Swiss MedLab 2008: plenary and parallel lectures

Selection of summaries, as submitted by the authors

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17PW12: New aspects of endocrine stress assessment

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Stress is an often used but bad defined term. It is either defined as resistance of an object against an effort or, in physiology, as a condition of disturbed balance or homeostasis. The first description of the term "stress" derives from Hans Selye who published three phases of "stress" in the Journal Nature 1936: 1) alarm phase, a reaction of our body to mobilize resources, 2) resistance phase, where our body tries to cope with the stressor and 3) exhaustion phase where reserves are depleted. One important question is whether and how we can measure the stress response of our body.

One of the most classical and important stress hormones is cortisol which is produced under the stimulus of corticotrophin-releasing hormone (CRH) from the hypothalamus and adrenocorticotropin (ACTH) from the pituitary. Cortisol circulates in blood largely bound to corticosteroid-binding protein and albumin, with the much smaller amount of unbound hormone responsible for its metabolic effects. In general, it is assumed that the biologically active level of cortisol to which tissues are exposed is free cortisol. Routinely available assays of adrenal function measure serum total cortisol and not the biologically active free cortisol, but it is usually considered that total cortisol broadly correlates with the biologically free fraction. However, it has been shown that in critically ill patients there is a fall in binding proteins such that total cortisol levels no longer accurately reflect the free fraction, and this could potentially cause misdiagnosis of adrenal function. Serum total cortisol levels after major surgery approach the levels during the acute phase of septic shock. In this context, major surgery can serve as a standardized model for studying acute and major stress. Previous studies have also demonstrated that, under the most stressful conditions, total cortisol levels in response to major stress are mirrored by similar changes in the response to a pharmacological dose of ACTH. It has therefore been assumed that responsivity to the ACTH stimulation test will reflect the pituitary-adrenal response to surgery, trauma or other types of stress, and can be used as a surrogate to decide on the appropriateness of corticosteroid replacement therapy in surgery or acute illness. Our results, however, show that in major stress the relative increase in free cortisol is significantly more pronounced as compared to the increase in total cortisol. Furthermore, after ACTH stimulation, total cortisol levels increase to a similar extent as compared to major stress, whereas free cortisol levels in major stress increase to a significantly greater extent after surgical stress as compared to after ACTH stimulation. This suggests that the ACTH test does not adequately anticipate the free cortisol levels needed during severe stress.

Another less well known but equally important stress hormone is vasopressin. Vasopressin has important hemodynamic and osmoregulatory effects. During stress, vasopressin is a potent synergistic factor of CRH as a hypothalamic stimulator of the hypothalamo-pituitary-adrenal axis. Copeptin is co-synthesized with vasopressin, directly mirroring vasopressin levels, but is more stable in plasma and serum. Interestingly, copeptin levels show a more gradual increase with increasing stress levels as compared to cortisol. Copeptin levels mirror moderate stress even more subtly as compared to cortisol levels. Copeptin might thus provide a novel tool to mirror the individual stress level at the hypothalamic level.

Stress hormones have recently been shown to be useful predictors for outcome in different diseases. In lower respiratory tract infections, total and free cortisol levels as well as copeptin levels were better predictors of survival as compared to usually measured parameters like C-reactive protein, body temperature or leukocyte count. In patients with acute ischemic stroke, copeptin levels were independent predictors for three months outcome and were able to improve the prognostic accuracy of the clinical scoring systems. In this context, measurement of stress hormones might be useful for a better risk stratification of patients with various diseases.

17PW13: Novel approaches in the assessment of fertility

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Fertility is the ability of people to produce offsprings. It is therefore dependent upon the health and environment of both partners, and thus represents a measure reflecting their joint health. Fertility is generally believed to be declining worldwide, but objective measures and epidemiological comparisons are flawed by a confusing terminology as well as loose definitions. Regardless of this debate, the demand for reproductive assisted technologies is rising in western countries, underlying the need for a better assessment of the fertility potential of couples seeking assistance.

Plasma anti-mullerian hormone (AMH) correlates well with the number of follicles in rodents, and recent data have demonstrated that it can be used as a marker of ovarian reserve in women. AMH usefulness in that setting is notably increased by the fact that its levels are probably not dependent upon the cycle stage, although

some debate about the latter point exists in the current literature.

In infertile or subfertile men, asthenozoospermia remains one of the most common findings and in the vast majority of cases, its aetiology remains obscure. Plasma FSH, and more recently inhibin B levels, have been useful to predict the success of sperm retrieval rate, but new developments in understanding sperm cell biology are urgently needed. The role of sperm proteomics will probably increase rapidly in this context. Indeed, in the absence of gene transcription, sperm cell function is depending essentially upon post-translational modifications of their protein complement. Sperm sample proteomic analysis has not yet made its way to the clinic, but bears the potential to provide both novel insights into sperm function as well as potential biomarkers of male factor infertility.

Notwithstanding such advances in the biological workup of either female or male infertility, the clinician should remain aware of the outmost importance of a global approach of these couples. Indeed, a single anomaly will rarely be the only culprit, and coordinate changes in metabolic and lifestyle factors seem to play an increasingly recognized role. Thus, a state of the art approach to the infertile couple will include the most comprehensive etiologic diagnosis of the problem, including potential individual and environmental parameters such as smoking, hypertension or obesity.

17PW21: Thinking big: finding novel genes influencing risk of type 2 diabetes and obesity

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Until recently, progress in identification of the genetic variants influencing predisposition to common forms of diabetes and obesity has been slow. However, recent advances in genetics, genomics and informatics have transformed the situation, with researchers now able to undertake well-powered scans designed to detect association signals across the genome.

For T2D, the six genome-wide association studies performed have extended the number of loci harbouring common variants implicated in diabetes-susceptibility into double figures. Amongst the novel loci identified by this process include variants in/around genes which encode beta-cell zinc transporters (SLC30A8) and putative regulators of beta-cell mass (CDKN2A/B and CDKAL1). One of these new-found loci, mapping to the FTO gene, influences individual T2D-risk through a primary effect on fat mass, making this the first common variant known to influence weight and individual risk of obesity. More recent efforts at large-scale meta-analysis have identified a further seven T2D-susceptibility genes, as well as other genes with an impact on body mass and risk of diabetes.

