GPI relies on the presence of antibodies at titers >40 units or >99th percentile. Persistent positivity of laboratory assays is important suggesting an interval of at least 12 weeks between the two positive tests. According to the 2006 criteria for the classification of APS, a single positivity of any of the three aPL tests mentioned before fulfill the serologic criteria. More recently, however, several studies have shown that the risk of thrombosis increases with the number of positive tests in APS patients and also in asymptomatic carriers of persistent aPL. The concept of triple positivity (LA/aCL/aβ2GPI) conferring a higher risk for thromboembolic events (first or recurrent thrombosis) and pregnancy losses has been proposed taking into account several retrospective and prospective studies. A number of studies have also suggested that testing for other aPL than those recommended in the last APS criteria may help to identify the syndrome. Among them, antibodies to prothrombin and the complex of prothrombin with phosphatidylserine have been proposed. Taken into account this increasing knowledge, it is now proposed that the definition of APS should include a different clinical risk to develop APS-related events: 1) definite APS in patients with triple aPL positivity (high risk group), 2) probable APS in patients with double aPL positivity (medium risk group) and 3) non-APS in patients with single aPL positivity (low risk group).

**COLABIOCLI Symposium Bleeding and thrombotic disorders: evaluation by haemostasis laboratories**

**HAEMOPHILIA AND VW DISEASES**

J. Pereira[1]
[1]*Department of Hematology-Oncology, School of Medicine, Catholic University of Chile*

Hemophilia A and B are X-linked, recessive, hemorrhagic diseases caused by deficiencies of factor VIII and IX, respectively and the degree of hemophilia severity is based on the coagulation factor activity level. The initial diagnosis of hemophilia patients depends on the demonstration by the laboratory of the factor deficiency as well as the identification of the respective disease severity. The demonstration of factor inhibitors will result in additional complex treatments, which highlights the key role played by the laboratory in the management of hemophilia patients. Preanalytical, analytical and postanalytical issues, common to all coagulation tests, also affect laboratory assessment of hemophilia. Most of the preanalytical variables (transport and storage) will result in inaccurate results but not important changes in factor levels. Analytical variables include among others, assay variability, ability to determine low factor levels, one-stage vs. two-stage factor assay and reagents choice. Factor inhibitor testing is also affected by several variables such as methodology (Nijmegen vs. Bethesda), type of plasma used and interpretation criteria.
Von Willebrand disease (VWD) is an autosomical dominant bleeding disorder affecting primary hemostasis, caused by quantitative or qualitative defects in von Willebrand factor (VWF). VWD type 1 comprises mild to moderate quantitative deficiency of VWF protein. Type 3 VWD, is characterized by absence of the protein and qualitative defects in VWF are classified as type 2 VWD and divided into four variants (A, B, M, N). To confirm the diagnosis of VWD in patients with clinical suspicion, laboratory testing is needed. The most typical screening panel includes VWF antigen (VWF:Ag) to quantify plasma VWF, and the VWF ristocetin cofactor assay (VWF:RCo) which determines the function of VWF. FVIII:C usually measured as well, since VWF is a carrier of FVIII. VWF multimeric pattern is performed to distinguish type 2 variants. From a laboratory standpoint, the diagnosis of type 1 VWD ( 80% of patients), constitutes a non-solved challenge due to the many acquired and genetic determinants of the FVW concentration and the lack of generalized consensus on cut-off values used to establish a definitive diagnosis of type 1 VWD.

**IFCC's Committee-Evidence Based Laboratory Medicine Symposium EVIDENCE BASED LABORATORY MEDICINE IN DECISION MAKING: A VALUE-BASED BUSINESS PERSPECTIVE**

## VALUE OF BIOMARKERS IN DIABETES CARE: MARRIAGE OF BETTER HEALTHCARE AND BUSINESS OPPORTUNITY

D. Aslan[1]
[1]*Pamukkale University Medical Faculty Department of Medical Biochemistry*

As seen in the clinical guidelines on disease management, several biomarkers such as HbA1c and LDL-cholesterol can be used as quality indicators for determining the quality of healthcare delivered. The Diabetes Complications and Control Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that the incidence of complications (outcomes) of diabetes such as retinopathy and nephropathy was related directly to the HbA1c levels. The LDL-cholesterol levels are also related to the cardiovascular disease (CVD), and it is shown that reductions of LDL-C levels are helpful in treating CVD. The reduction of such test results to the specified levels can be considered as successful outcomes, and the test results of patients can be used as standards for determining the care quality. The overall healthcare status can be driven from the test results analyzed in the structured manner.
An observational study based on the real-life test results can be established by the systematic approach (Ask, Acquire, Appraise, Apply, Assess - 5A) of the Evidence-Based Laboratory Medicine (EBLM). The findings from the study can be analyzed based on the Total Quality Management (TQM) approach for making the business case for improving healthcare quality.
In this talk, the findings from our multicenter cohort study on diabetic patients, and the test results of diabetic patients collected by the structured programs will be shared in the context of the EBLM approach together with the European Foundation of Quality Management (EFQM) Excellence Model as a business management approach. The adherence of the recommendations from the clinical practice guidelines on the diabetes management will be discussed from the context of healthcare services stakeholders, such as policy makers, government, healthcare providers such as healthcare organizations, clinicians, laboratory personnel, payers and patients.

**IFCC's Committee-Evidence Based Laboratory Medicine Symposium EVIDENCE BASED LABORATORY MEDICINE IN DECISION MAKING: A VALUE-BASED BUSINESS PERSPECTIVE**

## SHOULD WE LICENSE THIS TEST? PRACTICAL USE OF EVIDENCE BASED LABORATORY MEDICINE (EBLM) TECHNIQUES

R.H. Christenson [1]

[1]*Universty of Maryland School of Medicine, University of Maryland Medical Center, Baltimore, MD 21201*

Partnerships between industry and academics are essential for making innovations broadly available for benefiting public health and individual patients. A major challenge is determining which innovations show great promise versus those with merit, but showing less potential. Prioritizing candidate innovations for further characterization, potential licensing, development, refinement and adaptation to formats available for wide dissemination is a major task.
The A6 methodology consists of ASKing the clinically relevant question; ACQUIRing the evidence; APPRAISing the information; ANALYZing the evidence; APPLYing the findings in practice and AUDITing diagnostic effectiveness. The example of a new biomarker for stroke diagnosis will be presented. Asking the right question is vital for evaluating a new diagnostic. Using the acronym 'PICOT' is particularly useful. 'P' signifies (P)opulation or (P)atient; the population in the example has signs and symptoms of ischemic stroke. 'I' is for (I)ndicator or test; here 'I' is a new test for stroke diagnosis. 'C' specifies the (C)ontrol group, i.e. patients with conditions that mimic stroke. 'O' signifies the (O)utcomes of interest and 'T' signifies the (T)iming of diagnostic testing.
Considerations for designing a pilot trial to evaluate the diagnostic effectiveness of the novel stroke marker will be presented. This will be a randomized controlled trial developed using the A6 methodology to determine if this is the right test, for accurately identifying the right patient at the right time for administering therapy proven to improve outcomes. EBLM tools for formulating the clinical question, specifying the needed diagnostic performance and determining the number of patients needed to properly power the study for reliable pilot results are all part of this example.
Collaboration is necessary for assuring broad availability of innovations to benefit public health and individual patients. EBLM tools can assist innovators in focusing the clinical application of their discoveries. These tools can also assist industry decision makers in diligently examining the potential of novel innovations. An example involving a stroke marker illustrates practical use of EBLM processes.

**IFCC's Committee-Evidence Based Laboratory Medicine Symposium EVIDENCE BASED LABORATORY MEDICINE IN DECISION MAKING: A VALUE-BASED BUSINESS PERSPECTIVE**

## HOW INDUSTRY SHOULD UTILIZE EVIDENCE BASED LABORATORY MEDICINE

C. Price[1]
[1]*University of Oxford*

The goal of Evidence-Based Laboratory Medicine (EBLM) is the use of the best evidence for investigations in making decisions about the care of individual patients. Two of the key drivers in the delivery of healthcare today are improving the quality of care, and value-for-money. Meeting these objectives involves collaboration between a number of stakeholders - from researcher through to patients. One of the tools in this complex relationship is the value proposition.
The value proposition is a statement of the benefits that the provider will offer to the customer, along with the approximate price it will charge for those benefits. Whilst it is a concept used for many years in the commercial world, it can be applied in healthcare – including laboratory medicine. The complexity in healthcare arises because there are a number of customers and stakeholders, and each may have a differing perspective of what is required and the value of what is offered. The key attributes of a value proposition are (i) identifying the customer, (ii) the customers need, (iii) the product or service, (iv) the benefits of the product or service to the customer, (v) the cost of the product or service to the customer, (vi) the competition, and (vii) the proof to substantiate the claims.
Looking at this from an EBLM perspective, clearly the patient is the customer and key stakeholder, but other stakeholders, e.g. carers and care provider organisations, will also benefit from the use of laboratory medicine services. The product is that part of the care pathway in which the test result is used; the benefit will only be achieved if the result is acted upon. The benefit can then be described in clinical, operational and/or economic terms as an outcome. Competition can be considered in both clinical and economic terms - the best outcome, and the best cost, i.e. value-for-money. The proof to substantiate the claims lies in the evidence of clinical and cost effectiveness.
EBLM is the foundation of the value proposition in laboratory medicine and the language for dialogue between industry, healthcare providers, payers and patients.

**How ISO 15189 has influenced laboratory testing?**

S. Ehrmeyer[1]

[1]*University of Wisconsin School of Medicine and Public Health*

How ISO 15189 has influenced laboratory testing?
The need for quality test results is readily agreed upon by all. Unfortunately, things do "happen" to affect test quality! To ensure quality patient test results that are accurate, reliable and timely, internationally recognized laboratory standards, such as ISO 15189:2012, link quality and adherence to a series of requirements. These standards, based on good laboratory practices, recognize that quality encompasses much more than evaluating just the analytical phase of testing. Laboratories following ISO must take a broad, comprehensive view of testing. This view starts with a quality management system that addresses the many management and technical processes that ultimately impact the entire testing process and test quality. As a consequence, those involved must expand their focus beyond the analytical phase of testing to ensure that the many requirements for the total testing process are implemented. Self-regulation through internal audits is an important component of these quality standards and requires laboratories to ensure that their activities conform to the standards' directives. When inconsistences are found, quality improvement must be instituted. Recognized and accepted ISO standards provide appropriate directives for laboratories to generate universally accepted quality test results.

**How ISO 15189 has influenced laboratory testing?**

## DEVELOPMENT OF ACCREDITATION OF MEDICAL LABORATORIES IN EUROPE. DO WE NEED IT TO BECOME OBLIGATORY

W. Huisman[1]
[1]*EFLM*

The first quality systems for medical Laboratories were developed between 1990-2000. Differences existed between the countries and within Europe the EC4 developed its Essential Criteria. The first international standard was published in 2003: ISO15189.Its acceptance in Europe was a gradual process. However, today it is seen as the standard. It helps laboratories to set up a robust quality system. It indicates which aspects should be taken care of in relation with competence. The third edition of this standard was edited in 2012. Its structure and wording are clearer. It includes ict and ethics in the text itself. It makes a clear discretion between validation and verification. In its use it is more focused on preventing errors by stressing the importance of a risk analysis and continuous improvement. For specific aspects the TC212 of ISO, is now working on specifications related to pre-examination, measurement uncertainty and risk analysis.
The importance of accreditation according to ISO15189 is recognized by some authorities. In France it is demanded by law for all examinations. In Belgium for molecular biology, and in Latvia in hospital laboratories. In Rumania tests are only reimbursed when the laboratory is accredited. And in those countries, which had set up their national system, a transition to 15189 has started. But does it need to become mandatory?
For testing most substances clients can choose between accredited or not accredited laboratories. For medical examinations a patient can not choose, especially not for stat tests. Already in 2000 ILAC stated that medical tests are different from others because " they deal with human life and their results have a big impact on human health care ".
Authorities in a country should offer the medical care which fulfills the requirements of the patients. Just as indicated in the standard. For that reason they should consider to make accreditation obligatory. The same holds true for the reimbursement of the service .
To avoid an over exaggeration of the demands during assessment calibration of the assessors should take place. An inquiry in 2013 by the Health Care Committee of the EA has shown the present situation. It stays important that the exact demands are related to the purpose.

**How ISO 15189 has influenced laboratory testing?**

## ACCREDITATION IN LABORATORY – NEW ISO15189:2013 BENEFITS AND NEGATION FOR PATIENT SAFETY

T. Zima[1]
[1]*Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

At the beginning of the 21st century is one of the priorities in laboratory medicine improvement of quality management system for patient safety including accreditation. People are asking why do we need accredited labs ? Accreditation is a good way to demonstrate competence of the laboratory, is a tool to recognize laboratories world-wide, leads to high standard of services for clients.
The strategic documents of IFCC and EFLM support accreditation of labs and recognised that ISO 15189:2013 encompasses all the assessment criteria specified in the policy of quality. The first version was issued in 2003 and the last version in 2012 is oriented on process approach with detailed division with clearly defined requirements.
The processes for selecting, evaluating and transfer of samples and results between the labs and referral laboratories are precisely described with responsibilities of both sites. Evaluation and audits (4.14.) contain new chapters leading to better interactions with clients (user feedback) and staff of laboratory (4.14.4.). Risk management (4.14.6.). is important part to identify the potential risk for patients safety and laboratory processes with aim to eliminate them. The labs shall describe the quality quantitative indicators with regular reviewing at all laboratory processes. For personnel is important to precise job description with competencies and responsibilities with evaluation of effectiveness of continual professional development. Precise determination of biological reference intervals and critical values are important for correct communications connected to patient safety. Now, laboratory information management (5.10.) and ethical conduct (4.1.13.) are normative and they are showing the impact of these topics for patients.
Czech Republic has 265 accredited labs in all areas and reassessment according to new ISO which started in winter 2013 and 4 labs have passed successfully.
The accreditation of labs improves facilitation of accurate and rapid diagnostics, efficiency of treatment and reduction of errors in the laboratory proces. Accreditation is not about the best one, but about the one with the system of standard procedures with aim to improve the quality and patient safety. Supported by RVO MZ CR VFN64165.

**Established and emerging markers of renal function - "Chronic Kidney Disease – Best laboratory practice"**

**CREATININE, CYSTATIN C AND URINE ALBUMIN – ANALYTICAL UPDATE**

J. Delanghe[1]
[1]*Ghent University*

creatinine, cystatin C (cys C), and micro-albumine are key parameters for assessing renal function. In order to improve their clinical utility, standardisation efforts are ungoing.
- NIST has issued SRM 3667 creatinine urine reference material which provides an initial step for a urine creatinine reference system. A new cys C calibrator (ERM-DA 471/IFCC), was released in 2010, which enables an assay-independent, GFR-prediction equation. SRM 2925 is a primary certified reference material for use with higher order reference measurement procedures for albumin. It is a recombinant human albumin solution that will be characterized. A commutability study has not been done. NIST SRM 3666 is a matrix certified reference material that is in preparation. It will be albumin in frozen human urine.
Discussion: It is desirable to develop a single cys C equation that can be promoted worldwide. CKD-EPI Cystatin C Equation group proposed a new equation using standardized cys C (variables: age, gender, cys C) and report a 2nd equation using both cys C and creatinine. The combined equation showed an improved performance. No real improvement vs. the creatinine equation was seen using the cys C equation alone. Cys C equation did not perform well at either very high or low eGFRs. The cys C only equation was not more accurate than the combined one, but may be useful when race cannot be specified.
The utility of candidate reference materials for use in standardization of albuminuria methods is being assessed. Bachmann's albuminuria harmonization paper provides compelling evidence that standardization is needed. Median differences between the largest pos. and neg. biases vs IDMS were 37-45%. Biases mostly exceeded ±10%. Mean biases ranged from -35% to 34% at 15 mg/L. Bias was the major source of disagreement among routine measurement procedures.
New cys C based eGFR equations are a further step towards better eGFR estimation. Establishing reporting cut-offs for urine albumin will be difficult when using non-standardized assays. A lower cut-off and stratifying results by gender is to be considered for albuminuria. It will be several years before standardization of urine albumin occurs. Standardization efforts will improve agreement among results.