However, the variants so far identified explain only a small proportion of overall susceptibility to these conditions. To find further loci, our plans include: (a) combining data from multiple genome-wide scans, and performing very large-scale replication studies, to increase the power to detect variants of smaller effect; (b) undertaking genome-wide surveys of structural variants; and (c) initiating targeted deep resequencing efforts to trap low frequency, intermediate penetrance variants that may have escaped attention by classical linkage and association approaches. To characterise loci already found, we are resequencing the genomic intervals of interest in cases and controls, and have initiated comprehensive fine-mapping efforts. Finally, we have begun to translate these new discoveries into information of relevance to improved clinical management of diabetes and obesity, by focusing on the epidemiological, physiological and pharmacological consequences of the susceptibility variants.

17PW31: Microbiology: recognition, reconnaissance, reconoscimento

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Surveillance of medical testing has been regulated by the legislator. One major purpose of the pertinent legislation is regulation for the benefit of the patient, ie 1) to guarantee a standard of quality of testing and 2) to promote improvement of that quality.

Any medical testing is, as medical treatment or medication are, subject to the statutory health insurance and to other, in part cantonal legislation. However, among all medical analytics, testing for transmissible diseases have been assigned a special status by 1) The Law on Epidemics (EpG) and 2) The Ordinance for Microbiologic and Serologic Laboratories (LabV). Thus particular conditions under which such services may be conducted and reimbursed have been defined. These conditions demand formal, organisational, educational and technical prerequisites to be fulfilled, and they set the rules laboratories shall adhere to. If laboratories do so, they receive upon application, licences to conduct the medical-microbiology analytical services. At the same time, legislation does also define surveillance of said analytical services and appoints the responsible health authorities.

At present the Federal Office of Public Health (FOPH) is responsible for surveillance of microbiology diagnostic laboratories, while Swissmedic is responsible for surveillance of screening for transmissible diseases in transfusion / blood banking, manufacture of blood products and in transplantation. Swissmedic, in addition conveys inspections not only in the latter but also on behalf of FOPH in the former.

For medical testing in general the standards of quality are defined by the "criteria to run a medical analytical laboratory" (KBMAL) by QUALAB, a consensus organisation of interest groups (insurers, service providers etc.). However, for microbiology laboratories the LabV is legally binding. This ordinance contains the "Good Practice in Microbiology and Serology Laboratories". The presentation will touch on the key elements of good practice and it will address the major issues of federal licensing and of surveillance.

17PW33: Medical laboratories: cantonal conditions

C. Robert; Service du pharmacien cantonal, Etat de Genève, Switzerland

In Switzerland, cantons are the authorities in charge of the supervision of health institutions (eg hospitals, pharmacies, ML). They lay down the conditions to be fulfilled to obtain a license to run these institutions and they

set the rules of operation. For all institutions operating under health insurance federal statutory, cantons must also take into account the demands of federal laws (LAMal).

LAMal acknowledges different types of ML. In Geneva, ML must obtain a cantonal license when:

– performing tests other than these concerning basic cares or
– performing tests upon request of a third party.

In 2008, there are 26 private laboratories, one hospital laboratory type C, one hospital laboratory type B, three pharmacy laboratories licensed in Geneva.

To obtain the license, the owner and the medical laboratory director ("chef de laboratoire") must submit a formal request to the cantonal pharmacist department. This application must enclose documents (eg copies of diploma of the medical laboratory director and testing managers ("responsables d'analyses"), blueprints of the premises). Prior to licensing, the cantonal authority conducts an inspection to assess compliance with the applicable requirements. The license will only be issued upon the recommendation of the cantonal pharmacist. The license is submitted to fees.

Among cantonal conditions to run a ML, the following are the most important ones:

Regarding quality assurance:

– The laboratory must implement a system for quality assurance.
– It must show proof of mandatory quality controls.

Regarding staff:

– The medical laboratory director and the testing managers must hold a federal degree in medical or phar-

maceutical science (or a diploma accepted by LAMal) and FAMH (or FMH) in the field of medical analysis.
- The medical laboratory director is allowed to manage only one site.
- The testing managers are allowed to work in only one site.
- The medical laboratory director must ensure full compliance with rules and regulations and confidence and must instruct personnel herein.
- At least 50% of the technical staff must have completed an acknowledged professional training, eg biomedical scientist ("technicien en analyses biomédicales").

Regarding premises and equipment:
- Facilities must conform with their purposes and equipment with professional regulations.

In addition, the Canton has decreed rules concerning the medical analysis prescription, the transfer of medical analysis orders to other medical laboratories and the duration of storage of some documents, eg medical reports.

17PW34: Regulatory framework of human genetic testing

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The Federal Law on Human Genetic Testing and the Ordinance on Human Genetic Testing (OHGT) have entered into force as of April 1st, 2007. These regulations provide the legal framework for the protection of human dignity and personality with regards to genetic data, to the prevention of abuses, to the prescription and performance of genetic testing and to the ensurance of a high quality of genetic testing. The presentation will focus on the requirements for laboratories seeking federal licence (ie authorization) for genetic testing and the authorization process in particular. Specifically, laboratories conducting cytogenetic and molecular genetic tests on humans as well as human genetic screening have to comply with, among others, the following requirements (according to the OHGT): 1) the appropriate qualification of the head of the laboratory, 2) the appropriate qualification of laboratory staff, 3) the compliance of the laboratory with state-of-the-art of science and technology, 4) quality assurance, and 5) the submission of a concept for human genetic screenings to the Federal Office of Public Health (FOPH). Applications for authorization are filed to the FOPH. The scope of the licence depends on the qualification of the laboratory head (FAMH-title for specialization). According to this qualification, specific tests are admitted as listed in the Ordinance to the OHGT of the Federal Department of Home Affairs. The presentation will also include informations about the obligations of laboratories, exemptions from authorization and the status of the current authorization process.