**Established and emerging markers of renal function - "Chronic Kidney Disease – Best laboratory practice"**

## EGFR EQUATIONS – THE THEORY AND THE PRACTICE

G.R.D. Jones[1]
[1]*SydPath, St Vincent's Hospital, Sydney, Australia*

Glomerular Filtration Rate (GFR) is a key parameter in current international guidelines for the diagnosis, staging and monitoring of chronic kidney disease. As formal measurement of GFR is expensive and time consuming, routine clinical practice relies on estimates of GFR (eGFR) which are most commonly based on serum creatinine with additional information from patient age, sex and race (in some settings). The most commonly used equations, MDRD and CKD-EPI, are based on large data sets with mathematical optimisation to produce the best relationship between eGFR and formally measured GFR. These equations are optimised for the "average" people which were included in the studies used to establish them. Thus individual patients who differ in relevant factors from the centre of the population for a given age and sex can expect to have deviant results. These factors may be the amount of muscle relative to the body surface area, the size of the kidneys, the volume of distribution, the rate of creatinine secretion in the tubules or other factors. For most people these factors contribute to a variation of the eGFR of up to +/- 30% relative to measured GFR. Attempts to reduce this variation have not proved particularly effective, suggesting a component of random variation which is not easily accounted for.

In clinical practice it is necessary to be aware of limitations of the equations where significantly erroneous results may be produced. The first limitation is clinical settings where there equations are known to not be valid. These include age <18, pregnancy, dialysis and rapidly changing renal function. Less marked limitations include extremes of muscularity (lean or muscular) and vegetarian diet. Any effect on serum creatinine measurements such as drug interferences will also produce aberrant results.

An important area for understanding eGFR results is the effect of body surface area (BSA) normalisation. for the eGFR equations are produced already normalised for a BSA of 1.73m2 to reduce the effect or patient size on the frequency of CKD diagnosis. For large subjects the GFR without BSA normalisation (ie in mL/min) is higher than the equation result (in mL/min/1.73m2) and the converse for smaller patients. This difference can be important for drug dosing decisions.

**Established and emerging markers of renal function - "Chronic Kidney Disease – Best laboratory practice"**

## THE UPDATED KDIGO GUIDELINE FOR CHRONIC KIDNEY DISEASE

E. Lamb[1]
[1]*East Kent Hospitals University NHS Foundation Trust, Kent and Canterbury Hospital, Canterbury, Kent, CT1 3NG, United Kingdom*

The last decade or so has witnessed a succession of national and international guidelines in chronic kidney disease (CKD), beginning with the publication of the United States' National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) in 2002 [1]. The work of NKF K/DOQI has now been subsumed by an international organisation, Kidney Disease Improving Global Outcomes (KDIGO). In 2013 KDIGO published updated guidance on the identification, classification and management of kidney disease [2]. This guideline largely builds on what has gone before, but there are important new inclusions relevant to laboratory medicine. As would be expected, there is a large focus on the mainstays of diagnosis; glomerular filtration rate (GFR) and proteinuria. There is updated guidance regarding the equations that should be used for estimating GFR, with inclusion for the first time in such a guideline of a specific role for cystatin C. Albuminuria measurement is recommended as the preferred mode of detection of proteinuria and, critically, the threshold of clinical significance is lowered to the level of what has previously been termed 'microalbuminuria'. Throughout there is an emphasis on standardisation, acknowledging that for test results to be useful in the context of guidelines they should produce the same result, within clinically meaningful limits, in all laboratories in which they are measured. There is little doubt that these thoughtful international guidelines will influence future laboratory practice.

References

1. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1-266.
2. Kidney Disease Improving Global Outcomes. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int 2013;3(1):1-163. http://www.kdigo.org/clinical_practice_guidelines/pdf/CKD/KDIGO_2012_CKD_GL.pdf

**Biomarkers for neurodegenerative diseases**

## THE PROMINENT ROLE OF LABORATORY BIOMARKERS IN THE DIAGNOSIS OF ALZHEIMER'S DISEASE

S. Bernardini[1]
[1]*Dept. of Experimental Medicine and Surgery, University of Tor Vergata, Rome, Italy*

The diagnosis of "probable" Alzheimer's Disease based on the clinical observation of specific symptom of progressive cognitive impairment has become an old and almost obsolete term in recent years, that is no more useful neither in clinical trials nor in routine clinical setting. The decrease of β amyloid level (Aβ42) and increase of both total and phospho tau (T-Tau and P-Tau), detectable in cerebrospinal fluid (CSF), reflect the pathological events provoking the degenerative processes in the brain (the neuritic plaques and neurofibrillary tangles), defining the typical AD profile in the CSF. The inclusion of these biomarkers of neurodegeneration in the diagnostic guidelines has been suggested as supportive features for a definite in vivo diagnosis of AD. Actually, changes in CSF biomarkers can even predict the development of AD processes in preclinical stages in apparently healthy individuals, because pathological processes of AD start years before the onset of cognitive symptoms. New biomarkers are also emerging which may be useful for prognosis, disease progression, development and monitoring of new treatments and for understanding the pathologic processes underlying AD. Notwithstanding, the analysis of CSF biomarkers is full of pitfalls, and a great effort is needed for the standardization of analytical and pre-analytical factors, and to establish the normal range, since the measurements of CSF biomarkers are variable between studies and laboratories. All these aspect hinder the diffusion of CSF analysis of AD biomarkers, that is still limited to few specialized centers, and do not encourage the development of networks for centralized diagnosis of AD. In this new scenario, the Laboratory of Biomarkers could represent the point of reference for achieving standardization of analytical and pre-analytical procedures, for defining early, differential diagnosis and for developing new diagnostic and therapeutic strategies.

**Biomarkers for neurodegenerative diseases**

## DIAGNOSTIC AND THERAGNOSTIC CEREBROSPINAL FLUID BIOMARKERS FOR ALZHEIMER'S DISEASE

K. Blennow[1]
[1]*Clinical Neurochemistry Lab, Sahlgrenska University Hospital, Mölndal, Sweden*

Lumbar puncture is a routine procedure in clinical medicine. A set of Alzheimer's disease (AD) CSF biomarkers have been developed and evaluated in numerous studies. These include total tau (reflecting neuronal degeneration), Aβ42 (reflecting Aβ deposition in plaques), and phosphorylated tau (probably related to tangle formation). These biomarkers have high diagnostic accuracy for AD dementia and can differentiate AD from normal aging and several important differential diagnoses, and also to identify prodromal AD cases in mild cognitive impairment (MCI) cohorts.
CSF samples are easily sent from any clinic to a clinical laboratory performing the CSF biomarker assay, but absolute levels have been found to vary between laboratories and also between batches of ELISA kits. Therefore, several standardization initiatives have been initiated, including the IFCC Work Group (WG) for CSF proteins. The aim of this WG is to develop a Certified Reference Material (CRM), i.e. a large CSF pool with certified CSF biomarkers levels, and Reference Measurements Procedures (RMP), i.e., mass spectrometry-based "Golden Standard" methods for the biomarkers. This will be important to harmonize levels, and allow uniform cut-off levels for the biomarkers. Biotech companies have also initiated efforts to produce new validated high-quality assays and transfer biomarker assays to fully automated lab analysers. The between-lab and longitudinal performance of the assays are monitored by the Alzheimer's Association's worldwide Quality Control (QC) program for CSF biomarkers.
Taken together, these efforts will ascertain a high quality of the AD CSF biomarkers, and will enable their large-scale introduction in clinical diagnostic routine. This will be highly important to enable early diagnosis of AD the day we have effective disease-modifying drugs.
New developments include novel assays to monitor synaptic function and degeneration, such as SNAP-25 and neurogranin. This type of biomarkers may be important to monitor the effect of novel drugs on synaptic function, and might serve as surrogate markers for clinical improvement. Last, new ultra-sensitive techniques serve as the tools to measure the AD biomarkers in blood samples, which may have a position as screening tools at the primary care level.

**Biomarkers for neurodegenerative diseases**

## NEED FOR THE STANDARDIZATION OF ANALYTICAL AND PREANALYTICAL STEPS (CSF ALZHEIMER DISEASE BIOMARKERS)

A. Perret-Liaudet[2], S. Lehmann[1], I. Quadrio[3]

[1] *(2) French Society of Clinical Chemistry (SFBC): neurodegenerative disease working group for analytical guidelines; coordonators A. Perret-Liaudet.and S. Lehmann . (3) Clinical Biochemistry and Proteomic department, Institute of Research in Biotherapy, U*

[2] *(1) Neurobiology, Clinical Biochemistry of East-Hospitals, Hospices Civils de Lyon - Claude Bernard University, Lyon, France (2) French Society of Clinical Chemistry (SFBC): neurodegenerative disease working group for analytical guidelines; coordonators*

[3] *(1) Neurobiology, Clinical Biochemistry of East-Hospitals, Hospices Civils de Lyon - Claude Bernard University, Lyon, France; (2) French Society of Clinical Chemistry (SFBC): neurodegenerative disease working group for analytical guidelines; coordonators*

CSF biomarkers are currently used as an aid in diagnosis of Alzheimer Disease (AD) using Tau, pTau181 proteins, Aβ1-42 peptide or Aβ1-42/ Aβ1-40 ratio. Due to important between-centre variability, there is no consensus cut-off values for a CSF AD signature. This variability can be explained by analytical and pre analytical factors. The last initiative of standardization became last year in the frame of the BIOMARKPAD project (JPND initiative).

Pre-analytical confounders

CSF sampling is a major critical issue: Using atraumatic needles with low diameter is preferentially recommended. Due to the hydrophobic character of amyloid peptides, choosing a sample tube is a crucial point. We have driven a study comparing generic Polypropylene tubes showing significant variations in Ab42 levels. If it is recommended to centrifuge samples rapidly after sampling, a spinning conditions study is running (BIOMARKAPD). The time delay between sampling and freezing is not a crucial issue as finally it was defined a delay up to 5 days. The use of intermediate plastic tubes must take into account the adsorption of amyloids. The length of storage, the temperature conditions and the number of thawing/freezing cycles must be controlled, the last recommendations permitting to ensure stability of AD CSF biomarkers.

Analytical confounders

On the basis of the Alzheimer's Association external quality control programme we know that measurements of AD biomarkers vary between centres. This part of study is still running ( BIOMARKAPD). We give two examples. The instructions present in the kit insert protocols can be interpreted with a high degree of freedom for crucial steps as for example the "Room Temperature (18-30°C)".The adsorption of amyloids peptides was shown to have significant influence onto the levels of concentration using pre-analytical plates.

Effect of standardization on the cut-off.

In the frame of the SFBC we analysed differences in biomarker outcomes before and after harmonization CSF collection tubes in AD diagnosis resulting in a modification of the predictive value of Ab42. The cut-off shifted from 500 to 700 ng/l.

In conclusion, standardization is running but revalidation of cut-offs after harmonization in the pre-analytical and analytical phases is needed.

**Cancer and laboratory medicine**

## THE FAILURE OF PROTEIN CANCER BIOMARKERS TO REACH THE CLINIC: WHY, AND WHAT CAN BE DONE TO ADDRESS THE PROBLEM?

E. Diamandis[1]
[1]*Mount Sinai Hospital*

Abstract
Title: The failure of protein cancer biomarkers to reach the clinic: Why, and what can be done to address the problem?
Topic: Cancer in Laboratory Medicine (NYM15)
Cancer biomarkers are widely used for patient diagnosis, monitoring and providing response to treatment. Currently, a handful of cancer biomarkers are used in the clinic. All of these biomarkers have been discovered before 1985. Despite spectacular advances in Clinical and Laboratory Medicine the last few years and the introduction of powerful analytical techniques such as microarrays, whole genome sequencing, proteomics and other 'omics', very few, if any, new cancer biomarkers have been recently discovered. It is now evident that the optimistic view that new cancer biomarkers will revolutionize the practice of Oncology, has not been realized. It is interesting to ask as to why biomarkers are failing to reach the clinic.
In this presentation, I will analyze the reasons behind these failures by providing published examples.
There are three reasons for biomarker failure to reach the clinic: a] fraud, which is rare; b] false discovery and c] weak clinical performance. With false discovery, the original claims for a biomarker are failing after validation, due to pre-analytical, analytical, post-analytical and bioinformatic shortcomings. While many biomarkers reported in the literature represent true discovery, many of these biomarkers are not suitable for clinical-decision making, due to either low specificity, low sensitivity or low prognostic/predictive value.
In trying to predict the future, we could hypothesize that novel cancer biomarkers, with clinical characteristics that are suitable for decision-making either do not exist, or that such biomarkers escape discovery at present, due to limitations of the available analytical techniques.

**Cancer and laboratory medicine**

## CIRCULATING TUMOR CELLS AS BIOMARKERS IN CANCER PATIENTS

K. Pantel[1]
[1]*University Medical Center Hamburg-Eppendorf, Department of Tumor Biology*

Sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood at the single cell level. CTC can be distinguished and enriched from the surrounding leukocytes by either physical properties (e.g., density and size) or biological properties (e.g., expression of epithelial proteins such as EpCAM or cytokeratins) (Pantel et al., Nat Rev Cancer 2008; Parkinson et al., J Transl Med 2012). CTC/DTC are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach et al, Nat Rev Cancer 2011). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers such as the actin bundling protein plastin-3 (Yokobori et al., Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, in vivo capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the “fishing” for CTCs from approx. 1.5 liters of blood within 30 minutes (Saucedo-Zeni, Int. J. Oncol. 2012). CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res., 2013). Interstingly, the subset of EpCAMlow, CD44high, CD47+, c-Met+ CTCs obtained from the peripheral blood of breast cancer patients might represent metastasis-initiator cells (Baccelli et al, Nature Biotech. 2013). Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient. This information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

**Cancer and laboratory medicine**

## DIVERGENT AND COORDINATE REGULATION OF THE UNFOLDED PROTEIN RESPONSE PATHWAYS BY ANDROGENS IN PROSTATE CANCER

W. Håkon [7], F. Saatcioglu[3], A. Yke Jildouw [4], S. Margrethe [4], S. Xia [4], T. Martina [4], J. Yang [4], N. Hatice Zeynep [4], Z. Sen [4], M. Ian G. [2], F. Ladan [8], R. Paul [8], R. Bjørn [6], D. Håvard E. [1], H. Gökhan S. [5]

[1]*Center for Cancer Biomedicine, Department of Informatics, University of Oslo, Oslo, Norway; Institute for Cancer Genetics and Informatics, Oslo University Hospital, Oslo, Norway;*

[2]*Centre for Molecular Medicine Norway, University of Oslo; Department of Urology, Oslo University Hospital, Oslo, Norway; Department of Cancer Prevention, Institute of Cancer Research, Radium Hospital, Oslo, Norway*

[3]*Department of Biosciences, and Institute for Cancer Genetics and Informatics*

[4]*Department of Biosciences, University of Oslo*

[5]*Department of Genetics and Complex Diseases, Harvard School of Public Health, Harvard University, Boston, MA, USA*

[6]*Institute for Cancer Genetics and Informatics, Division of Pathology, Oslo, Norway;*

[7]*Institute for Cancer Genetics and Informatics, Division of Surgery, Oslo University Hospital, Oslo, Norway;*

[8]*The Vancouver Prostate Centre, 2660 Oak Street, Vancouver, British Columbia, Canada*

The unfolded protein response (UPR) is an in-built homeostatic mechanism to maintain endoplasmic reticulum (ER) function. Here we show that the canonical UPR pathways in prostate cancer (PCa) cells are directly and divergently regulated by androgens which are critical for PCa survival. Androgen receptor (AR) bound to gene regulatory sites and activated the IRE1$\alpha$ branch, but simultaneously inhibited PERK signaling. IRE1$\alpha$ knockdown significantly increased apoptosis of PCa cells in vitro and its expression was decreased in human xenografts upon androgen withdrawal and regression. Moreover, IRE1$\alpha$ inhibition or its target XBP1, profoundly inhibited PCa cell growth in vitro as well as tumor formation in preclinical models of PCa in vivo. Consistently, AR and UPR gene expression were concomitant in human PCa wherein spliced XBP1 expression was significantly upregulated compared with normal prostate. These data suggest that targeting of IRE1 signaling may have utility as a novel therapeutic approach in PCa.