17PW41: Genetic heterogeneity of myeloproliferative disorders

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A number of oncogenic somatic mutations have been identified in myeloproliferative disorders (MPD) in the past few years. Among these JAK2–V617F is most frequent, followed by mutations of the thrombopoietin receptor (MPL-W515L, MPL-W515K) and JAK2 exon 12 mutations. In addition, deletions on chromosomes 20q (del20q) occur frequently in MPD and other myeloid malignancies. To explore the genetic aberrations present in MPD patients at higher resolution than offered by classic cytogenetics, we performed microarray karyotype analysis in a series of patients with variable presence of JAK2 and MPL mutations. About half of the analyzed patients exhibited loss of heterozygosity (LOH) in at least one chromosomal region. Several patients had complex karyotypes with two and more regions with LOH. Uniparental disomy on chromosomes 9p, 1p, and 14q represented the largest proportion of LOH detected followed by deletions on chromosome 13q, 20q, 12p, and 7q. To validate the presence of these defects, we performed LOH analysis in a cohort of 400 MPD patients and determined the frequency of these aberrations. The presence of genetic markers (LOH) in individual patients offers an opportunity to decipher the clonal composition of the stem/progenitor and terminally differentiated cell compartments. To determine the clonal composition of progenitor cells, we genotyped individual clones of BFU-Es and CFU-GMs in a series of 20 patients. We observed a remarkable clonal heterogeneity at the progenitor cell level. The presence of clonal heterogeneity in MPD has profound implications for therapeutic strategies since development of therapy resistance may simply be a matter of selection of clones least susceptible for myelosuppressive effects of drugs. In fact, we observed changes in the clonal composition of patients treated with interferon alpha. In one patient a selection of a clone positive for del20q and homozygous for MPL-515L was observed and linked to acquisition of interferon alpha resistance. In summary, our results provide evidence of the genetic heterogeneity of MPD and demonstrate how this heterogeneity affects the outcome of myelosuppressive therapy.

17PW43: Current methods for the clinical evaluation of platelet function

S. Panzer; Medical University Vienna, Austria

Primary haemostasis involves a tightly controlled cascade of events where under flow conditions activated platelets play a key role. Vascular damage induces the interaction with vWF, and collagen under high shear conditions, leading to platelet activation which then – through release of numerous activators – propagates platelet adhesion to the vascular tissue, platelet aggregation and formation of the platelet plug. In the past, platelet function was addressed in most instances to assure platelet haemostatic capacity to control bleeding. Only in more recent years, the role of platelet activation became better understood in thromboembolic disease. Treatment with anti-platelet drugs has further enhanced the demand to test platelet function in order to control anti-platelet drug effects, thereby balancing between the induced increased risk for bleeding and thrombosis.

Nowadays, a variety of tools are available to test platelet function ex vivo. All these test systems are based on platelet aggregation and are evaluated in com-

parison to light-transmission aggregometry (LTA), which is regarded the "gold standard". However, LTA is not even close to physiological conditions. The need to separate platelets from the other cellular components, thus losing the physiological environment, and the lack of high shear are the major shortcomings. Technically, whole blood systems are advantage because they do not require preparations of the platelets. Thus, any whole blood system tests not platelets alone, but also their interaction with all the other elements in the blood. This appears closer to physiological conditions, is easier to handle and can even be developed to a point of care system. This review will focus on currently commercially available whole blood systems to estimate platelet function, thereby differentiating between those systems that test platelet function under close to physiological conditions under high shear, and those which do not. Thereby our own experience shall be presented.

17PW51:
Value of MS Imaging to Biomedical

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Imaging by MALDI MS proved over last few years to be a valuable method in a variety of different arenas, including medical and biomedical research, and clinical diagnostics. While the application horizon is constantly being expanded, the demand for robust and reliable methods grows simultaneously. It is no longer sufficient to provide colorful images from a single sample, instead accurate result for a large number of samples have to be generated. Results presented here include new methods for sample preparation, tools and hardware for image acquisition and applications thereof to actual research activities at Novartis.

17PW52:
The role of mass spectrometry in therapeutic drug monitoring

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Therapeutic Drug Monitoring (TDM) is an important tool for guiding drug therapy in a wide range of different indications. The development of immunoassays for the quantification of drugs and drug metabolites is costly. Especially for new drugs TDM always has to be done by chromatographic methods, at least at the beginning of their introduction into the market.
For a long time this has been done by HPLC coupled to an UV or fluorescence detector using self-made reagents or commercially available reagent kits. In the last few years HPLC coupled to mass spectrometry (LC-MS) has entered into clinical laboratories, first in research orientated laboratories, in recent times also in pure routine institutions. In addition, the companies selling reagent kits are more and more offering kits especially dedicated to LC-MS analysis. This enormous increase in the use of mass spectrometry in clinical laboratories has happened despite the fact that mass spectrometric detectors are much more expensive than UV or fluorescence detectors. The reasons for this development will be depicted and examples of well established procedures will be shown.
Nevertheless, also mass spectrometry is not the magic bullet avoiding incorrect peak identification and some caveats have to be considered if drugs and drug metabolites are analysed by LC-MS. The most important reasons for erroneous results will be demonstrated and precautions will be presented, how to avoid them.

17PW53: Screening in clinical toxicology and mass spectrometry

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In clinical, forensic and doping controls. the compounds which have to be analysed for general toxicology screening are mostly unknown. The analytical strategy must be efficient, extensive and reliable because thousands of drugs and toxic substances are on the market worldwide.
Among the analytical techniques available to the toxicologists, chromatographic methods coupled to mass spectrometers are the most powerful, characterized by high specificity and high information content which can be used for both qualitative and quantitative analysis.
In the last 30 years several chromatographic methods with various detection systems were used in screening procedures; GC/MS from the end of 1970 and HPLC/UV, especially with the introduction of the REMEDI system beginning of 1990, proved their efficiency in clinical laboratories and largely contributed in the development of toxicology in university hospitals.
From the year 2000 and with the continuous development of the HPLC/MS/MS systems able to bypass the well known limitations of both LC/UV and GC/MS techniques, there is a growing interest in establishing toxicology screening procedures by LC/MS/MS.
The relevant results obtained with these powerful new systems and the development of identification softwares, very promising potentialities exist to help implementing robust and reliable LC/MS/MS methods for the clinical toxicology screening.

17PW54: Development of a multi-target screening analysis using LC-MS-MS as an alternative to the Remedi® method

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Introduction: The aim of this work is to develop a method to detect and identify a wide range of compounds in clinical toxicology by using LC-MS/MS as an alternative method to the Remedi® (BioRad) instrument.
The work includes the development of the analytical process with establishment of a data base. Detection, separation and sample preparation are the main process steps.
Method: A multi-target screening (MTS) procedure is developed using a hybrid triple quadrupole linear ion trap mass spectrometer (3200 QTrap, Applied Biosystem) for the simultaneous detection and identification of 300 important drugs in one single run using liquid chromatography/tandem mass spectrometry (LC/MS/MS) technique.
The samples are analyzed after a solid phase extraction (SPE).
The off-line SPE process is automated

and if necessary, it could be modified to be on-line.
The LC-MS/MS analysis is an information dependent acquisition (IDA) experiment. This one contains a multiple reaction monitoring (MRM) as survey scan followed by an enhanced product ion (EPI) scan as a dependent scan.
Finally, the drug identification is carried out by a library search with SmileMS, a newly developed software (GeneBio) based on EPI spectra similarity.
Results: Practicability, limit of detection, matrix effect and process efficiency will be presented.
Conclusion: The preliminary comparison of LC-MS/MS, GC-MS and Remedi suggests that the present LC-MS/MS technique is efficient and complementary to GC-MS and immunological tests. It's a help to enlarge the drugs detected in clinical toxicology.
The threshold limit to classify the product as identified or suggested need to be determined in the near future as well as the reliability of the method.
Furthermore, parallel studies are required to improve the robustness of the process between different brands of instruments.

17PW62: Rules for evaluation of haematology blood films in the External Quality Control

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Rules for evaluation of haematology blood films depend on the exigences given by the kind of participants of external quality control assessment (ECA). They are also related to the frequency and level of difficulty of abnormal blood smears sent out by the quality control center (QCC). A minimum of conformity has to be defined in a systematic, prospective and commonly agreed manner by each QCC conducting ECA for all or subgroups of participants. Rules are made up of definitions of target values in quantitative assessments and, related to that, of the tolerance for conformity (ie % white blood cell differential) as well as of the selection of items in qualitative or semiquantitative assessments (ie blood cell abnormalities) that have to be met by the participants (all or subgroups of them), while preventing successful hits merely by chance. In special situations modifications of both, quantitative and qualitative criteria of conformity, have to be made by taking into account the biological relevance of disagreed findings (rare and equivocal cases, respectively). A general judgment by the participants, whether an ECA trial is normal or abnormal, should prevent false positive assessments of a normal blood smear even if formally predefined quantitative and qualitative criteria have been met. Performance of participants should be documented in relation to other participants and should be weighed over a defined period of time to meet the requirements.

17PW63: Present situation of quality assessment for allergy in Switzerland

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The objective of reliable, quality controlled, results delivered by each laboratory performing in vitro specific IgE assays is realistic and achievable. This implies that the supplied material is adequate for all assay systems, either those quantitative performed by specialized laboratories or those simple, qualitative performed by specialists with rapid "in office" immunoassays.
In Switzerland, in the past years the situation has evolved from self adherence to quality scheme in the context of laboratory accreditation to compulsory participation to the external quality assessment (EQA) schemes. Successful participations to EQA are now mandatory for individual analysis reimbursement.
Analytes of clinical immunology and allergology are only performed in specialized medical laboratories and will not be assessed by EQA scheme in Switzerland due to the number of participants. The first coordinated Swiss scheme for autoimmunity and lymphocyte's phenotype analysis has recently been organized by the CSCQ in collaboration with UKNEQAS.
In basic allergy diagnosis, the specific context of Switzerland, where specialized medical laboratories, as well as allergologists and paediatricians perform in vitro allergen specific IgE determinations has to be taken into account. The Swiss Centre for Quality Control organizes an allergen specific IgE EQA scheme performed by more than 100 participants, that compares quantitative methods with rapid "in office" immunoassays: the same sera are analysed for specific IgE to birch pollen, cat dander and peanut with all methods and the results are reported in class for comparative analysis. The first results from this scheme are satisfactory, but they indicate some heterogeneity in the results of the rapid "in office" immunoassays. The performances of the failing methods clearly improve over time, showing that the regulatory pressure on the real performance in practice is an efficient way to improve quality of used assays at term.

17PW64: Quality needed for microbiology laboratories

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The main task of a microbiological laboratory is the detection of infectious agents in human or animal samples. Furthermore, the susceptibility of bacteria must be determined to give to the clinicians adequate information for the antibiotic therapy. Internal quality controls are crucial for the control of diagnostic processes to identify bacteria and their resistance mechanisms. The external quality control allows the participants to compare their performance with other laboratories and to see new bacteria and new resistance mechanisms that have not been recognized in their own laboratory. Therefore, external quality control has also a teaching effect. Bacteria with special mechanisms of resistance can appear in every microbiological laboratory independent from the size of the laboratory. Sometimes, the bacteriology of urine samples is considered as a small bacteriology; any technician without specific formation and even without supervision by a microbiologist is supposed to manage a microbiology laboratory in a small hospital. How-

ever, a great – even increasing – part of all methicillin resistant Staphylococcus aureus and Enterobacteriaceae with an extended spectrum beta-lactamase is first detected in the urine. The cooperation with the hospital hygiene is needed in such cases. Microbiological laboratories must be recognized and must participate to an external quality control by law. Unfortunately, the supervision of microbiological laboratories is not well regulated in Switzerland and must be approved.

Thursday, September 18

18PL1: Microfluidics technology for a future diagnostics

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For clinical diagnostics as well as biochemical research it is the ultimate goal to be able to analyse all substances in body fluids, cell contents and breath, holistically and in real time. Chemical and biosensors can be employed for rapid and continuous measurements of specific analytes in a complex matrix. However, because of their specifity one sensor is needed for each analyte, which makes this approach insuitable for the detection of a huge number of analytes like the proteome.

The two-dimensional polyacrylamid gel electrophoresis (2D-PAGE), for instance, is a well-established method in proteomic research since its capability for the comprehensive detection of all proteins. However, this method is very time and labour expensive. Here, miniaturisation can step into the gap. As the scaling laws predict a ten-fold miniaturisation in one dimension leads to a one-hundred-fold increase in separation speed. At the same time, analysis steps can be parallelised for higher reliability of the diagnosis. Additionally, only a thousandth part amount in sample and reagent volume is needed. This reduced amount in sample means less stress for the patient and also more safety for the operator.

Several miniaturized chemical analysis systems and operational modules have been developed in the last years and will have high inpact in future research and diagnostics.

18PL3: New methods for the detection of bacteraemia

J. Bille; Clinical Microbiology and Infectious Diseases, University Hospital, Lausanne, Switzerland

Bacteraemia and/or septicaemia constitute a frequent and severe infection, particularly in intensive care units, with a high mortality (30%). Early treatment, in particular early antibiotherapy, is essential for prognosis.

Microbiological investigations are important to determine the aetiology and establish the antibiotic susceptibility profile. Blood cultures are still the cornerstone of this diagnosis, even if they have some limitations due to the patient (variable bacterial loads), to microbial determinants (various doubling time), or related to technical determinants (volume of blood, delay of incubation).