**New developments in hemostasiology**

**CHRONIC LIVER DISEASE: NEW DEVELOPMENTS IN LABORATORY TESTING**

P.M. Mannucci[1]
[1]*Scientific Director, IRCCS Cà Granda Maggiore Policlinico Hospital Foundation, Milano, Italy.*

Chronic liver disease is characterized by an impaired synthesis of coagulation factors. This complex defect is conventionally documented through the measurement of individual coagulation factors, or through the prolongation of global tests such as the prothrombin time (PT) and the activated partial thromboplastin time (APTT). On the other hand, it is known that these tests are not predictive of bleeding in patients with cirrhosis. Recently, it has been surmised that the PT and APTT might be inadequate to reflect the balance as it occurs in vivo especially in cirrhosis, a condition where the naturally-occurring anti-coagulants protein C, antithrombin and tissue factor pathway inhibitor (TFPI) are reduced in parallel with pro-coagulants. The balance of pro- and anti-coagulants in cirrhosis was found to be normal when assessed in terms of thrombin generation monitored over time in the presence of thrombomodulin. This happened notwithstanding that PT and APTT were prolonged. All the above observations are consistent with the views that the coagulopathy in liver disease is more a myth than a reality, and that the culprits for the bleeding problems seen in these patients should be searched elsewhere. Together with severe thrombocytopenia, other potential candidates responsible for bleeding are hemodynamic alterations subsequent to portal hypertension, endothelial dysfunction (reduced vascular tone), bacterial infections and renal failure.

**New developments in hemostasiology**

**QUALITY IMPROVEMENT IN THE HAEMOSTASIS LABORATORY: THE ROLE OF EXTERNAL QUALITY ASSESSMENT**

P. Meijer[1]
[1]*ECAT Foundation*

One of the major challenges in the coagulation laboratory is to reveal the cause for a prolonged APTT.
A prolonged APTT can either be caused by a clotting factor deficiency, a specific or non-specific inhibitor or heparin. But even combinations of these phenomena exist. It is therefore important that the laboratory should have a proper diagnostic strategy to reveal the potential cause for a prolonged APTT.
From an electronic survey of approximately 1000 laboratories throughout Europe we know that about 20% was not able identify the correct cause for a prolonged APTT.
Surveys and projects organised by external quality assessment programmes may assist laboratories to understand potential problems better, to assess the analytical quality of particular laboratory testing as well as to initiate processes for improvement.
With the use of an algorithm for the laboratory diagnosis of a prolonged APTT the importance of a systematic approach will discussed. In addition examples will be given of potential pitfalls, results from external quality assessment surveys and solutions for problems.
Special attention will be paid to a project that was organised by the ECAT Foundation to reveal the causes for the wide between-laboratory variation in Factor VIII inhibitor testing. With a step-by-step approach we were able during this project to reduce the between-laboratory variation from 40-50% to 10-20%. This project clearly shows the role external quality assessment programmes may play in quality improvement.

**New developments in hemostasiology**

**FACTOR XIII DETERMINATION: WHY, WHEN AND HOW?**

L. Muszbek[1]
[1]*University of Debrecen, Clinical Research Center, Debrecen, Hungary*

Coagulation factor XIII (FXIII) is a zymogen consisting of two catalytic A and two carrier/inhibitory B subunits (FXIII-A2B2). It becomes converted into an active transglutaminase (FXIIIa) in the last phase of coagulation cascade by thrombin and Ca2+. The main function of FXIIIa is to strengthen fibrin by cross-linking its chains and to protect it against fibrinolysis by covalently attaching $\alpha$2 plasmin inhibitor to fibrin. FXIII is also involved in wound healing, and in maintaining pregnancy. Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiency. The former is usually a very severe bleeding diathesis and due to the high frequency of intracranial bleeding, it needs life-long substitution therapy. Auto, or alloantibodies against one of the FXIII subunits usually result in very severe acquired FXIII deficiency. Milder deficiencies might be due to consumption coagulopathy or to decreased synthesis.
The aim of FXIII determination is: 1/ to diagnose inherited or acquired FXIII deficiency, 2/ to establish the severity of deficiency and indicate if there is a need for substitution, 3/ to monitor the effectiveness of prophylaxis or therapy, 4/ to detect and measure neutralizing autoantibody.
FXIII determination is indicated: 1/ in the case of bleeding when coagulation and platelet function screening tests are normal or, if abnormal, but do not explain the bleeding. Delayed umbilical bleeding in infants and intracranial bleeding in children represent strong suspicion. 2/ when bleeding diathesis and habitual abortions are combined, 3/ when bleeding is associated with extracorporeal circulation or major surgery, 4/ to monitor the efficiency of FXIII supplementation.
Reported guidelines for FXIII determination emphasize the following points: 1/ neither clot solubility test nor FXIII antigen assay is recommended for screening FXIII deficiency; a quantitative functional assay based on ammonia release or on amine incorporation into a protein should be the first-line test, 2/ in the case of ammonia release assays a plasma blank is to be measured and subtracted from the results, otherwise in the low activity range FXIII activity is overestimated, 3/ the results of amine incorporation assays might be influenced by FXIII-A Val34Leu polymorphism.

**AFCB Symposium Inborn errors of metabolism in the AFCB region**

## A PILOT STUDY ON AN EXPANDED NEWBORN SCREENING PROGRAM IN PALESTINE

S. Khatib[1]
[1]*Medical School, Alquds University, Palestine*

Until this day newborns in Palestine are being screened for phenylketonuria and congenital hypothyroidism only. However, a number of other metabolic diseases have been recognized amongst the population, including amino acid and urea cycle disorders, organic acidemias and fatty acid oxidation disorders. The present study was conducted as an initial phase of a project that involves investigating the possibility of finding amino acid and urea cycle disorders in newborns throughout the West Bank of Palestine. In addition, newborn reference ranges for a number of amino acids and urea cycle intermediates will be established. Other phases of the project will look into organic acids and fatty acid oxidation disorders.

A cross-sectional observational study design was used and the study was conducted in all 12 districts of the West Bank. A pilot study was done before the actual study took place. Convenience sampling was used to recognize the newly born participants in the study. Information regarding date of birth, weight, sex, district and parent kinship was recorded. The sample size was 4240 and an informed consent forms were collected from all parents. The study blood collection cards were collected over a one year period by Ministry of Health staff in parallel to the routine collection for the existing newborn screening program. The study cards were shipped to the University of Liege Human Genetics Department in Belgium for analysis using tandem mass spectrometry (MS/MS).

Statistical analysis showed a significant relationship between newborn weight and levels of some amino acid and urea cycle intermediates. The odds ratio analysis showed no effect of gender and parent kinship on the metabolite levels. However, there was a significant difference between some amino acid levels and the districts. The reference range for each amino acid and urea cycle intermediates tested was calculated based on the non-parametric percentile method as indicated by CLSI C28-A3.

Based on our established reference ranges for each amino acid and urea cycle intermediate the results showed that 22.5% of the tested samples (955) had at least one analyte level in the upper 2.5% of the population.

**AFCB Symposium Inborn errors of metabolism in the AFCB region**

## LYSOSOMAL STORAGE DISORDERS IN MOROCCO

L. Chabraoui[1], H. Talbaoui[1], J. Puech[3], B. Benhamou[4], Y. Kriouile[4], R. Froissart[2], C. Caillaud[3]
[1]*Biochemistry Laboratory, Ibn Sina Hospital, University Mohammed V Souissi, Rabat, Morocco*
[2]*Centre de biologie et pathologie Lyon-Est, maladies hereditaires du metabolisme, Lyon France*
[3]*Laboratoire de Biochimie, metabolomique et proteomique, Hôpital Necker Enfants Malades, Paris France*
[4]*Pediatric service 2, Children hospital of Rabat Morocco*

Lysosomal storage diseases (LSDs) are a family of almost 50 distinct diseases caused by enzyme deficiencies which result in the accumulation of non-metabolized complex molecules within lysosomes of various body cell types. We distinguish sphingolipidoses, mucopolysaccharidoses, oligosaccharidoses and glycogenosis type II. They are transmitted as autosomal recessive inherited diseases except the Hunter, Fabry and Danon diseases whose transmission is linked to the X chromosome.
In Morocco we are interested in these conditions since 1991. We developed a protocol for urinary screening of Mucopolysaccharidoses (measurement of glycosaminoglycans and their separation by electrophoresis). We then installed the enzymatic assays for definite diagnosis. Finally, we carried out molecular studies for some diseases.
We diagnosed 189 cases of MPS corresponding to 115 MPS I, 10 MPS II, 34 MPS type III, 23 MPS IVA, 1 MPS IVB and 6 MPS VI. The diagnosis of sphingolipidoses is much more difficult because the clinical symptoms are less suggestive. Since 2003 we diagnosed 88 cases: 28 cases of Gaucher, 13 cases of Niemann-Pick A/B, 12 cases of metachromatic leukodystrophy, 9 cases of GM1 Gangliosidosis, 9 cases of Sandhoff, 4 cases of Tay-Sachs, 7 cases of Fabry, 2 cases of Krabbe disease and 2 cases of ceroid lipofuscinosis. We also diagnosed 5 cases of Pompe disease.
Regarding genetic studies we have shown that for the MPS I, the P533R mutation has a founder effect; it represents more than 90% of all mutations. We also found that most of our patients with Gaucher types II and III are carrying the common mutation, L444P. However for Fabry, Hunter and Morquio IVA disease we identified some common mutations and new private mutations.
Our results underline the importance of LSDs in Morocco where inbreeding is common.
Although each disease is rare, LSDs as a group are relatively common. They should have a frequency that is approximately 1 case per 6,000 births.
Early recognition of symptoms and timely diagnosis are essentials and require good co-operation between Pediatricians and Biochemists specialized in the diagnosis and management of LSDs. Identification of causative mutations allowed providing adequate genetic counseling to the carrying families.

**AFCB Symposium Inborn errors of metabolism in the AFCB region**

## DIAGNOSIS AND MANAGEMENT OF THE AMINOACIDOPATHIES IN TUNISIA

N. Kaabachi[1]
[1]*Biochemistry Laboratory - Rabta Hospital – Tunis, Tunisia*

In Tunisia, after eradicating transmissible diseases and malnutrition the diagnosis of aminoacidopathies (AA) began in 1987. Therefore objective of the study was to determine the resources available for their diagnosis, the most frequent deficits,their characteristics and their management.
From 1987 to 2013, the laboratory received 20 000 samples accompanied by a request sheet for biochemical diagnosis that included the history and the suggestive clinical signs for an inherited metabolic disease.
The techniques used were: fluorimetry, ion exchange chromatography and gas chromatography / mass spectrometry.
The patients diagnosed numbered 743, divided into 496 AA (67%) and 247 organic aciduria cases (33%). Among the AA cases, 39% (193) corresponded to phenylketonuria (PKU) and 61% (303) to other types of AA (303).
The most frequent AA after the PKU were: MSUD 79 cases (16%), type1 tyrosinemia 75 cases (15%), nonketotic hyperglycinemia 43 cases (9%), abnormal urea cycle 37 cases (7.5%). The estimated incidence of PKU was 1/7631 and that of MSUD 1/13716.
Around two third of patients (62%) came from pediatric services. Only 24% were diagnosed during the neonatal period. Consanguinity and family history were present in respectively 62% and 41% of cases.
The majority of patients diagnosed with AA treatable were transferred to the Department of Pediatrics at The Rabta hospital. Since 1998, 73 patients with PKU were treated with controlled phenylalanine diet, 18 cases were detected in the neonatal period based on previous fraternal cases and 55 patients were diagnosed on clinical signs with a median age at diagnosis of 34 months.
For type 1 tyrosinemia , out of 70 patients diagnosed, 42 were treated by low protein diet only or not treated at all but they all deceased and 28 patients received NTBC and controlled diet; among them, 20 patients grew normally without complications.
The other treatable aminoacidopathies were managed by a controlled protein diet and specific vitamins.
Given the high frequency of AA, it is imperative to improve their diagnosis and management and to establish the systematic neonatal screening for PKU.

**AFCB Symposium Inborn errors of metabolism in the AFCB region**

**ORGANIC ACIDURIAS, INHERITED METABOLIC DISEASES**

M. Shaarawy[1]
[1]*Faculty of Medicine, Cairo Universiy , Cairo, Egypt*

Sequential sounds of fatty acyl Co A dehydrogenation are catalyzed by long, medium and short chain acyl Co A dehydrogenases (LCAD, MCAD and SCAD respectively). Genetic defects in each of these enzymes have been described as well as defect in their common cofactor, electron transfer flavoprotein (ETF) in addition to carnitine deficiency. Pattern of abnormal fatty acids metabolism seen in urinary organic acid analysis indicate which enzyme (s) may be defective. SACD is diagnosed by increased urinary ethyl malonic acid. MCAD is diagnosed by increased urinary adipic acid and hexane glycine. LCAD is diagnosed by increased urinary dicarboxylic and 3-hydroxy dicarboxylic acids. LCAD is diagnosed by lactic acid acidosis and increased urinary 3-hydroxy dicarboxylic acid. Systemic L-carnitine deficiency is characterized by hypoglycemia and hyperammonemia. Gas chromatography and mass spectrometry (GC-MS) is the method of choice for organic acid analysis

LCAD was detected in 1/4000 new born. Published data revealed that the overall incidence of diseases involving organic aciduria was estimated to be 24 per 100.000 live births or 1 in 1400 births.

Organic aciduria is clinically important for differential diagnosis of inherited metabolic diseases, especially in patients with family history of this genetic disorder, to avoid potential manifestations affecting major organ system

**IFCC Symposium Immunodeficiences - A cutting edge workshop representing top research and novel diagnostic options**

## DIAGNOSIS OF PRIMARY DEFICIENCIES IN LYMPHOCYTE CYTOTOXICITY

Y. Bryceson[1]
[1]*Karolinska Institutet*

Cytotoxic lymphocytes, encompassing cytotoxic T lymphocytes (CTL) and natural killer (NK) cells play an important role in control of infections and malignancy. Together, they can selectively kill aberrant cells through directed release of granules containing perforin and other cytolytic effector molecules. Their activity also contributes to immune homeostasis, preventing excessive immune activation. Thus, deficiencies in cytotoxic lymphocyte function are not only associated with susceptibility to infection or malignancy, but are also linked to hyper inflammatory syndromes. Aided by clinical and laboratory evaluations, mutations in genes required for the development or function of NK cells and, in many cases, CTL, are being elucidated and linked to disease. Measurement of NK cell-mediated lysis of target cells devoid of MHC class I expression has represented a gold standard for assessment of cytotoxic lymphocyte activity. More recently, however, the advent of multiparametric flow cytometry has provided means to characterize developmental stages and quantify NK cell functions in greater detail and with increased sensitivity. I will summarize emerging concepts relevant to the understanding of cytotoxic lymphocyte development and function and exemplify how insights may be used for the diagnosis of primary immunodeficiency syndromes.

**IFCC Symposium Immunodeficiences - A cutting edge workshop representing top research and novel diagnostic options**

**CLINICAL GENE THERAPY FOR X-LINKED CHRONIC GRANULOMATOUS DISEASE**

J. Reichenbach[1], C. Brendel[2], G. Santilli[3], U. Siler[1], R. Seger[1], A. Thrasher[3], M. Grez[2]
[1]*Div. Immunology, University Children's Hospital Zurich*
[2]*Georg Speyer Research Institute Frankfurt*
[3]*Molecular Immunology Unit, UCL Institute of Child Health*

X-linked chronic granulomatous disease (X-CGD) is a primary immunodeficiency caused by mutations in the CYBB gene encoding the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalytic subunit gp91phox. Patients suffer from recurrent life threatening infections with bacteria and fungi, often requiring bone marrow transplantation. In case no matched bone marrow donor is available, the only alternative treatment option is gene therapy of autologous hematopoietic stem cells.
A recent Swiss-German clinical trial for X-CGD using a gamma-retroviral vector has demonstrated clear therapeutic benefits in four patients although complicated by enhancer-mediated mutagenesis and diminution of effectiveness over time due to silencing of the viral long terminal repeat. In collaboration with other centers in Europe a new lentiviral SIN (self-inactivated) gene transfer vector for X-CGD has therefore been developed to improve efficacy and safety. In this vector expression of the therapeutic transgene gp91phox is mediated by a chimeric promoter - a synthetic fusion of two myeloid promoter elements derived from the CathepsinG and the cFes gene regulatory regions.
This vector results in high levels of gp91phox expression and normal NADPH oxidase activity in committed myeloid cells and granulocytes from transduced human X-CGD CD34+ cells.
Based on these results the chimeric vector was selected for large scale GMP-production in a joint effort between labs in Zürich, Frankfurt, London and Paris aiming at a multicenter clinical gene therapy trial phase I/II. First patients (pediatric and adult) are planned to be treated by 2014 in Zürich in this EU-FP7 funded trial.