Molecular detection of microorganisms in blood at time 0, when blood is drawn, is very appealing but quite challenging. Molecular methods have been used mainly with blood cultures at time of positivity, to speed up (4–6 h) the identification, or to detect some molecular determinants of resistance such as the gene mecA for methicillin resistance among staphylococci or Van A/B for glycopeptide resistance among enterococci.

One of the most clinically relevant indications to use molecular methods in positive blood culture bottles at detection time is the rapid differentiation between S. aureus and coagulase neg. staphylococci, as well as the search for mecA gene. Other approaches than PCR involve in situ hybridization methods.

Detection of bacteria or fungi in the blood at time 0 has been tried for over 10 years, often as narrow spectrum (Neisseria, mycobacteria, Aspergillus), or more recently as broad-range PCR. The method is still confronted with many problems linked to the limited amount of bacteria, the potential presence of dead organisms and of contaminants during the venipuncture, and the presence of inhibitors.

The first commercially available PCR system to be used as broad-range PCR

directly in blood specimens (Septifast Roche) has been evaluated in a few clinical settings so far (oncohaematologic neutropenic febrile patients, ICU patients). Septifast detects in a shorter time (5–6 h) as many or often more bacteria and fungi than blood cultures, some being potentially pathogens, other probable contaminants. The gold standard of positive blood culture should be challenged, of course. The system seems particularly promising when patients have previously received antibiotics, for the detection of fungi, and in patients persistently febrile while on antibiotics. Before we can really evaluate the benefits of this new system additional studies in different clinical settings are needed. The current version is very cumbersome. Many developments using chips micro arrays, nanotechnology, or calorimetry, are currently in development.

Today, classic automated blood cultures are still largely used, with many post-detection improvements aimed to speed-up the results both of identification and susceptibility testing. The ultimate goal is to establish in less than one hour the microbial etiology and susceptibility pattern of a sepsis episode using a simple POCT at the bedside.

18PW11: Detecting Group B streptococci colonization in pregnancy – culture, short turn-around-time or point-of-care testing?

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Background: Early onset neonatal sepsis due to group B streptococci (GBS) causes severe morbidity and mortality in newborns. While different preventive strategies to identify women at risk are recommended, the optimal strategy depends on the incidence of GBS sepsis and on the prevalence of anogenital GBS colonization. The aim of this study was to evaluate the performance of the "GeneXpert real-time PCR" (GeneXpert GBS, Cepheid, Sunnyvale, CA) for the detection of GBS carriers at the onset of labour, and to compare it to the results obtained by culture.

Methods: Pregnant women who had given informed consent were prospectively enrolled in the study. At the onset of labour two swabs from the outer third of the vagina and the anal region were taken from each study participant. One swab was cultured (overnight enrichment in Todd-Hewitt broth, followed by subculturing onto blood agar plates incubated for 24 h at 35 °C in 5% CO_2) and the other was tested by the "GeneXpert real-time PCR" which integrates all the processing (extraction-amplification-detection) in 75 minutes. Sensitivity and specificity of GeneXpert GBS was assessed against culture as the reference, and time-to-result and ease-of-use were evaluated.

Results: To date, 135 women have been enrolled in the study. The anogenital GBS colonization rate was 18% (culture). Five patient specimens (4%) gave inconclusive PCR results (eg inhibition due to excess mucus) and were excluded from further analysis. The "GeneXpert real-time PCR" test had a sensitivity of 87%, a specificity of 97%, a positive predictive value of 87% and a negative predictive value of 97%. GeneXpert was easy to use, and the test results were available within 75 minutes.

Conclusions: The "GeneXpert GBS real-time PCR" test had a short turn-around-time and was easy to use. It proved to be sensitive and specific in detecting GBS carrying women at the onset of labour, and may thus have a role to play as a point-of-care test that can be performed at the onset of labour by the staff of maternity clinics. This question will be addressed during the second part of the study.

18PW12: Human papilloma virus: new possibilities for testing/ vaccination

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Infection with high-risk (HR) types of Human papilloma virus (HPV) is the necessary cause of ano-genital cancers, particularly of the uterine cervix. Cytological screening, where implemented, efficiently diminishes the incidence of this cancer. However, the recent availability of two highly efficient prophylactic Virus-like-particle (VLP) based HPV vaccines (Cervarix®, from GlaxoSmithKline and Gardasil®, from SPMSD, Merck) that target the two major HR types, 16 and 18, may change the preventive strategies. Because these vaccines are close to 100% efficient only if administered before HPV infection, ie prior the onset of sexual activity, and because they cannot prevent the 20–30% cervical cancers caused by other HR types, screening remains mandatory. However, HPV DNA testing, which is already used in conjunction with liquid cytology, may become a major player. The only FDA approved HPV DNA test (HCII from Digene) that detects HR HPV types as a whole has shown utility in detecting clinically relevant HPV disease. Nevertheless, more sensitive HPV type-specific DNA test will be necessary when dealing with epidemiology and vaccine surveillance. Disappearance of the vaccine HPV types in the cytological samples will be the first sign of vaccine efficacy and it will be critical to verify that HPV DNA type substitution does not occur following vaccination. Also, as Gardasil is a quadrivalent vaccine that targets, in addition to HVP16 and 18, the two major low risk (LR) HPV types, 6 and 11, responsible of 90% of genital warts, LR HPV DNA testing may become of interest. Efforts of the WHO HPV labnet towards standardization and validation of type-specific HPV DNA test will be presented.

Serological test based on VLP or neutralization assays based on pseudovirions have been developed to assess vaccine immunogenicity in the vaccine trials. Those tests do not have clinical implications as natural HPV infection only results in low and un-sustained seroconversion in about half of infected women. However, these tests are of interest for assessing vaccine lots variability or durability of vaccination, for establishing age of vaccination in female populations where genital samples cannot be taken, as well as for assessing general vaccine survey in countries where vaccine registry are not available. Another important issue will be to establish a reliable immunological correlate of protection. Serological tests are not commercially available and standardization studies are ongoing in the WHO HPV labnet. Overall,

both DNA and serological HPV tests will be useful to evaluate vaccine safety and effectiveness in general but also in individual that may have received fewer vaccine doses than recommended, different VLP vaccines or administration with another vaccine.

18PW21: Recombinant allergens modify the food allergy concept

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The primary tools for the diagnosis of food allergy are skin prick testing (SPT) and in vitro determination of food specific IgE. Both diagnostic procedures are mainly based on the use of food extracts. Many commercial food extracts, however, lack appropriate biological standardization or a precise adjustment of the content of individual allergens for instance due to degradation of allergenic proteins during the extraction procedure [1]. This is particularly the case in pollen related food allergy since Bet v 1 homologous food proteins are prone to degradation. Inadequate quality of food derived allergen extracts often results in a low sensitivity of the extract based diagnostic procedure [2–3]. Moreover, even with well prepared food extracts, positive SPT or specific IgE results are frequently observed in the absence of clinical symptoms of allergy due to the phenomenon of subclinical sensitisation or allergen cross-reactivity.

It cannot be expected that the use of recombinant allergens will resolve the dilemma of sensitization not accompanied by clinical allergy, but improved clinical sensitivity is a realistic goal to be achieved.

Recombinant allergens in the diagnosis of food allergy might be used in different ways:

1 Utilizing the traditional extract based diagnostic approach but to determine additionally specific IgE to recombinant food allergens that are underrepresented in the commercial food extract.
   This approach proved to be valid in birch pollen mediated soy allergy. When Gly m 4, the Bet v 1–related molecule in soybean, was applied for diagnostic purposes as an ImmunoCAP reagent, determination of specific IgE to Gly m 4 improved the diagnostic sensitivity in patients with combined birch and soy allergy from 45% (soybean extract-based test) to 96% [4].
2 Enhancing the sensitivity of the extract based diagnostic approach by supplementing the extract with the recombinant allergens, that are underrepresented in the extract (spiking of extracts). Spiking of hazelnut extract for instance by r Cor a 1, the Bet v 1 homologue in hazelnut, increased the diagnostic sensitivity from 84% (pure extract) to 100% (spiked extract) [5].
3 Determination of specific IgE to a whole panel of allergens derived from one source, which enables assessment of the sensitization profile in individual patients, a concept that has been defined as "Component Resolved Diagnostics" (CRD).
   CRD might detect associations between subpopulations of specific IgE antibodies, measured by the use of individual allergen components, and clinically relevant aspects of the allergic disease such as severity of the allergic response or geographic differences in the sensitisation patterns. A recently published review provides a comprehensive summary on studies regarding CRD in food allergy [3].
4 Determination of specific IgE to mixes of recombinant allergens derived from one source, ie to a highly standardized "artificial" extract.
   In a recent study in cherry allergy an ImmunoCAP containing a combination of recombinant cherry allergens (rMIX), ie rPru av 1, rPru av 3 and rPru av 4, proved to have a sensitivity of more than 95% whereas the natural extract ImmunoCAP displayed a sensitivity of just 35% [6].

In conclusion: Recombinant allergens are becoming increasingly available and can be used to map, characterize and exploit the clinical significance of individual IgE reactivity profiles.

18PW22: In vitro allergy diagnosis: should we go with the flow?

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Introduction: Upon challenge with allergens, basophils up-regulate the expression of activation markers. These alterations can be analysed by flow cytometry in the basophil activation test (BAT).

Technical issues: Ideally, BAT is performed within 3 hours after sampling in preservative and endotoxin free tubes. As such, tubes can be kept at room temperature. The BAT can be done on whole blood or on isolated cells.

Basophils stimulation is dependent on the conditions, requiring optimal temperature and incubation times, as well as correct composition of buffers. Prewarming of blood and reagents significantly enhances onset and magnitude of anti-IgE mediated CD63 up-regulation.

Negative control stimulation, to assess spontaneous expression of the activation markers, implies incubation of the cells with stimulation buffer. Generally the positive control is a polyclonal or monoclonal anti-IgE antibody. An alternative positive stimulant is monoclonal anti-FceRI antibody. In the absence of positive control stimulation, it is impossible to interpret negative results of allergen challenges. In these "non-responders" the BAT is lost as a diagnostic tool and for study purposes they should be recorded as false negatives. About 5–10% of the individuals tested fail to respond with regard to IgE-mediated basophilic activation. False-negative BAT might also be due to other causes (recent exposure to the allergen with temporary refractoriness of the cells and/or transiently reduced levels of allergen-specific circulating and membrane-bound IgE). Sensitivity of the BAT might decrease over time. False negative results can also have technical causes (improper handling and storage, challenge of the cells with poorly identified allergens containing cytotoxic/inhibitory components, application of cytotoxic stimulation concentrations). Allergen-specific phosphorylation of p38 MAPK and upregulation of CD63 and CD203c is generally not restricted to a single stimulation concentration. It implies different stimulation concentrations that span several log scales. This offers the opportunity to restrict BAT experiments to an "optimal" concentration discriminating patients from control individuals.

Most studies have applied an anti-IgE to identify basophils in peripheral blood. It has been argued that monocytes and denditic cells that also express FceRI might interfere with the BAT and that exposure of basophils to anti-IgE prior to allergen stimulation may alter their responsiveness. However, average cell-surface FceRI expression on monocytes is low. Alternatively, anti-IgE applied for basophil characterisation does not elicit cell activation. In traditional BAT, cells are exposed to an anti-IgE for flow cytometric characterisation only after the stimulation phase and additional activation by anti-IgE is avoided by fixing, cooling or adding EDTA containing buffer.

In peripheral blood, CD203c expression is exclusively and constitutionally expressed on the surface of resting basophils. Therefore, it can be used as a marker for basophil identification. However, spontaneous expression of CD203c by resting cells can be weak, hampering clear identification of the cells. Alternatively, CD203c expression is quickly upregulated upon contact of the basophil with allergen and offers the advantage to study basophil activation without additional staining. Alternative basophil characterisation markers are the chemoattractant receptor-homologous molecule expressed by Th2 lymphocytes and the eotaxin CC chemokine receptor-3. As these markers are not lineage specific, it is mandatory to apply an additional marker such as anti-CD3 to differentiate with TH2 lymphocytes. Some groups have used the combination of anti-CD123 and anti-HLADR to characterise basophils in peripheral blood. The most commonly used markers in the BAT are CD63 and CD203c. Up-regulation of CD63 is generally bimodal with a subpopulation of cells that express CD63 with a high intensity vs. a population with lower CD63 expression. Up-regulation of CD203c is generally less prominent but often occurs in almost all cells.

It appears phosphorylated p38 MAPK to constitute a novel marker for flow-assisted allergy diagnosis.