**IFCC Symposium Immunodeficiences - A cutting edge workshop representing top research and novel diagnostic options**

**COMPLEMENT DEFICIENCIES: CLINICAL IMPACT AND DIAGNOSTIC STRATEGIES**

M. Kirschfink[1]
[1]*Institute of Immunology, University of Heidelberg, Germany*

Complement as a major component oft the innate immune system has a crucial role in the protection against infections. By orchestrating the immune response, complement substantially contributes to homeostasis. However, complement may turn against healthy tissue with severe consequences if not properly controlled. Complement deficiency cases comprise about 5 to 10% of all primary immunodeficiencies. As ‚experiments of nature' they have significantly contributed in defining the role of complement in host defence.
There is great variation in the spectrum of disorders associated with complement deficiency dependent on which complement protein and activation pathway is affected. Genetic deficiency of any early component of the classical pathway (C1q, C1r/s, C2, C4) is often associated with autoimmune diseases, esp. with SLE due to the failure of clearance of immune complexes and apoptotic materials and impairment of normal humoral response. Individuals, deficient of properdin and of the terminal pathways (C5 to C9) are highly susceptible to meningococcal disease, indicating that its cytolytic property is of particular importance in the host defense against Neisseriae. Deficiency of C1 Inhibitor, either inherited (hereditary angioedema, HAE) or acquired, results in episodic angioedema. Mutations affecting the regulators factor H, factor I, or CD46 and of C3 and factor B leading to severe dysregulation of the alternative pathway have been associated with renal disorders, such as atypical hemolytic uremic syndrome (aHUS) and less frequent with membranoproliferative glomerulonephritis (MPGN).
The diagnostic approach leading to the identification of a complement deficiency involves a multistep process that starts with functional screening of each activation pathway and proceeds in specialized laboratories with the characterization of the defect at functional, protein and molecular level. Careful handling and storage of blood samples is of critical importance for meaningful complement analysis. Leading international diagnostic complement laboratories have assembled for quality assurance and further development of analytical tools (http://www.iuisonline.org/iuis/index.php/quality-assessment-and -standardization-committee.html ).

**IFCC Symposium Standardization of common endocrine tests**

## PROGRESS IN STANDARDIZATION OF THYROID FUNCTION TESTS

L. Thienpont[1]
[1]*Laboratory for Analytical Chemistry, Faculty of Pharmaceutical Sciences, Ghent University*

The IFCC Committee for Standardization of Thyroid Function Tests (C-STFT) aims at equivalence of laboratory testing results for thyroid hormones by standardization. The focus of this presentation will be on free T4 and TSH measurements.

For standardization of the measurand free T4, the C-STFT made available an IFCC endorsed international conventional reference measurement procedure (RMP). It is based on equilibrium dialysis isotope dilution-liquid chromatography/tandem mass spectrometry. For the measurand TSH, it is generally recognized that developing a RMP is still technically unlikely. Therefore, the C-STFT proposed an alternative approach for 'harmonization' rather than standardization. It is based on the use of a surrogate RMP, i.e., the all-procedure trimmed mean (APTM), statistically calculated from method comparison (MC) data. With these 2 tools, the C-STFT first assessed the quality and comparability of assays, then investigated the feasibility and impact of standardization/harmonization. This was done in 3 MC studies (Phase I-III) in cooperation with the in-vitro diagnostic (IVD) industry. According to the step-up approach, Phase I and II used samples from apparently healthy volunteers, Phase III clinically relevant ones.

The Phase I and II MCs showed quite good quality of the assays, but confirmed considerable inter-assay differences. Simultaneously, they demonstrated the feasibility of aligning the results by recalibration of the assays to the RMP (free T4) or APTM (TSH). The Phase III MC showed that all FT4 assays were strongly negatively biased to the RMP over the clinically relevant measurement range. For TSH, the inter-assay comparability was reasonable in the mid-concentration range, but worse for pathophysiological concentrations. Again, recalibration was able to eliminate the inter-assay differences. The impact of recalibration on the numerical results was particularly high for FT4.

The C-STFT is ready for standardization/harmonization of FT4 and TSH measurements from a technical point of view. The IVD manufacturers agree to prepare for this process (Phase IV), however, without direct implementation. Indeed, the impact of recalibration urges to carefully prepare all stakeholders before implementation.

**IFCC Symposium Standardization of common endocrine tests**

**ENSURING COMPARABLE VITAMIN D MEASUREMENT WITH DIFFERENT ASSAYS**

C. Sempos[1]
[1]*National Institutes of Health, Office of Dietary Supplements, Bethesda, Maryland, 20852 USA*

Vitamin D Standardization Program (VDSP) is an international effor that was developed to promote the standardized laboratory measurement of serum total 25-hydroxyvitamin D [25(OH)D] worldwide . Laboratory measurement of 25(OH)D concentration is used to assess an individual's vitamin D status. However, there is substantial variation in 25(OH)D measurement within and among the different assays whether commercially available or developed by individual laboratories. Such assay variation confounds clinical assessment and it makes the pooling of 25(OH)D research results for the specific purpose of determining dose-response and/or clinical cutpoints problematic, at best. 25(OH)D assay standardization is, therefore, essential to the development of evidenced-based clinical and public health guidelines for vitamin D. The VDSP was established in 2010 by the NIH Office of Dietary Supplements in collaboration with National Institute of Standards and Technology (NIST), CDC, Ghent University, eight national health surveys and an international group of researchers. VDSP has developed a standardization system consisting of NIST and Ghent University reference measurement procedures (RMP), NIST standard reference materials (SRMs 972a & 2972), CDC's Vitamin D Standardization Certification Program, CAP & DEQAS accuracy-based performance testing schemes and study designs for standardizing 25(OH)D results measured in the past. Performance criteria of CV 10% and Bias 5%, have been established. Standardization amounts to calibrating routine 25(OH)D assay values to the true concentration as based on the reference methods. The presentation will be used to describe this effort including what labs can do now to begin the standardization effort and VDSP research efforts to further improve laboratory measurements in the future. The major point of emphasis is that the VDSP has an effective program to standardize the laboratory measurement of 25(OH)D now. Join us in this effort.

**IFCC Symposium Standardization of common endocrine tests**

## DEVELOPMENT OF A REFERENCE MEASUREMENT SYSTEM FOR PARATHYROID HORMONE

C.M. Sturgeon[1]
[1]*Royal Infirmary of Edinburgh*

Renal physicians strive to maintain parathyroid hormone (PTH) concentrations for patients with chronic kidney disease (CKD) within guideline limits, but poor method comparability means there is serious risk of clinical misinterpretation. External quality assessment scheme data consistently demonstrate differences in method bias of up to 50%. This represents a major challenge to patient safety with significant potential for under or over-treatment. The IFCC Scientific Division Working Group for PTH is developing a reference measurement system with the aim of reducing this unacceptable variation in patient results. This requires a well-validated reference measurement procedure (RMP) and a reference panel of sera and plasma with values assigned using the RMP.

Raising awareness of the clinical implications of method-related differences in PTH among both clinicians and laboratorians is a pre-requisite for the success of this project and is being addressed in a variety of ways. Establishment of a well-characterized panel of serum and plasma samples of defined clinical provenance that will enable manufacturers to determine appropriate reference intervals and clinical decision points is an integral part of the project that will also provide an invaluable clinical resource. A systematic review of the literature has been undertaken to establish pre-analytical requirements for PTH measurement as this information is required to define the stringent requirements for the reference panel.

Commutability of the recently established International Standard (IS) 95/646 for PTH1-84, a suitable candidate for re-standardization of PTH methods, is being investigated. A mass spectrometric candidate reference measurement procedure for PTH is currently being evaluated. Concurrently, evidence-based recommendations on clinical requirements and performance goals for measurement of PTH are being developed.

The support and participation of the clinical and scientific communities together with the diagnostics industry are key to the success of this project, which and will enable more equitable implementation of the evidence-based recommendations essential for optimal care of patients with CKD-MBD as well as meaningful comparison and interpretation of national and international audit data.

**IFCC Symposium Standardization of common endocrine tests**

## HOW TO ACHIEVE COMPARABLE RESULTS ACROSS LABORATORIES

I.S. Young[1]
[1]*Centre for Public Health, Queen's University Belfast, UK.*

The achievement of comparable results across laboratories is an important goal which facilitates the delivery of consistent, high quality healthcare to patients. This is particularly critical where patient mobility results in management of chronic conditions by multiple health care providers, or where joint management occurs in primary and secondary health care. In addition, diagnostic or treatment guidelines which are dependent on decision limits or therapeutic targets assume that comparable results are available from different laboratories.
Development of a full reference measurement system to which all manufacturer's assays are traceable with help to ensure standardization of a measurand, leading to comparability of results between laboratories. In the absence of full assay standardization, harmonization of assays may be possible, giving comparable results even in the absence of some elements of a full reference measurement system.
Trueness based external quality assurance schemes using commutable samples provide an important mechanism to ensure that comparability of results is maintained following successful standardization or harmonization.
Individual laboratories have an important responsibility to ensure that assays which they use are traceable to a reference measurement system where possible, and in addition to ensure appropriate internal quality control and participation in suitable external quality assurance schemes for all assays.

**ORPHEUS Symposium PHD training in clinical chemistry, clinical biochemistry, and laboratory medicine where are we heading?**

## AACC'S COMACC PROGRAM: POST GRADUATE EDUCATION IN LABORTORY MEDICINE IN THE UNITED STATES

R.H. Christenson[1]
[1]*University of Maryland School of Medicine, Baltimore, MD USA*

Laboratory medicine's future depends on properly training and assuring the competency of entry level individuals through education, mentoring and professional development. The AACC facilitates this through its Commission of Accreditation in Clinical Chemistry and laboratory medicine (ComACC) program. ComACC assures Lab Medicine competency by accrediting and inspecting the quality of training programs, providing recognition of accredited programs and attracting talented individuals to the field.

ComACC has an expert board of 8 commissioners that continuously evaluates and updates the needs of lab medicine trainees. A team of qualified inspectors examines and evaluates new programs after 2 years; established programs are inspected every 5 years. A checklist is used as an evaluation tool that queries institutional support, curriculum content, instructional techniques and fundamental concepts in laboratory management, organization and operation. The inspection delves into specific training documentation in laboratory safety, specimen collection and processing, principles of analysis and lab techniques. Inspected teaching techniques include development of specific objectives in each training area; utilization of pre- and post-tests are considered an effective means of teaching and training. Documentation by periodic formal evaluation and feedback to trainees regarding their progress is essential. The inspection team also meets with the institution's top administrators, lab administrators, academic leaders and practicing clinicians in various specialties to gain their perspective of the training program.

There are currently 26 ComACC-accredited 2-year training programs for post graduates fellows. These programs are currently training 49 individuals, about 2 fellows per program. The major issue limiting training is funding; many programs use research funds for financial support.

A dynamic, well-organized and professional accrediting system, dedicated to establishing standards and assuring the sustained quality of training is critical for a competent workforce. The number of current number of trainees in ComACC programs is likely not sufficient to replace individuals leaving the field, mainly due to challenges in funding.

**ORPHEUS Symposium PHD training in clinical chemistry, clinical biochemistry, and laboratory medicine where are we heading?**

**RECENT TRENDS IN PHD TRAINING- A GLOBAL VIEW**

G. Güner Akdogan[1]
[1]*School of Medicine, Dokuz Eylül University-IZMIR-TURKEY*

The concept of the PhD degree as a "research training under supervision", has been developed in the nineteenth century and since then has been propagated to the whole world. The recognition of PhD diploma globally implies that the holder of PhD diploma has fulfilled all the criteria to obtain the PhD title. However, the criteria may vary according to countries, even according to universities within the same country. Perhaps, the most important variable is the quality of PhD research work that is dependent on a large variety of factors. PhD training in Europe, as well as in other parts of the world, constitutes the main connection between the higher education and the research area. Undergraduate students are required to understand what others have discovered and PhD students are required to do their own discoveries. PhD training has an additional consequence: PhD is not any more responsibility of a supervisor and a PhD candidate student (or alternative term, PhD candidate), but also the responsibility of the university. Consequently, so called structured PhD programmes are developed in most countries. Besides research, such programmes consist of focused courses (usually no more than two semesters) which should support the PhD candidate to be more efficient in research as well as to attain different so called "transferable skills". PhD candidates and graduates constitute the basis of scientific research; they are resources for future researchers and research, and, with transferable skills, they are expected to be pillars for a knowledge-based society. With this understanding, standards for PhD training have been developed globally and a significant project accomplished in 2012 by ORPHEUS-AMSE-WFME, "Standards for PhD Education in Biomedicine and Health Sciences" constitutes an excellent work on the standards required for basic elements of PhD training: research environment, outcomes, admission policy and criteria, PhD training Programme, supervision, PhD thesis, assessment, and structure.

**ORPHEUS Symposium PHD training in clinical chemistry, clinical biochemistry, and laboratory medicine where are we heading?**

## PHD TRAINING IN LABORATORY MEDICINE – CONNECTION TO MEDICAL SPECIALIZATION

T. Zima[1]
[1]*Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

The role of Universities is changing in the last decades from classical Humbolt traditional role – science and education to implementation of „the third role" of Universities. This role is tightly connected to the society and of course, to communication and cooperation with industry. The curricicula should implement not only the scientific knowledge, but also non-scientific skills – communication and commercial awareness. The students should be prepare for real life in changing world.
PhD programmes form the third cycle of higher education. However, the core component of this part is the advancement of learning through original research ,which makes the third cycle different and unique from the Bachelor and Master cycles. In particular, PhD programmes in clinical biochemistry and laboratory medicine are based primarily on original, hands-on research in laboratory. As the mobility between countries is increasing, there is a need for standards and requirements for PhD programmes and proper education with quality assurance are thus becoming of increasing importance in the internationalisation of higher education .
One of the main task of the specialist in clinical biochemistry and laboratory medicine is the direction and supervision of a laboratory department in a hospital or health service. He or she must have the ability to apply biological knowledge to clinical requirements. Apart from providing a competent laboratory service, this specialist must be able to function as a consultant to clinical colleagues. Appropriate knowledge of laboratory management and scientifically based quality assurance procedures are important,too.
Charles University has accredited many PhD programmes focusing on biomedicine and medical schools have clinically oriented PhD programmes connected to university hospitals which are comparable for medical specialization and for PhD programme in biomedicine. Other task for our comunity is connection between PhD education and professional specialization for MDs and how combine these both trainings/education.
There must be tight cooperatoion and harmonisation with two key documents UEMS-section Laboratory medicine - Standards for training specialists and EFLM –EC4 European syllabus for postgraduate training for whole Europe.