Clinical applications: The BAT proved sensitive and specific for the diagnosis of several IgE-mediated allergies including inhalant allergens, hymenoptera venom allergy, natural rubber latex allergy, food allergies and drugs. In drug allergy the BAT contributed to establish the individual therapeutic alternative and/or allowed identification of cross-reactive structures. In venom allergy, it was demonstrated the BAT to take the sting out of difficult cases that yield equivocal or negative sIgE and/or skin test results and to monitor treatment.

Another application comprises demonstration of functional auto-antibodies IgG against the high affinity IgE receptor or against IgE itself in patients with auto-immune chronic urticaria.

18PW23: How internet-based allergen platforms and multiplex allergy testing improve our Inowledge on IgE-mediated diseases

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For more than a century allergy research has been dedicated to the discovery of allergenic sources which cause IgE-mediated diseases. This has involved a step by step process moving from identification of raw material causing allergic reactions (ie house dust), to organisms and tissues as triggers (ie pollen, fruits, mites, fungi). Raw materials, organisms and their tissues have been used and are still in use for diagnostic and therapeutic purposes as allergenic extracts. Although great efforts have been made by manufacturers and authorities to control allergenic extract composition, the best definition for an allergenic extract is still "an unpredictable mixture of allergenic and non allergenic compounds".

In the last forty years the knowledge on protein structures (allergens) causing IgE-mediated diseases is dramatically increased. The finest proteomics approaches and the molecular biology methodology lead us to identify more than 1400 allergenic compounds, having several degrees of biochemical, immunochemical, and clinical characterization (www.allergome.org). We are now acquiring information mostly about primary and secondary protein structures, unless numerous tertiary and quaternary complex structures have been reported so far. Some allergenic molecules have been further characterized in terms of IgE binding epitopes, either linear or conformational (mimotopes). The availability of proteomic microarray systems for IgE detection based on purified allergenic molecules is creating a wealth of knowledge and increasing the characterization level of each allergen, and their relevance within the allergic population and for each allergic disease. All these tools are helping to define the "allergenicity" property of any compound. In fact, strong criteria to define the allergenicity feature of a molecule are still lacking, and the introduction of new proteins into genetically modified organisms and the use of novel biological drugs require the highest effort to "predict" such feature.

This increase in the knowledge of potentially allergenic molecules, requires a systematic organization and a clear definition of the criteria for defining what comprises an allergen and when an IgE-binding structure is causing an allergic disease. To address the need for bringing sense and organisation to the increasing amount of data on potential allergens we need to consider some critical aspects of the IgE immune response, report on current diagnostic and epidemiological tools used for allergic disease studies and the need to implement them, and lastly discuss the usefulness of novel biotechnology, information technology and microtechnology tools at this regard.

18PW31: Organic acids determination by GC/MS

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Organic acids analysis together with amino acid analysis is an important tool to investigate inborn errors of metabolism and to diagnose organic acidurias. Oraganic acids are metabolic intermediates of amino acids, fatty acids and carbohydrates metabolism and also of some xenobiotics metabolism. They represent a large class of very different compounds of low molecular weigh (<600). Development of capillary gas chromatography coupled to mass spectrometry allowed separation and identification of more

than 250 organic acids in only one run. This kind of analysis is not really expensive but fastidious requiring several steps: extraction of organic acids from biological fluids, derivatisation, chromatographic separation, identification and quantification. Two methods are available for extraction: solvent extraction or solid phase extraction (SPE). Solvent extraction (aethylacetate or aethylacetate + aethylether), the most popular method, is fast and not expensive but gives variable extraction yield for different organic acids, poor precision and reproducibility. SPE is not so fast and much more expensive, but allows good recoveries (near 100%) and a better precision. After extraction organic acids are not enough volatile to be separated by gas chromatography and require a derivatisation step where the different functional groups are transformed into ester or ether derivatives. Two methods are usually proposed: trimethylsilylation or methylation. Silylation is fast, safe and gives only one derivative as compared to methylation. Silylated derivatives can separated by gas chromatography using a capillary column. Capillary column usually used is 30 m length, 0.25 mm in diameter and coated with a liquid phase chosen for the polarity of compounds to analyse. These columns have a very high number of theoretical plates and allow a high resolution. When this powerful separating method is associated to mass spectrometry technology identification of the different compounds is much easier and avoid mistakes. Today two technologies are proposed for mass analysis: quadrupole or ion trap, with the possibility to combine tandem mass analysis. Identification will be facilitated through the use of mass spectrum libraries, either commercial or home libraries. Home libraries are often required because mass spectra of many metabolic intermediates are not available in commercial ones. Knowing ion composition of a mass spectrum of each compound it will easy to quantify a specific metabolite by choosing a specific ion. Quantification on a specific ion can be done either by extraction of this ion from all ions obtained after scanning of the whole chromatogram or by using the «Selected Ion Monitoring» (SIM) method. SIM method allows great sensitivity and precision. Concentrations of less 1 µM can be determined by this technology.

Organic acids analysis is usually done to diagnose (post and prenatal) inborn errors of metabolism and so it is very important to recognize and to good knowledge of the different metabolic intermediates (mainly the unusual ones) which can make a diagnosis sure.

18PW32: Simultaneous quantification of homocysteine, methionine, cysteine in human plasma using LC/MS/MS: a combination which may help to explain disorders in metabolism of sulphur-containing amino acids

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First, we developed an LC/MS/MS method for total homocysteine (tHcy) determination in order to be able to measure this amino acid as a biochemical marker in vascular disease [1] but sometimes we were aware that the knowledge of the other sulphur amino-acids could help us to interpret a high value of homocysteinaemia. Therefore we decided to add methionine (Met) and total cysteine (tCys) in the LC/MS/MS method.

Homocysteine, cysteine, methionine stock solutions were prepared from homocystine, cystine and methionine. Deuterated homocysteine (Hcy-d4) and deuterated cysteine (Cys-d2) stock solutions were prepared from homocystine-d8 and cystine-d4 (Cambridge Isotope Laboratory). Standards (included in each batch of samples) at concentrations of 0, 3.73, 7.45, 14.9, 29.8, 49.7, 74.5, 99.3 and 149 µmol/L for tHcy and 0, 8.25, 16.5, 33, 66, 110, 165, 220 and 330 µmol/L for tCys and 0, 3.35, 6.7, 13.4, 26.8, 44.7, 67, 89.3, and 134 for Met were prepared in acidic aqueous solutions. Sample preparation was carried out by adding 100 µL of diluted internal standards (~50 µmol/L of homocysteine-d4 and ~ 80 µmol/L of cysteine-d2) to 100 µL of plasma, or quality control, or standard. Complete reduction of plasma disulfides was accomplished by the

addition of 30 µL of a freshly prepared solution of dithiothreitol (DTT) at 500 mmol/L. After mixing, the reduction step was performed at room temperature for 15 min. Proteins were precipitated by the addition of 200 µL of a solution of 1 ml/L formic acid and 0.5 mL/L trifluoroacetic acid in acetonitrile and incubated 10 minutes on ice to improve precipitation. After centrifugation at 13 000 g for 5 min, 200 µL of the clear supernatant was transferred to an autosampler vial.