**Learning 2Lead Consultants (L2L symposium ) CONTINUOUS QUALITY IMPROVEMENT - A PRACTICAL APPROACH TO ACHIEVE OPERATION EXCELLENCE**

**QUALITY PLANNING AND TEAMWORK**

E.A. Frank[1]
[1]*Bio-Chem Diagnostic Laboratory, Mysore, India*

"When you fail to plan you plan to fail." Quality planning is a key to operational excellence. The lab needs to have a well thought of plan to assure the quality of the total process and to implement it in an efficient manner. A step wise approach of how to plan quality and implement it based on CLSI guidelines will be discussed

**Learning 2Lead Consultants (L2L symposium ) CONTINUOUS QUALITY IMPROVEMENT - A PRACTICAL APPROACH TO ACHIEVE OPERATION EXCELLENCE**

**IS MY LABORATORY PERFORMANCE AT ITS BEST? - QUALITY MONITORING AND QUALITY IMPROVEMENT**

E.A. Frank[1]

[1]*Bio-Chem Diagnostic Laboratory, Mysore, India*

Quality is not an end in itself but rather a daily necessity of meeting the need of the client. How does one monitor quality, how does one assure continuity of efficiencies and accuracy of the total testing process? How does one improve daily to give better service to the next patient? How do we continually improve? These topics will be discussed based on the CLSI guidelines

**Learning 2Lead Consultants (L2L symposium ) CONTINUOUS QUALITY IMPROVEMENT - A PRACTICAL APPROACH TO ACHIEVE OPERATION EXCELLENCE**

**WORK FLOW CONCEPTS AND PROCESS MAPPING**

E.A. Frank[1]

[1]*Bio-Chem Diagnostic Laboratory, Mysore, India*

Diagnostic laboratories have very complex operations with both technical and non-technical working together to deliver an accurate report . Very often, due to the highly unique level of operation, each department on the workflow tends to be inward focused and has very little interaction with the related specialities. This talk will focus on working through the different steps starting from the physicians request, the client the sample flow, and how the departments need to work in tandem to ensure fast accurate reports. We will focus on how to map the process and identify the bottle neck and work on improving efficiency

**Learning 2Lead Consultants (L2L symposium ) CONTINUOUS QUALITY IMPROVEMENT - A PRACTICAL APPROACH TO ACHIEVE OPERATION EXCELLENCE**

**DEVELOPING AND DERIVING VALUE FROM QUALITY INDICATORS**

M. Plebani[1]

[1]*Department of Laboratory Medicine, University-Hospital of Padova, Italy*

Quality indicators (QIs) are fundamental tools for enabling users to quantify the quality of all operational processes by comparing it against a defined criterion. QIs data should be collected over time to identify, correct, and continuously monitor defects and improve performance and patient safety by identifying and implementing effective interventions. According to the International Standard for medical laboratories accreditation, the laboratory shall establish and periodically review QIs to monitor and evaluate performance throughout critical aspects of pre-, intra-, and post-analytical processes. However, while some interesting programs on indicators in the total testing process have been developed in some countries, there is no consensus for the production of joint recommendations focusing on the adoption of universal QIs and common terminology in the total testing process. A preliminary agreement has been achieved in a Consensus Conference organized in Padova in 2013, after revising the model of quality indicators (MQI) developed by the Working Group on “Laboratory Errors and Patient Safety” of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The consensually accepted list of QIs, which takes into consideration both their importance and applicability, should be tested by all potentially interested clinical laboratories to identify further steps in the harmonization project.

**Learning 2Lead Consultants (L2L symposium ) CONTINUOUS QUALITY IMPROVEMENT - A PRACTICAL APPROACH TO ACHIEVE OPERATION EXCELLENCE**

## "TO ERR IS HUMAN". ERROR MANAGEMENT

M. Plebani[1]

[1]*Department of Laboratory Medicine, University-Hospital of Padova, Italy*

Clinical laboratories play a vital role in patient care, but many diagnostic errors are associated with laboratory testing. The past decades have seen sustained improvements in analytical performances but the error rates, particularly in pre- and post-analytical phases is still high. Although the seminal concept of the brain-to-brain laboratory loop has been described more than four decades ago, the awareness about the importance of extra-analytical aspects in laboratory quality is a recent achievement. According to this concept, all phases and activities of the testing cycle should be assessed, monitored and improved in order to decrease the total error rates and thereby improve patient safety. In the interests of patients, any direct or indirect negative consequence related to a laboratory test must be considered, irrespective of which step is involved and whether the error depends on a laboratory professional (e.g. calibration or testing error) or a non-laboratory operator (e.g. inappropriate test request, error in patient identification and/or blood collection). Data collected in various clinical settings demonstrate that many diagnostic errors are associated with laboratory testing. In particular, errors are due to inappropriate test request and/or result interpretation and utilization. Collaborations between laboratory professionals and other care providers, namely clinicians and nurses, are needed to achieve the goal of improved patient safety.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

**THE PERSPECTIVE OF HBA1C NOW THE IFCC REFERENCE MEASUREMENT PROCEDURE IS IN PLACE**

C. Weykamp[1]
[1]*Queen Beatrix Hospital, Winterswijk, the Netherlands*

HbA1c is a key-analyte in the medical laboratory. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on Standardization of HbA1c developed a Reference Measurement Procedure (RMP) which is implemented in a global network of 16 reference Laboratories (7 in Europe, 7 in Asia, and 2 in the Americas). According to the consensus statement of IFCC, International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD), and American Diabetes Association (ADA), the RMP is the only valid analytical anchor for HbA1c. The standardization efforts of the IFCC as well as the harmonization efforts of the National Glycohemoglobin Standardization Program (NGSP) in US raised a lot of exposure and put pressure on manufacturers to improve the test. With the result that in 20 years the interlaboratory CV in External Quality Assessment/ Proficiency Test (EQA/PT) programs came down from 22% to 3%. This dramatic improvement led to a paradigm change in the diagnosis of diabetes. Whereas fasting plasma glucose or the oral glucose tolerance test used to be the test of choice, an International Expert Committee concluded in 2009 that HbA1c is the gold standard for diagnosis and screening of diabetes.
Although HbA1c is indeed accepted as gold standard in many countries there are still many questions to be answered. Clinical decision limits for diagnosis of diabetes and normoglycemia are very close: studies show that a positive bias of only 1 mmol/mol does already give rise to an increase of 40% diagnosed diabetics. Use of HbA1c for diagnosis requires a higher quality than for monitoring therapy. But what should be the criteria? And are present EQA/PT programs fit for purpose: are their acceptance ranges tight enough, are the samples commutable and reliably targeted? What about the performance of Point Of Care instruments and the monitoring of their performance? And finally a fundamental biochemical question: does HbA1c reflect mean glucose levels or are differences in a (sub) normal population (partly) dependent on the erythrocyte lifespan? These topics will be discussed to highlight the perspective of the HbA1c assay now the standardization of this analyte is in place.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

## POCT FOR GLUCOSE - A NEW APPROACH FOR IMPROVED EXTERNAL QUALITY ASSESSMENT

G. Schumann[1]

[1]*Calibration Laboratory D-K-15117-02, c/o Medical School Hannover, Institute of Clinical Chemistry, D-30625 Hannover, Germany*

Determination of glucose in whole blood is performed frequently using point of care (POC) mobile devices (MD). Material of investigation is whole blood from the finger pulp or from the earlobe. Measurement results are expressed as glucose concentrations in plasma. Required traceability is performed by manufacturers using patients' samples in method comparisons. Preferred designated comparison methods are enzymatic procedures. Traceability to a primary reference material is provided by calibration with a higher order reference procedure e.g. isotope dilution (ID) gas chromatography (GC) mass spectrometry (MS). Calibration of POC-MD is very complex, and the end user ought to rely on proper calibration of POC-MD. The end user has only access to non-commutable material provided by the manufacturer for internal quality control (IQC). Whole blood for reference analyses has the disadvantage of lacking stability and small sample volume. Therefore, external quality assessment (EQA) is also limited to non-commutable control material, and EQA is based only on group mean values for the different POC-MDs. Suited tools for effective IQC and EQA of POC-MD are lacking.

An experimental design for the selective investigation of venous whole blood and plasma out of it – only one blood sample per voluntary donor (n = 20) - was applied to five randomly chosen POC-MDs (Accu-Check Performa, Onetouch UltraEasy, StatStrip Xpress-I, ContourXT, HemoCue 201 DM) and to routine procedures for plasma and for whole blood collected in a capillary (both on Cobas 6000, Roche Diagnostics). Traceability chains to ID-GC-MS were established and controlled using certified reference materials. The linearity (4 mmol/L to 20 mmol/L) of five POC-MDs was investigated by us of pooled human plasma. Proven traceability of the glucose concentration in venous whole blood, and given linearity of the measurements in plasma samples were observed for all five investigated POC-MDs. Manufacturers should be asked to evaluate and to disclose the signal ratio for the glucose concentration in whole blood and resulting plasma. This could be an important step for IQC and EQA. Plasma based commutable control materials with certified glucose concentrations could be introduced to quality management for improved POC-MD.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

## TEN YEARS EXPERIENCE WITH INTERNATIONAL PROFICIENCY PANELS FOR MOLECULAR DIAGNOSTICS

V. Haselmann[1]
[1]*Institute for Clinical Chemistry, Medical Faculty Mannheim of the University of Heidelberg, Germany*

The field of molecular genetic diagnostics is steadily gaining more attention in today´s healthcare systems all over the world. This is due to the fact that molecular genetic testing is no longer used for the diagnostics of hereditary disorders only but also plays an important role in the field of phamacogenetics and oncology. As the indications to perform a molecular genetic analysis are increasing, the number of different test being performed and the kind of methods used are steadily growing as well. This in combination with the fact that a certain molecular genetic test is usually only performed once in a lifetime, is making the diagnostics but also its quality assessment even more challenging. Therefore external quality assessment programs (EQAs) are absolutely necessary and laboratories should participate in order to guarantee a high performance quality.
One provider for molecular genetic EQA programs is the German Society for Clinical Chemistry and Laboratory Medicine (DGKL) and its Reference Institute for Bioanalytics (RfB). Over ten years now the RfB is offering different EQAs for the quality control of DNA-isolation (DI survey), DNA-sequencing (SQ survey) as well as for genotyping (FV ring trial). Since then the number of participating laboratories and the number of genotypes being offered is steadily increasing. Based on more than 5000 genotypes determined within each FV survey, the experiences derived display that considerable variations exist in DNA genotyping methods and reasons for wrong determinations. These can be assigned to different types of errors including for example analytical ones that can be caused by rare sequence variations specific for a certain method used. Our experience shows that these EQAs are a helpful tool to reveal strengths and weaknesses in both – technique and proficiency. In addition our results emphasize the need for mandatory EQA programs in order to fulfill the aforementioned rising requirements in the field of molecular genetic diagnostics.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

**ROLE OF CIRCULATING FREE DNA (CFDNA) FOR LABORATORY MEDICINE**

M. Neumaier[1]
[1]*Inst. for Clinical Chemistry, Medical Faculty Mannheim, University of Heidelberg, Germany*

Recent years have highlighted the fact that DNA in plasma is an analytical substrate more stable than anticipated. Indeed, minute amounts of DNA circulating in the bloodstream can be found in healthy individuals. More recently, good evidence has accumulated that other nucleic acids (NA) like mRNA species and micro-RNAs are present in blood and can be found in association with different disease conditions.
Particularly, prenatal diagnostics and diagnostic strategies in oncology call for NA-testing.
Technological advances allow multiplex analysis of NA circulating in the bloodstream. Next to quantitative measurement of gene expression that can be done by standard DNA microarrays, the use of massive parallel sequencing will further revolutionize the dimensionality of information that can be retrieved from a single blood draw. Next to the analysis of predesigned gene panels, e.g. gene mutation panels, de-novo sequencing may unravel unknown defects that may be important for cancer progression and metastasis in a highly individualized fashion. While the primary tumour is amenable to surgical resection, distant metastatic foci often are not. Accordingly, tumour profiling carried out by the histopathologist needs to be complemented by “liquid profiling” carried out in the clinical diagnostic laboratory. This will allow to characterize changes in the genetic make-up of the tumour that will be critical for therapeutic stratification of cancer patients. Laboratory medicine has a long-standing reputation for excellence in bioanalytics and rigorous quality standards that are prerequisite to assure reliability and precision in this new area of testing of bodily fluids.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

**CREATININE - A KEY MEASURAND OF CLASSIC AND NEW FIELDS IN EQA**

C. Dr., Ritter-Sket[1]
[1]*Reference Institute for Bioanalytics (RfB)*

In the field of laboratory medicine creatinine is a classical biomarker for assessing renal function and along with the estimated glomerular filtration (eGFR), it is useful for diagnosing and stratifying kidney disease.
Furthermore, creatinine is - beside bilirubin and the international normalized ratio for prothrombin time (INR) - a key measurand for the calculation of the MELD score.
The MELD score is used to assess the severity of chronic liver disease and is decisive for the placing of a patient on the transplant list.
Tests for creatinine in clinical routine laboratories base upon different methods (enzymatic reaction, Jaffé-Reaction). However, independent of the methods, the reagents and the laboratory where such testing is performed the results for one and the same sample have to be comparable.
In order to demonstrate the comparability, the results of participants of external quality assessment schemes are evaluated on the basis of target values traceable to methods and materials of highest metrological order.
The certification of the target values is performed by calibration laboratories, accredited to ISO 17025 and ISO 15195.
The reference method for creatinine is based on the principle of isotope dilution mass spectrometry (IDMS). The use of a specific detection method (GC-MS, LC-MS), an elaborate sample preparation and the calibration of all volumetric and gravimetric steps of a reference method assure highest accuracy (precision and trueness) of the results and allow the calculation of the expanded uncertainty.
The implementation of this system improves the quality in clinical diagnostics and ensures a better medical attendance of the patients.

**The German Common Society of Clinical Chemistry and Laboratory Medicine (DGKL) Symposium Challenges and issues of external quality assurance in laboratory medicine**

## PROMOTING QUALITY OF MOLECULAR DIAGNOSIS OF HEREDITARY DISEASES: THE NEED FOR CONTINUED QUALITY IMPROVEMENT

N. Wolstenholme[1]
[1]*EMQN*

With the speed of change in the field of clinical molecular genetics and the complexity of the results generated, clinicians referring samples for molecular genetic results require not only a full and accurate genotype but also an appropriate clinical interpretation of the results presented. The European Molecular Genetics Quality Network (EMQN) promotes quality in genetic testing worldwide by offering External Quality Assessment (EQA) schemes to its 1300 members. These activities include 32 disease specific, 4 molecular pathology, and 4 technique specific EQA schemes. The basic principle behind EQA is to send validated DNA samples, with mock clinical cases, to participating laboratories. Participants use their routine protocols and return reports which are anonymously reviewed by a panel of assessors. The EMQN EQA schemes have developed to test not only the ability of laboratories to generate accurate genotypes but also to interpret the data in the light of mock clinical scenarios. Most molecular genetic results are qualitative in nature and consequently the assessment of genotyping accuracy is quite straight forward. Some elements of interpretation, such as clinical utility are universal; however others such as analytical sensitivity and positive and negative predictive values may vary according to the methodology chosen as well as the geographical location of lab. When considering advice for further testing and implications for other family members, local practices in reporting have evolved to address the needs of local clinicians and vary considerably between countries and between labs within a country. Consequently EMQN report evaluation is based upon agreed current best practice and is achieved as a consensus between experts from more than one country. Numerical evaluation of the report is applied; however EMQN places a high emphasis on continued education and will offer advice to participants in a non-punitive manner. The primary aim is to be educative, to help laboratories improve their analysis protocols and reporting. Examples and the learning outcomes from these activities will be described, along with discussion on how to meet the future challenges that massively parallel sequencing presents to diagnostics and EQA provision.

**ISOBM/EGTM Workshop TUMOR MARKERS IN CLINICAL PRACTICE: AN UPDATE**

## BIOMARKERS IN OVARIAN CANCER, PAST, PRESENT AND FUTURE

M.J. Duffy[1]
[1]*St Vincent's University Hospital and University College, Dublin*

Ovarian cancer is one of the most lethal cancers affecting women, being the 4th most common cause of tumor-related death in women and the most lethal gynecological malignancy. In order to improve outcome, we need sensitive and specific biomarkers for the early detection of the disease, sensitive markers for monitoring therapy and predictive markers for identifying patients likely to benefit from specific therapies. Although over 50 biomarkers have been proposed for ovarian cancer, the 2 most investigated are CA 125 and HE4. Indeed, CA 125 and HE4 have been shown to be the most sensitive biomarkers for the early detection of ovarian cancer (1). Despite this, neither marker can be recommended for screening for ovarian cancer in asymptomatic women outside the context of a randomised controlled trial (2,3). Preoperative levels of CA 125 in combination of transvaginal ultrasound or combined measurement of CA 125 and HE4 (as in the ROMA algorithm) may aid the differentiation of benign and malignant pelvic masses. For differentiating benign from pelvic masses, HE4 has been reported to be more specific than CA 125 (4). Furthermore, combined measurement of CA 125 and HE4 (as in ROMA) appears to be superior to CA 125 alone, in the differential diagnosis of pelvic masses. Serial levels of CA 125 during chemotherapy for ovarian cancer are useful for assessing response to treatment. Other serum biomarkers proposed for ovarian cancer include OVA1, OVX1, transthyretin, osteopontin, transferrin, CEA, CA 15-3 and mesothelin. Currently however, only CA 125 and HE4 are in clinical use.

Key references

1. Cramer et al, Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res 2011;4;365.
2. Duffy MJ, et al, CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. Int J Gynecol Oncol 2005;15:679.
3. Sturgeon et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Clin Chem 2008;54:e11.
4. Escudero JM, Auge JM, Filella X, Torne A, Pahisa J, Molina R. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. Clin Chem 2011;57(11):1534-44.

**ISOBM/EGTM Workshop TUMOR MARKERS IN CLINICAL PRACTICE: AN UPDATE**

**TUMOR MARKERS IN LUNG CANCER: A TOOL FOR EARLY DIAGNOSIS?**

R. Molina[1], R.M. Marrades[1], J.M. Augé[1], J.M. Escudero[1], N. Viñolas[1], N. Reguart[1], J. Ramirez[1], X. Filella[1], L. Molins[1], A. Agustí[1]
[1]*Hospital Clínic Barcelona*

Lung cancer (LC) is the most frequent and fatal human cancer. Its prognosis is directly related to early diagnosis. In this study, we hypothesized that a combined panel of six serum tumor markers (TM) is useful in the diagnosis of patients with clinical suspicion of LC and evaluated the possible utility in early diagnosis.
We investigated the diagnostic utility of a combined panel of six serum TM (CEA, CA125, SCC, CYFRA 21-1, NSE and ProGRP) in 3,644 consecutive individuals referred to our institution because of the clinical suspicion of LC.
Using standard clinical procedures, LC was confirmed in 2,259 patients (1,876 with NSCLC (83%) and 383 with SCLC (17%)) and excluded in 1,429 individuals (38%)). The sensitivity of the TM panel investigated here for the diagnosis of LC was 90.0%, its specificity 81%, its NPV 85% and its PPV 89%. In patients with early stages of LC these figures were, respectively, 73%, 81%, 91% and 69% for NSLC stage I/II and 96%, 81%, 99% and 36%for intra-thoracic SCLC. Finally, two TM (NSE and ProGRP) significantly differentiate NSCLC from SCLC (AUC 0.903 and 0.856, respectively).
This study is the first to show that a panel of six serum TM can be useful in the clinical management of LC. It may do so either as a first step in the screening of high risk populations (its high sensitivity and NPV can help to exclude LC) and/or through the diagnostic assessment of patients with a clinical suspicion of LC (high specificity, high PPV), even in early stages of the disease. In addition, the six serum TM show a significant capacity to differentiate NSCLC and SCLS, which may require different therapeutic strategies.

**POCT: its impacts on patients and laboratories**

## A NEW POC PARADIGM: CONTINUOUS GLUCOSE MONITORING TO ENABLE ACCURATE CONTROL OF GLYCEMIA

D. Bruns[1], J. Boyd[1]
[1]*University of Virginia*

Improvements in the control of glycemia have depended on advances in measurement of glucose. Measurements initially were made with urine test strips, which provided semi-quantitative results that reflected blood glucose concentrations above the renal threshold for glucose and with a considerable time lag. Currently, glucose meters and blood gas analyzers provide rapid measurement of glucose in samples of whole blood, with measurements in settings such as intensive care units (ICUs) made every 1-4 hours. Ideally, future methods for glycemic control will use frequent analyses of glucose analogous to the physiologic control processes. Indeed, devices for near-continuous glucose monitoring (CGM) are now appearing, with measurements made every 5-15 minutes.

We have undertaken Monte Carlo simulations to model the control of glycemia when meters or CGM devices are used to support tight-glucose-control protocols in over 100,000 modeled ICU patients. In each modeled patient, insulin infusion rates were determined during 100-200 hours of monitoring by one of two protocols (Yale and University of Washington). The studies show that, regardless of the treatment protocol, the frequency of glucose measurements is a major determinant of the (1) rate of hypoglycemia, (2) rate of hyperglycemia and (3) variability of glucose in the modeled patients. All three of these measures of glycemic control, which are known predictors of patient outcomes, are improved with CGM measurements made every 5 or 15 minutes compared with control found when measurements are made hourly or less frequently. Moreover, the impact of measurement imprecision was markedly blunted when measurements were made every 5-15 minutes rather than hourly or less often. By contrast, the effects of analytical bias on control of glycemia were not lessened by frequent measurements, highlighting the continuing need for accuracy of glucose measurement. We conclude that CGM devices have the potential to markedly improve glycemic control in ICU patients and to improve patient outcomes as suggested by studies of tight glucose control that have used hourly glucose measurements.

**POCT: its impacts on patients and laboratories**

**WHAT IS IMPORTANT IN QUALITY CONTROL FOR POC INSTRUMENTS**

S. Sandberg[1]
[1]*Noklus, Bergen, Norway*

Internal- (IQ) and external quality control (EQA) are integrated parts of the analytical quality system in larger laboratories. It is also recommended that POC users should perform IQ and participates in EQA schemes. However, there seems to be little information about how this should be performed in practice and little evidence that these activities are useful.
The literature is searched for information on the usefulness of IQ and EQA for POC users as well as information about how it should be carried out.
Data will be present from some control activities that have proved to be useful, and it will also be shown that both IQ and EQA routines for POC users varies very much.
It is important that the way quality control for POC instruments are performed is critically judged and more research in this area is needed. Systems used in larger hospital laboratories should not be automatically implemented without showing that is useful.

**POCT: its impacts on patients and laboratories**

## PAST, CURRENT AND CONTINUING CONTROVERSIES IN MONITORING GLYCEMIC CONTROL IN CRITICALLY ILL PATIENTS

M. Scott[1]

[1]*Washington University School of Medicine*

The evolution of glucose meter use in critically ill patients along with the progression of more stringent accuracy guidelines will be reviewed. The use of POC glucose meters in hospitals became “standard of care” in the mid to late 1980s without evidence that they improved clinical outcomes or length of hospital stay. However, in the early part of the 21st century the studies of Van den Berghe et. al. showed that using frequent POC glucose testing and IV insulin to maintain tight glycemic control (TGC) improved mortality, morbidity and length of stay outcomes for patients in critical care settings regardless of whether they had diabetes. As a result of these studies TGC in critical care settings became common across the globe with most institutions using POC glucose meters in their protocols. From 2008 – 2012 a meta-analyses and several prospective studies, including NICE-SUGAR, suggested that TGC was harmful to critical care patients for several outcomes; most strikingly severe hypoglycemic events. Almost immediately, professional societies and critical care practitioners raised the “target” values for glycemic control in critically ill patients by 30 – 40 mg/dL. What may not have been appreciated is that the original Van den Berghe studies used laboratory quality blood gas analyzers to measure glucose at the POC whereas most of the meta-analysis studies and the NICE-SUGAR study mainly used glucose meters for their TGC protocols. This raised the obvious question of whether glucose meters were up to the task of maintaining TGC in critical care settings. It also raised awareness of the interferences of glucose measurement with meters that are present in many critically ill patients. For these and other reasons, including improved meter technology and sophisticated modeling studies demonstrating necessary meter accuracy, both ISO and the Clinical Laboratory and Standards Institute (CLSI) issued new accuracy requirements for glucose meters in 2013. In the United States the Food and Drug Agency (FDA) began to reexamine the accuracy requirements for glucose meters at a public meeting in 2010 and in January 2014 issued separate draft guidances for consumer and hospital use glucose meters.

**Bone metabolism and osteoporosis**

**BONE MARKERS IN PATIENTS WITH CKD**

E. Cavalier[1]
[1]*University Hospital of Liège, Clinical Biology Department*

Assessing bone turnover is a key diagnostic tool in the global management of chronic kidney disease-mineral and bone disorder (CKD-MBD). Because bone biopsy is invasive and cannot be repeated in clinical practice, and because bone histomophometry is less and less available due to a lack of specialized laboratories, different bone markers are routinely used for the monitoring of bone turnover of CKD patients.
Among them, Parathyroid hormone (PTH) is still the most used, including in clinical trials studying therapies of CKD-MBD. By far, PTH is however, sensu strictu, not the best bone biomarker both from a physiological point of view (PTH is acting in calcium metabolism and only indirectly on bone) and a biological point of view (low stability, high Least significant change value, presence of fragments potentially interfering with the assays, lack of standardisation and reference range problems). In this context, we can only agree with the KDIGO recommendation to measure phosphatase alkaline and especially its bone isoform (b-ALP) which can be considered as a true “bone biomarker”. The biological profile of b-ALP is also probably better than PTH, even if not unquestionable. Indeed, b-ALP immunoassays can also cross-react with the liver form of the enzyme, there is also a lack of standardization and of correctly established reference ranges. Some other bone biomarkers, like the N-terminal propeptide of type I collagen (PINP), the tartrate resistant acid phosphatase, isoform 5B (TRAP-5B) or even the β-crosslaps or CTX have emerged as potential new markers of interest. Their place in the diagnostic and monitoring of bone turnover still needs to be further studied. In this lecture, we will focus on the strengths and weakness of these markers in the context of CKD-MBD.

**Bone metabolism and osteoporosis**

## NEW DEVELOPMENTS IN BIOLOGICAL MARKERS OF BONE METABOLISM

G. Patrick Garnero[1]
[1]*INSERM research unit 1033*

Over the last 20 years several biological markers of bone turnover have been developed with increased specificity and sensitivity. In osteoporosis clinical studies, the IOF-IFCC have recently recommended the measurements of serum type I collagen N-propeptide (PINP) and the crosslinked C-terminal telopeptide (serum CTX) as markers of bone formation and bone resorption, respectively. However these markers have some limitations including a lack of specificity for bone tissue, their inability to reflect osteocyte activity or periosteal apposition and they are all protein-based. To address some of these limitations, new developments in markers of bone metabolism have been recently achieved. These include assays for periostin, a matricellular protein preferentially localized in the periosteal tissue, sphingosine 1-phosphate, a lipid mediator which acts mainly on osteoclastogenesis and the osteocyte factors such as sclerostin and FGF-23. Recent studies have shown an association between the circulating levels of these biological markers and fracture risk in postmenopausal women or elderly men, although data require confirmation in additional prospective studies. Finally, recent preliminary studies suggest that the measurements of microRNA in circulating cells may represent a novel class of biological markers in osteoporosis. It is foreseen that with the use of genomics and proteomics, new markers will be developed to ultimately improve the management of patients with osteoporosis.

**Bone metabolism and osteoporosis**

## MARKERS OF BONE TURNOVER IN THE DIAGNOSIS AND THERAPEUTIC FOLLOW-UP OF OSTEOPOROTSIS

M.J. Seibel[1]
[1]*University of Sydney*

Laboratory tests play an important role in the assessment and differential diagnosis of metabolic bone diseases. The molecular characterization of the skeletal matrix has resulted in the development of biochemical markers that reflect either bone formation or bone resorption. Apart from serum total alkaline phosphatase (sTAP), markers such as bone specific alkaline phosphatase (sBAP), osteocalcin (sOC) and the N-terminal collagen propeptide of type I collagen (PINP) are useful to assess bone formation rates. Bone resorption can be measured by a number of serum and urine markers, including the pyridinium crosslinks and their high molecular weight derivatives, NTX-I and CTX-I.
Biochemical markers of bone turnover are non-invasive and, when applied and interpreted correctly, helpful tools in the assessment of metabolic bone disease, therapeutic efficacy and patient compliance. It should be borne in mind, however, that the measured marker levels represent the result of three partly independent processes: the frequency of remodeling activation (which varies over a wide range), the amount of bone resorbed and formed during each bone remodeling cycle (which varies over a narrow range), and the metabolism and elimination of the marker component itself.
Markers of bone turnover are of particular interest in patients with osteoporosis, where they may be used to predict bone loss and fracture risk as well as monitor therapeutic efficacy and patient compliance. Both resorption and formation markers have been shown to predict bone loss and osteoporotic fractures in larger cohorts of postmenopausal women. High rates of bone turnover are associated with future vertebral and non-vertebral fractures, independent of bone mineral density or other risk factors. Also, the use of bone turnover markers in monitoring therapeutic efficacy and outcomes has been well documented. Whether the measurement of bone markers is helpful in encouraging adherence and persistence with drug therapy is less clear. Also, the predictive power in the individual patient, and hence the role of bone markers in clinical practice is still under investigation. In this context, variability and standardization of bone markers remains to be a major issue of concern and controversy.

**New advances in prenatal and postnatal testing**

## NEW PHILOSOPHY OF FIRST TRIMESTER SCREENING IN PREGNANCY

P. Calda[1]
[1]*Charles University, First Medical Faculty, Prague, Czech Republic*

Over the last 20 years there has been a debate about the best model of aneuploidy screening in pregnancy: to simplify, the alternatives are a combined test in the first trimester or an integrated test using biochemical parameters over the first and second trimesters. The inclusion of objective measurements of risks of pregnancy pathologies in the algorithm of first-trimester screening was immense progress, as well as an argument for the predominant use of this examination: this meant that detection of pregnancy risks and fetus morphology (premature delivery, preeclampsia, intrauterine growth retardation) as early as at the end of the first trimester came to the fore, so detection of aneuploidies was thus no longer the most significant outcome of this test. Now we are witnessing the dynamic onset of a new method, Non-Invasive Prenatal Testing (NIPT) or Screening (NIPS). This is based on detecting cell-free DNA in the peripheral blood of the mother, which can be performed as early as the completed 10th week of pregnancy. Arguably, NIPT can now determine the risk of Trisomy 21 with 99% sensitivity, while the sensitivities are similarly high for Trisomies 18 and 13; it can also determine the sex of the fetus with 99% sensitivity. The potential of this method may be even greater, but there are not yet sufficient reliable data for its clinical use, for instance, to detect micro-deletion syndromes.

**New insights in quality management of total testing process**

**IMPACT OF PREVENTIVE AND CORRECTIVE ACTIONS IN QUALITY MANAGEMENT FOR TOTAL TESTING PROCESS**

Ö. Güzel[1]
[1]*Biruni Centro Laboratories*

Laboratories need to monitor and improve their procedures to deliver the correct test results ensuring quality in all phases of the test performance.
The purpose of the corrective and preventive action system is to collect information, analyze information, identify and investigate testing and quality problems and take appropriate and effective corrective and/or preventive action to prevent their recurrence.
Corrective action is an activity that should be taken to stop re-occurrence of non-conformities.
Preventive action is an activity that should give the opportunity to prevent the potential non-conformities.
Corrective action has to be started when a problem exists, if such a problem does not exist but there is a possibility for it, preventive action has to be taken.
Remedial action can easily be confused with corrective actions. This action is taken to correct the mistake.
Collection of non-conformities is the key process and should be well defined in quality systems.
All departments of laboratory and all steps of analyzing process, from customer complaints, feedbacks, internal-external audits and maintenance of quality system, laboratory has to prepare lists for possible nonconformities. Daily data should be collected. Weekly/monthly evaluation of collected data gives the opportunity for corrective and preventive actions.
Always consider that collected data must be useful for the purpose. If the data collected and classified correctly, this activity will help to identify the problems clearly. During classification, priority and significance of non-conformities should be evaluated.
Corrective action process starts with cause analysis. Any kind of mistake in this step may cause implementing wrong corrective action and does not avoid re-occurrence of non-conformities.
Impact of non-conformities on the laboratory work should be analyzed carefully. Possibility of re-occurrence and effect to a routine procedure should be determined.
of corrective actions should be recorded and monitored for effectiveness of corrective actions.
“Corrective and preventive action team” should be organized and the adverse affects to the processes or activities should be carefully evaluated and classified according to their severity and priority.

**New insights in quality management of total testing process**

**HARMONIZATION, QUALITY AND SAFETY IN THE TOTAL TESTING PROCESS**

M. Plebani[1]
[1]*Department of Laboratory Medicine, University-Hospital of Padova, Italy*

The path leading to quality and patient safety in laboratory medicine is infinite, since it must be ensured that each and every step in the Total Testing Process (TTP) is correctly performed, thus guaranteeing a valuable medical decision making process and effective patient care. Laboratory-associated error has a completely different meaning today than it did a century ago. At that time the term referred to defects in the analytical performance of the test itself, the so-called analytic phase. The new millennium has hailed a formidable improvement in the analytical phase with a ten-fold reduction in error rates, thanks to an improved standardization of analytic techniques and reagents, advances in instrumentation and information technologies, as well as to the availability of more qualified and better trained staff. In addition, this achievement is due to the development and introduction of reliable QIs and quality specifications for the effective management of analytical procedures. According to recent evidence, most errors fall outside the analytical phase, in fact the extra-analytical steps have been found to be more vulnerable to the risk of error. It needs, therefore, to evaluate all the steps in TTP, whether or not they fall under the direct control of laboratory personnel, with the ultimate goal being to improve, first and foremost, quality and safety for patients. Quality indicators (QIs) are fundamental tools for enabling users to quantify the quality of all operational processes by comparing it against a defined criterion. According to the International Standard for medical laboratories accreditation, the laboratory shall establish and periodically review QIs to monitor and evaluate performance throughout critical aspects of pre-, intra-, and post-analytical processes. A preliminary agreement on a possible list of QIs has been recently achieved after revising the model of quality indicators (MQI) developed by the Working Group on “Laboratory Errors and Patient Safety” of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The consensually accepted list of QIs, which takes into consideration both their importance and applicability, should be tested by all potentially interested clinical laboratories to identify further steps in the harmonization project.

**New insights in quality management of total testing process**

**QUALITY MANAGEMENT OF THE PRE-ANALYTICAL PHASE**

A. Simundic[1]
[1]*University hospital Center "Sestre milosrdnice", University Department of Chemistry, Zagreb (Croatia)*

Quality management in the pre-analytical phase is challenging and complex. It poses substantial demand in terms of human, financial and organizational resources to the laboratory management. It requires contribution by all involved stakeholders: laboratory professionals, medical doctors, nurses and patients, as well as hospital management and regulatory authorities. Nevertheless, ISO 15189 recognizes laboratory as responsible entity for managing the quality of pre-analytical phase, by carefully monitoring and continuous improvement of all respective processes and steps. According to ISO 15198, pre-examination processes include "all steps starting in chronological order from the clinician's request, including the examination requisition, preparation of the patient, collection of the primary sample, transportation to and within the laboratory and ending when the analytical examination starts".
Several important prerequisites need to be in place in order to establish and maintain an effective pre-analytical quality management system. Those issues shall be discussed and presented in this lecture. In brief, they include carefull risk management by detailed analysis of the processes, by defining the safe practice standards and best practice recommendations and consistently enforcing compliance to those, by establishing systems for error-detection and system performance, by initiating corrective and preventive actions and education and training of staff involved in this processes.
The initiatives need to be taken at several levels: by international professional associations in laboratory medicine, by national professional associations and at the level of individual laboratories. International associations should take the lead in defining and providing best-practice recommendations, national professional associations should assist in efficient distribution of those recommendations and individual laboratories should do their best to adhere to the guidance documents.

**New strategies in the diagnosis of hematologic diseases**

## FLOW CYTOMETRIC EVALUATION OF PLATELET MARKERS IN HEMATOLOGICAL DISORDERS

J. Kappelmayer[1]
[1]*Department of Laboratory Medicine, University of Debrecen, Hungary*

Flow cytometry is a versatile technique that plays a major role in the diagnosis and monitoring of variable diseases. In case of hematological malignancies, it is used in a multicolor setting, where numerous lineage or maturation specific markers are applied as backbones in staining panels. Compared to other markers, there are only a few reports that systematically investigate platelet markers in hematological disorders.
In AML, surface staining for various megakaryocyte/platelet glycoproteins (GP) like CD41/61 and members of the CD42 complex have been performed, and was found to provide false positivity in several types of non-M7 AML types. We introduced the utilization of the A-subunit of coagulation factor XIII (FXIII-A) into our routine acute leukemia panels. This protein is normally expressed intracellularly in megakaryocytes and monocytic cells in the bone marrow, and we found that it is useful for the identification of AML subtypes of monocytic or megakaryocytic lineage. In both types we found it more specific than conventionally used surface markers like CD14 or CD41. Furthermore, in a recent study we described that FXIII-A can also be used as a leukemia associated immunophenotype in about 40% of cases in pediatric ALL where its expression was found to be associated with a favourable prognosis.
Platelet surface markers that are constitutively expressed or are activation dependent (e.g. P-selectin, PAC-1, annexin V) are useful in identifying activated or primed platelets in cardiovascular disorders and in functional assays to detect the platelet activating effect of plasmas derived from patients with heparin induced thrombocytopenia. In the past decade, the identification of platelet GP together with annexin V staining has become routine in enumerating platelet microparticles in several thrombotic disorders. It is also advantageous to execute platelet GP determination on peripheral blood leukocyte subsets - primarily on myeloid cells - as this has been shown to be one of the most sensitive indirect platelet activation markers both in animal models and in ex vivo clinical samples.
Overall, the flow cytometric investigation of platelet/megakaryocyte markers are useful both in hematological malignancies as well as in patients with vascular disorders.

**New strategies in the diagnosis of hematologic diseases**

**MOLECULAR PROFILING OF MYELODYSPLASTIC SYNDROMES**

S. Ogawa[1]
[1]*Kyoto University*

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by varying degrees of cytopenias and a predisposition to acute myeloid leukemia (AML). With conspicuous clinical and biological heterogeneity in MDS, an optimized choice of treatment based on accurate diagnosis and risk stratification in individual patients is central to the current therapeutic strategy. Diagnosis and prognostication in patients with myelodysplastic syndromes (MDS) may be improved by high-throughput mutation/copy number profiling. To address this, a total of 944 patients with various MDS subtypes were screened for gene mutations and deletions in 104 known/putative genes relevant to MDS using targeted deep-sequencing and/or array-based genomic hybridization. A total of 2,764 single nucleotide variants (SNVs) and insertions/deletions (indels) were identified in 96 genes as high-probability somatic changes). A total of 47 genes were considered as statistically significantly mutated ($p<0.01$), which included 6 genes (TET2, SF3B1, ASXL1, SRSF2, DNMT3A, and RUNX1) mutated in >10% of the cases, followed by less frequently mutated genes, such as U2AF1, ZRSR2, STAG2, TP53, EZH2, CBL, JAK2, BCOR, IDH2, NRAS, MPL, NF1, ATM, IDH1, KRAS, PHF6, BRCC3, and ETV. Intratumoral heterogeneity was evident in as many as 50% of the cases. Mean variant allele frequencies showed significant variations among major gene targets, suggesting the presence of chronogenic hierarchy among these common mutations during clonal evolution in MDS. The mutation/deletion status of a set of genes could be used to build a clinically relevant prognostic system as independent variables from clinical parameters, which outperformed the IPSS-R. In conclusion, large-scale genetic and molecular profiling by targeted deep sequencing and array CGH not only provided novel insights into the pathogenesis and clonal evolution of MDS, but also helped to develop a potent prognostic model based on gene mutations and other clinical variables that could be used for risk prediction. Molecular profiling of multiple target genes in MDS is feasible and provides an invaluable tool for improved diagnosis, biologic subclassification and especially prognostication for patients with MDS.

**New strategies in the diagnosis of hematologic diseases**

**ADVANCES IN LEUKEMIA/LYMPHOMA IMMUNOPHENOTYPING: CONTRIBUTION OF THE EUROFLOW CONSORTIUM**

A. Orfao [9], J. Flores-Montero[9], V. van der Velden[4], S. Böttcher[8], J. Almeida[9], P. Lucio[6], E. Mejstrikova[5], T. Szczepanski[7], M. Brüggemann[8], L. Sedek[7], A.W. Langerak[4], J. Caetano[6], T. Kalina[5], Q. Lecrevisse[9], C.E. Pedreira[1], M. Lima[3], M. Martin[2], E.S. Costa[1], J.J. van Dongen[4]
[1]*COPPE/IPPMG, UFRJ, Rio de Janeiro , BR*
[2]*Cytognos SL, Salamanca, Spain*
[3]*Department of Hematology, Santo António Hospital, Porto, PT*
[4]*Department of Immunology, Erasmus MC, Rotterdam, The Netherlands*
[5]*DPH/O, Prague, CZ*
[6]*IMM, Lisbon, PT*
[7]*SAM, Zabrze, PL*
[8]*UNIKIEL, Kiel, DE*
[9]*University of Salamanca, Salamanca, Spain*

Immunophenotyping of leukemia and lymphoma represents one of the most widely used clinical applications of flow cytometry. However, advanced flow cytometry for diagnostic application needs innovation and standardization at many levels, particularly in case of multicolor flow cytometry, where large data sets are generated, which need fast and easy processing.
Based on this background, the EuroFlow Consortium defined several key objectives which mainly focused on: 1) new methods for detection of fusion proteins in acute leukemia for fast and easy classification; 2) standardization of flow cytometry; 3) development of new software tools for easy and reproducible data analysis; 4) design and evaluation of 8-color antibody panels for the diagnosis, classification and treatment monitoring in patients with different types of hematological diseases. A total of 14 different groups from 12 Public Hospitals and/or Universities and two small size biotech companies started and have been collaborating in the EuroFlow project since 2006.
Major achievements reached mainly include: i) immunobead assays developed for detection of the most relevant fusion proteins in cell lysates of acute leukemia cell samples at initial diagnosis; ii) standardization of virtually all steps of flow cytometric immunophenotyping; iii) new and innovative software tools to facilitate and promote more objective data analysis/interpretation; iv) comprehensive 8-color antibody panels designed and evaluated for the diagnosis and classification of all types of hematological malignancies. These antibody panels have been carefully attuned to each other so that the overall immunophenotypic profile of a given cell population can be compared with reference data sets of multiple normal and pathological samples stained with the same (or partially overlapping) antibody panels.
Current ongoing activities are mainly focused on: i) the construction of la large reference data base for the 8-color EuroFlow leukemia/lymphoma diagnostic antibody panels, and; ii) the design of 8-color minimal residual disease (MRD) tubes for monitoring of treatment effectiveness.

**AFCC Symposium Qualitative and quantitative PCR: HIV viral load and Eid**

## PERFORMANCE ANALYSIS OF HIV EARLY INFANT DIAGNOSIS (EID) AT ARTHUR DAVIDSON CHILDREN'S HOSPITAL POLYMERASE CHAIN REACTION (PCR) LABORATORY IN NDOLA ZAMBIA: A CRITICAL LOOK AT TURNAROUND TIME.

H. Lumano[1]
[1]*Zambia Prevention Care and Treatment Partnership (ZPCTII)*

Without early intervention, up to 40% of Human immunodeficiency virus (HIV) positive infants would die by the age of 2 years. Critical to the survival of HIV infected infants is access to early diagnosis and treatment. From 2007, the laboratory has ensured that reliable results are generated under quality measures and dispatched to district hubs within a short turnaround time (TAT). In spite of measures in the laboratory to reduce TAT to 2-5 days, Dry Blood Spot (DBS) specimen referral system experienced delayed delivery of results from district hubs due to logistical challenges. Survey conducted by Ministry of Health (MoH) and cooperating partners in 2008 showed average TAT of 6.2 weeks. To alleviate this problem co-operating partners, embarked on a pilot program to expedite the delivery of results by identifying suitable sites in which a Short Messaging System (SMS based) PCR results delivery system could be implemented. In July 2010, the laboratory began sending encrypted PCR results via SMS.
Data comparing pre-SMS system against post-SMS system results were reviewed from 21 facilities in 11 districts across 5 provinces. Testing data collected from ZPCTII laboratory and SMS server located at MoH.
A total of 1,876 results were delivered to the facilities and 95% were received in less than 3 weeks. Aggregated TAT reduced by 56% from 45.5 days pre-SMS system to 21 days post SMS system. 138 out 510 facilities have been activated as the rollout continues.
With mortality high among HIV-infected infants, a robust EID program is essential to allow access to life-saving drugs. Reducing TAT by 56%allows infants to accessearly treatment assuring improved quality of life.

**AFCC Symposium Qualitative and quantitative PCR: HIV viral load and Eid**

## UGANDA'S EARLY INFANTS DIAGNOSIS LABORATORY CONSOLIDATION IMPROVES HEALTH OUTCOMES TO HIV POSITIVE INFANTS

S.P. Rugera[1], C. Kiyaga[4], A. Opio[2], J.R. Aceng[3]

[1]*Chair, Research and Development, National Health Laboratory Technical and Policy Advisory Committee, Ministry of Health, Uganda and Department of Medical Laboratory Sciences, Faculty of Medicine, Mbarara University of Science and Technology, Uganda*

[2]*Commissioner, Department of Infectious Disease Control, Ministry of Health, Kampala Uganda*

[3]*Director General of Health Services, Minitry of Health, Uganda*

[4]*Head, Early Infant Diagnosis Programme, Central Public Health Laboratory, Ministry of Health, Uganda*

UNICEF estimates indicate that globally, one child is born with Human Immunodeficiency Virus (HIV) every one and half minutes. Without diagnosis and treatment, one third of HIV-infected infants will die before their 1st birthday, and almost half will die during their second year of life. Uganda has made progress in early detection of HIV among exposed infants by Polymerase Chain Reaction (PCR) using Dry Blood Spot samples,through the Early Infant Diagnosis (EID) programme, that augments HIV treatment strategies.

We did a desk review of available data on EID services in Uganda as well as Uganda health sector reports. Our personal experience and involvement in the EID services provision was also exploited.

Since inception of the Early Infant Diagnosis (EID) programme within the Central Public Health Laboratories (CPHL), Ministry of Health in Uganda in 2007, over 300,000 HIV-exposed infants have been tested. Cost cutting from $22.2 to $5 over-head cost per test, and reduction in turn around time (TAT) of 1 to 2 weeks from what used to be 2 to 3 months, was made possible through the consolidation of eight laboratories into one centralized laboratory and the creation of a sample transport network for the EID programme. Improved laboratory efficiency and reduction in TAT through laboratory consolidation lead to improved patient outcomes and patient retention. The number of HIV-positive infants that were initiated on Anti Retro Viral (ART) went up from 23 percent in 2009 to 57 per cent in 2012, an improvement of 34 per cent.

Uganda's innovative handling of the EID programme that reduced cost, improved efficiency, coordination and monitoring, provides fertile ground for improving EID services globally, especially in the developing economies where HIV remains endemic and yet resources are limited.

**AFCC Symposium Qualitative and quantitative PCR: HIV viral load and Eid**

## IMPLEMENTATION OF ISO 15189 STANDARDS FOR IMPROVEMENT IN QUALITY OF HIV VIRAL LOAD AND EID SERVICES IN KENYA

T.T. Gachuki[1]
[1]*National Public Health Laboratories-National HIV Reference Laboratory*

Human Immune Deficiency Virus (HIV) viral load assay is vital for effective management of infections, diagnosis, and assessment of treatment efficacy. The extreme sensitivity of Polymerase Chain Reaction based assays is a double edged sword as its clinical usefulness is attributed to this while pre-analytical contamination leads to errors, thereby requiring stringent quality measures. Laboratories in many countries in sub-Sahara Africa, including Kenya have had poor infrastructure and weak or non-existent laboratory quality management systems. The National HIV Reference laboratory (NHRL) has established a quality management system (QMS) to ensure analytical and service quality of HIV viral load and Early Infant Diagnosis (EID) operations.
NHRL started implementing the requirements of ISO (International organization for standardization) 15189 standard using the World Health Organization African Regional office stepwise tool for continual improvement in 2011. After a baseline survey personnel were sensitized on ISO and received training on Good Laboratory Practice, biosafety and internal auditing. Improvement projects were undertaken in all areas of the QMS.
Analytical quality: Access control reduced contamination, reducing reagent wastage and analytical delays. Procedures for all the processes set in place. Retraining of personnel and competency assessments reduced analytical variations and improved results quality. Inventory control eliminated reagent stock outs reducing delays of results. Equipment downtime reduced by regular preventative maintenance and placement on service contracts. Performance, strict monitoring of daily internal quality control and participation in proficiency testing has improved results quality. Customer services: Due to monitoring of turnaround time this has reduced from 1month to10 days. Customer confidence has been enhanced by entering into contracts, regular communication and direct emailing of results. Sample rejection rates reduced from 25% to 0.25% due to training on sample management. Regular internal audits assist in making improvements.
Implementation of ISO 15189 has led to major improvement in both analytical and service quality scopes of HIV viral load and EID services in Kenya.

**AFCC Symposium Qualitative and quantitative PCR: HIV viral load and Eid**

## DETECTION OF HIV DRUG RESISTANCE BY REAL-TIME PCR BASED METHOD

N. Ulenga[2], K. Phyllis[1]
[1]*Harvard School of Public Health*
[2]*Management and Development for Health (MDH), Tanzania*

The availability of active antiretroviral therapy (ART) to individuals infected with HIV in many African countries including Tanzania has greatly improved over the past 10 years as result of coordinated efforts of funding agencies and individual governments in rolling out HIV care and treatment. As the numbers of individuals receiving ART rises, the long- term clinical outcomes of ART among patients on treatment are not clear. In some countries data on patterns of HIV resistance to antiretroviral drugs is available but remain limited. Due to limited infrastructure and resources, HIV drug resistance testing is generally not available in resource-limited settings such as Tanzania. There is a need to adopt a cost effective approach to testing HIV drug resistance among individuals receiving ARV treatment in developing countries. The objective of our research was to evaluate an ultra-sensitive, real-time PCR based assay for the detection of HIV drug resistance mutations, which would also be cost-effective and sustainable.
We adopted the allele-specific real time PCR assay to detect HIV's single-base mutations. Pairs of DNA oligos for specific subtypes were designed to bind at the sites of mutations, and amplified by real-time PCR after ligation. The sensitivity of the assay was determined, and its performance was evaluated by comparing it to a commercially available, population-based sequencing assay, ViroSeq (Abbot).
Lig-Amp detected Y181C mutation in 215 out of 245 samples (87.7%), whereas ViroSeq identified 51%. M184V was detected in 201 out of 245 samples (82.0%) by Lig-Amp and 76% by ViroSeq. The K103N mutation was detected in 222 out 245 samples (90.6%) by Lig-Amp, while detected by ViroSeq in 40%.
We have demonstrated that, for the three mutations studied, in a patient population infected with diverse subtypes and recombinants, Lig-Amp is able to detect at least all mutations detected by ViroSeq in different subtypes, at a 10-fold lower cost. Lig-Amp could be important tool in HIV drug resistance surveillance, and patient ART management in resource poor settings.

**BCLF Symposium Balkan Clinical Laboratory Federation (BCLF) Symposium**

**WATER CHANNEL PROTEINS (AQUAPORINS AND RELATIVES) IN LABORATORY MEDICINE**

G. Benga[1]
[1]*"Vasile Goldis" Western University of Arad, Romania*

In 1985 Benga group in Cluj-Napoca, Romania,discovered th first water channel protein (WCP) in the membrane of the human red blood cell membrane. In fact a new concept was introduced, the WCP as a protein specialized in water transport. The first WCP was rediscovered in 1992 by Agre group in Baltimore, USA, and in 1993 it was called later aquaporin 1 (AQP 1). In subsequent years hundreds of WCPs have been discovered in organisms from all kingdoms of life. It was discovered that WCPs are a family of membrane proteins, belonging to the Membrane Intrinsic Proteins (MIPs) superfamily. WCPs family include three subfamilies: aquaporins, aquaglyceroporins and S-aquaporins.
WCPs in humans. Twelve WCPs were identified in the human body; out of these, seven are aquaporins (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), four are aquaglyceroporins (AQP3, AQP7, AQP9, and AQP10), whereas AQP11 and AQP12 are S-aquaporins. , having a great importance in a lot of physiological phenomena, as well as in pathological conditions. WCPs play very important roles in a variety of cellular processes in all organs of the human body.
WCPs in diseases .The malfunction of WCPs is linked to a lot of diseases. Some of these are water channelopathies (the congenital cataract due to mutations in AQP0 gene, the nephrogenic diabetes insipidus due to mutations in AQP2 gene, or neuromyelitis optica due to autoantibodies against AQP4). Others are diseases in which WCPs from various tissues are implicated, ranging from epilepsy, brain disorders, kidney diseases, cardiovascular diseases, gastrointestinal and hepatobiliary diseases, cancer etc. These diseases can be diagnosed in the specialized laboratories by various methods: analyses of genes, detection of AQPs in urine, detection of anti-WCPs autoantibodies in the serum, quantitation of WCPs in various tissues etc.
I believe that by discovery of WCPs a new domain of biomedical and natural sciences was opened, for which I suggested the term “aquaporinology”. This can be defined as the domain of sciences dedicated to the integrated approach of WCPs, as well as a chapter of Cellular and Molecular Biology.

**BCLF Symposium Balkan Clinical Laboratory Federation (BCLF) Symposium**

**SPECIFIC CELLULAR ELEMENTS OF URINE SEDIMENT IN VARIOUS KIDNEY DISEASES**

B. Glišić[1], N. Lalić[2]
[1]*Center of Medical Biochemistry, Clinical center of Serbia, Belgrade, Serbia*
[2]*Faculty of Pharmacy, Europian University, Novi Sad, Serbia*

Cellular elements that can be found in urine sediment are not just specific for the kidney, but are unique for specific part of kidney or for specific disease affecting kidney. Some of these cells found in urine of nephrological and kidney transplanted patients that had been analyzed in our laboratory will be discussed here.
Hematuria is very common finding. Distinguishing between kidney or lower urinary tract bleeding is important. Special form of dismorphic eritrocytes, known as acanthocytes are reliable tool for helping us make that decision. They can be identified as ring shaped eritrocytes from which one or more blebs protrude. Presence of more than 5% of acanthocytes is considered to be of glomerular origin.
Another type of specific cells that can point out to disorders regarding glomerular membrane are podocytes. Podocytes are visceral glomerular epithelial cells located on the outer surface of glomerular basement membrane. They function as molecular sieve. Different pathological conditions can induce podocyte injury resulting in podocyturia. They can be identified with fluorescence microscope after they had been treated with specific podocyte protein antibodies and fluorescein labeled antibodies.
Various forms of leukocytes that originate from kidney can also be found in urine. Eozinophils are found in acute interstitial nephritis, rapidly progressive glomerulonephritis, acute renal failure. Lymphocytes are considered to be early marker of acute rejection of renal allograft and can be identified using stains and cytological techniques. In the case of pyelonephritis, Glitter cells can be seen in urine sediment. Those are neutrophils containing cytoplasmic granules that exhibit Brownian movement.
Very important findings in the urine of patients with transplanted kidney are Decoy cells. Decoy cells are epithelial cells infected with Polyomavirus BK that can be seen in urine by using phase-contrast microscopy. They are characterized by enlarged nucleus, irregular chromatin pattern , nuclear inclusion bodies and cytoplasmatic vacuoles. Their appearance in urine sediment can arise suspicion to Polyomavirus BK-associated nephropathy, condition that affects immunocompromised patients and is considered to be significant post transplantation complication.

**BCLF Symposium Balkan Clinical Laboratory Federation (BCLF) Symposium**

**CYTOMICS - IMMUNOMICS**

K. Psarra[1]
[1]*Dept of Immunology - Histocompatibility, Evangelismos Hospital, Athens, Greece*

Cytomics concern the study of the functional relationships between the cell (cytome) and the metabolic pathways (proteomics – proteome) governed by the genetic control (genome – genomics). Some researchers relate cytomics to functional genomics. Cellular analysis tools like mulriparametric flow cytometry, multiplexed assays, image cytometry, computational resources for 3D imaging, bioengineering resources for cell manipulation as well as modern microscopy instrumentation are the main technical tools of cytome analysis and offer information about the present status (diagnosis) and future development (prognosis) of the disease. Bioinformatic tools are used in order to combine the information derived from high throughput systems towards understanding cell population heterogeneity in health and disease.

The human cytome project consisted of three levels: a) single cell study (cell cycle, biomolecules production, cell energy, organelles function), b) cell populations study correlated to genomics or proteomics study and c) cells within tissues concerning functional cell interactions.

The term 'immunome' corresponds to all the genes, proteins and cells taking part in immune responses. It excludes genes and proteins that are expressed in cell types other than in immune cells. Immunomics is a new discipline that uses high throughput techniques to understand the immune system mechanism. To better understand immune function and regulation immunomics projects should include specific immune cells at various stages of development, activation and disease. A lot of the studies concern genetics and evolution of MHC, TCR and Ig loci.

Immunomics is now leading to vaccine studies, including the prediction of immune responses to epitopes and informatics concerning the analysis of complex results derived from such experiments. Immunoinformatics not only helps in dealing with such a huge amount of data but saves time and work by modelling immune responses. Other scientific studies connected to immunomics concern B cells epitopes, cytokines and their networks, interactions between pathogens and their hosts, cancer, autoimmunity, infectious diseases.

It seems that although genomics reach their maturity, immunomics innovation will keep growing well into the 2020s.

**BCLF Symposium Balkan Clinical Laboratory Federation (BCLF) Symposium**

**COMMON URINALYSIS-CURRENT PRACTICES AND FUTURE PERSPECTIVES**

M. Shishenkov[1]
[1]*Military Medical Academy Sofia Bulgaria*

Introduction Urinalysis is one of the first tests, used in medicine, from uroscopy to nowadays qualitative and quantitative analyses.
Purpose: Development of routine application urinalysis through the decades and changes that were made due to the development of analytics and techniques in laboratory practice.
Urinalysis being a set of 10-12 chemical tests and microscopic analysis is about 12% of the routine laboratory workload in Bulgaria (2010). The main applications are diagnostics, treatment, monitoring, and screening of patients. The expectations towards analytical sensitivity of dry chemistry are raised. Protein testing is enough to prove and monitor proteinuria but for screening purposes among diabetics 10 to 20 times more sensitive tests are used. Calculation of ACR is recommended to compensate concentration differences. With increased ACR levels higher cardio-vascular risk is expected. Dry chemistry is used since the mid 20-th century, manually performed with uncertain result timelines. The optimal length is a series of 5-urine-samples for manual reading. Strip readers are more reliable. Sediment is the most time consuming. Aiming at better preanalytics we were surprised that HPF is not a reliable unit. HPFs (40x) diameters are between 250 and 800 µm; CV+/- 31%! If the surfaces of HPF-s are calculated the imprecision rises up to (+/-) 73 %! Applying number of cells/µl or number pro defined net area in a one way slide chamber is a hard to perform solution. Automation is the choice or we have to accept the urine sediment as qualitative approximate analysis.
The optimum result output and standardization in urinalysis is achieved by application of standardization and automation, including microscopic analysis. The strip reader completes 1 sample for 2.5 min, 10 for 15 min and 232 for 232 min. A person verifies the findings on a screen and selects samples for microscopic analysis. On the microscopic analysis the “common” sediment should be seen as a qualitative test.

**IFCC Symposium Clinical utility and standardization of emerging and less common tests**

## THE IMPACT OF STANDARDIZATION ON THE CLINICAL UTILITY OF BONE MARKER MEASUREMENT

H.A. Morris[1]
[1]*University of South Australia and Chemical Pathology, SA Pathology, Adelaide South Australia 5000 Australia*

The incidence of osteoporosis is high and with an ageing 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. Do 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?
Evidence from prospective studies was gathered through the PubMed database between the years 2000 and 2010 and the systematic review of the Agency for Healthcare Research and Quality up to 2001 and published by the IOF-IFCC Working Group on Bone Marker Standards for Osteoporosis in 2011.
7 bone formation assays and 8 bone resorption assays are available on automated clinical chemistry analysers and point-of-care devices. 22 studies examined the relationship between BTM levels and risk of subsequent fracture. 18 studies found that one or more BTM's was associated with risk of fracture. However 15 different markers were used in these studies and there was considerable heterogeneity with regard to prediction of the anatomical site of fracture. A large number of clinical trials of osteoporosis therapeutic agents have been assessed for prevention of fracture only 5 were identified to include the measurement of a BTM and these only included a sub-group of patients. Data from several studies suggest that the larger the fall in the BTM following commencement of treatment with an antiresorption agent the larger the reduction of risk of subsequent fracture.
Considerable debate remains as to the clinical utility of BTM levels for the individual patient. Future clinical trials should utilise measurements of serum β-CTx, to assess bone resorption, and serum PINP to assess bone formation to enlarge the experience and provide data to be incorporated in meta-analyses. The task of harmonizing these assays is underway. Preliminary data of a meta-analysis of serum β-CTx data indicate a significant relationship with increased risk of fracture.

**IFCC Symposium Clinical utility and standardization of emerging and less common tests**

## IS THERE A NEED FOR STANDARDIZATION OF MOLECULAR DIAGNOSTIC TESTING?

D.D. Payne[1]
[1]*American Pathology Partners*

Molecular Diagnostic Testing (MDT) provides information used to detect disease, monitor minimal residual disease, and guide treatment. In theory, standardization insures delivery of laboratory-independent diagnostic information used by health care providers to manage their patients' health.
Documents such as ISO 15189 provide recommendations for general laboratory testing but specific guidelines for MDT are also available (e.g. Clinical Laboratory Standards Institute).
Standardization of MDT should address the pre examination, examination and post examination phase [aka the total testing process (TTP)]. MDT standardization faces many challenges due to variability with specimen types, instrument platforms, reagents, and technologist skill. Even heavily standardized methods may still produce discordant laboratory results. Rapidly evolving technology further complicates the development of ISO Certified Reference Materials and guidelines.
Even with these challenges, a few successes for the standardization of MDTs have occurred in the areas of oncology and infectious disease testing. As MDT increases, additional standardization will benefit patient care.

**IFCC Symposium Clinical utility and standardization of emerging and less common tests**

## STANDARDIZATION IN AUTOIMMUNE TESTING

J. Sheldon[1]
[1]*St. George's Hospital, London*

The concepts of traceability and measurement of uncertainty in laboratory testing have been with us for decades. However, with the implementation of ISO 15189.2011, demonstrating traceability and uncertainty has become a requirement. A rational person, a scientists or most importantly a patient, would say this was appropriate; they may be surprised that it is not always the case! Autoimmune testing does have some standards and some assays are even reported in International Units but EQA repeatedly shows negligible agreement in results between kits, and patient results vary dramatically from one lab to another. This is a significant clinical risk.

The first step in establishing a traceable standard is defining what you are measuring. We are typically measuring IgG (antibodies) in serum and there is a reference material for IgG in serum (ERM-DA470k/IFCC). However, we are actually measuring serum IgG that is capable of binding to an (auto) antigen. The preparation of the antigen may vary from one method to another and the binding parameters of IgG to the antigen may vary between patients. The next step is the definition of the methods, but for autoimmune serology there is a multitude of variations on a basic heterogenous immunoassay. Robust traceability and measurement of uncertainty will require all these elements to be considered.

The IFCC Harmonisation of Autoantibody Testing Working Group is working towards materials for the harmonisation of 5 clinically significant autoantibodies. The aim is to assign values that are traceable to ERM-DA470k/IFCC and enable reagent producers and laboratories around the world to take the first step towards demonstrating traceability of their autoantibody tests and assessment of analytical uncertainty.

**IFCC Symposium Clinical utility and standardization of emerging and less common tests**

## HOW TO STANDARDIZE PROTEIN MEASUREMENT BY MASS SPECTROMETRY

S. Lehmann[1]
[1]*CHU Montpellier, France*

Mass spectrometry (MS) has demonstrated its clinical potential, for example in the field of small molecules. Recent improvements in technology allowed MS to perform the quantitative analysis of proteins in biological fluids. The future application of this technology in the clinical field is believed to be important in particular for the detection of novel biomarkers/isoforms that lack satisfactory immunochemical detection methods; or the development of panels of several relevant clinical analytes. These analyses need to reach a satisfactory level of analytical performance. In this context, the method to standardize protein measurement is of critical importance.
The different absolute quantification strategies generally relies on the principle of isotope dilution: a known quantity of a stable isotope labeled internal standard might be added at different steps of the analytical procedure workflow. If for small molecules and for peptide it is possible to generate primary standard reference material (SRM) with known concentration, for proteins which production relies in most cases on recombinant approach, this task seems out of reach. Alternative approach using synthetic labeled peptides or secondary SRM are however possible. To address these issue, we set up and tested clinical workflows to detect the apolipoprotein E (ApoE) in human blood and the tau protein in human cerebrospinal fluid.
For ApoE, serum samples from patients were digested using trypsin. Before analysis, samples were resuspended a buffer containing heavy internal standard peptides of ε2, ε3 and ε4 isoforms. Peptides were then detected using a nanoLC-MS/MS. For the tau protein, we used a precipitation method prior to digestion and recombinant isotope labeled protein standard to ensure quantitation. In both cases, protocols were optimized to control enzyme-protein ratio, incubation time, digestion buffer and treatment preceding the digestion step.
Standardization of protein measurement by MS is a problematic task linked to protein extraction/digestion workflow and the availability of adapted SRM. It is however possible to develop protocols that generate quantitative data with similar or even better quality than classical immunodetection methods.