An Applied Biosystems/MDS SCIEX API 3000™ Triple-Quadrupole Mass Spectrometer equipped with a TurboIonSpray® source was used. The source operated in positive ion mode at an ion spray voltage of +5500 V. The turbo gas (nitrogen) temperature was set to 250 °C at a flow rate of 7 L/min. Maximum sensitivity for tHcy, tCys, and Met was obtained by measuring product ions from the fragmentation of the protonated [M+H]+molecule in the positive ion mode. The multiple reaction monitoring (MRM) mode was used with the following transitions 136.1>90 and 140>94 for homocysteine and homocysteine-d4, respectively; 122>76 and 124>78 for cysteine and cysteine-d2, respectively; and 150>104 for methionine. Aqueous calibration curves were established to calculate the amount of homocysteine, cysteine and methionine in the sample on the basis of the integrated peak area ratio of homocysteine to homocysteine-d4, of cysteine to cysteine-d2, and methionine to homocysteine-d4.

The chromatography was performed on an Agilent HP 1100 system using a short Supelcosil CN column, 3 µm (33 × 4.6 mm) (Supelco, reference 58979). The column temperature was set to 20 °C. Autosampler injections of 5 µL were made using isocratic elution in 30:70 with 0.1% formic acid in water/acetonitrile at 1 ml//min. A split ratio 1:5 was used in the source. Total acquisition cycle was 4 min per sample.

Reference values of plasma tHcy, tCys and Met concentrations were determined in 31 healthy adults. The simultaneous assay of Met and tCys helps to distinguish between transulfuration and remethylation pathway deficiency when an hyperhomocysteinaemia is found. A few cases of this help will be detailed during the workshop.

18PW33: Quantification of small molecules in biological fluids using LC/MS/MS:application to metabolic diseases

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Recent progress in the field of mass spectrometry have position this method as a key tool for investigation of inborn errors of metabolism. The advent of "soft-ionization" techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization using heated nebulizer (APCI) has dramatically simplified the use of mass analyzers in liquid chromatography.

New systems, especially tandem mass spectrometry are now widely used to analyse families of compounds in biological fluids that share a common chemical structure. Tandem mass spectrometry (MS/MS), in a very generic description, is a process in which an ion formed in an ion source is mass-selected in the first stage of analysis, reacted, and then the charged products from the reaction are analyzed in the second stage of analysis. In the case of chemically related compounds that generate a common ion product, it is possible by selecting this ion in the second stage to build a metabolite profile of the parents' ions that have been analysed in the first stage. The example of acylcarnitines will be presented. For this type of analysis, the combination of two mass spectrometers with an interposed collision cell characterises MS/MS as an analytical technology on its own and not just as a more specific detector for HPLC compared with conventional techniques.

However, it must be warn that it is not a universal solution. In many cases, limitation by the biological matrix (matrix effect) and isobaric molecules makes a chromatography separation and the used of stable isotopes as internal standards compulsory. In LC-MS/MS, liquid chromatography is rather used for sample preparation but not for complete resolution of compounds of interest. Ideally, each analysed molecule should have its own stable isotope as internal standard. Examples will be given to illustrate these phenomenons.

In routine use, tandem mass spectrometry methods are far more rugged compared to conventional chromatographic techniques and enable high-throughput analyses with limited manual handling steps however the instrument technology is complex and required a minimum of knowledge and real involvement of the biochemist or the assistance by specialised engineer.

18PW34: MS/MS neonatal screening for genetic and metabolic disorders

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Robert Guthrie's seminal work in the 1960s laid the foundation for newborn screening as we know it today. Although his bacterial inhibition for the detection of phenylketonuria (PKU), maple syrup urine disease (MSUD) and other inborn errors of metabolism have been replaced by enzymatic tests (PKU) or partly discontinued (MSUD), dried blood spots (DBS) on filter paper continue to be preferred sample used for population-based newborn screening.

The introduction of the analysis of acylcarnitines and amino acids by electrospray ionisation (ESI) tandem mass spectrometry (MS/MS) to population-based neonatal screening has tremendously increased the number of detectable inborn errors of metabolism amenable to intervention (defects in amino acid degradation and the urea cycle, defects in organic acid degradation, defects of the carnitine cycle, fatty acid oxidation and ketone body synthesis and guanidinoacetate methyltransferase deficiency).

Expanded newborn screening with MS/MS provides the means to detect a large number of metabolic disorders in a single analytical run. The disorders chosen to be included in European newborn screening programmes differ considerably. Given that all European populations have a common genetic background, the reason for these dif-

ferences cannot be explained by major differences in disease prevalence but only by different approaches to the estimation of risks and benefits. Interestingly, each programme claims to be based on the screening principles that were first developed by Wilson and Jungner.

In the most restrictive European countries, only MCADD is screened in addition to PKU and all metabolites that are not necessary to detect these two disorders are not analysed or not reported. In other European countries, twenty disorders are screened. The rationale behind inclusion or exclusion of a respective disorder is far from clear in most cases. In some case this restriction may impose an ethical dilemma. The opposite approach was adopted in the ISA, with 29 disorders being screened for as primary targets in many states and 25 more as secondary targets.

Last but not least, analytical "problems" have still to be solved for some metabolites and disorders. However, the inclusion of second-tier strategies will allow screening for some of these disorders with a close to 100% sensitivity and simultaneous high specificity.

Common projects for quality control and assessment, as well as expert medical judgement, are urgently needed at a European level and should result in standardization of screening panels and the spectrum of metabolites analysed, as well as in suggestions for analytical procedures, follow-up management and proficiency and quality testing.

Carefully weighed arguments are needed since patient organizations, opinion leaders and politicians are pressing to proceed with expansion of neonatal screening. As well, additional groups of disorders including lysosomal storage disorders and X-linked adrenoleukodystrophy are already at the doorstep awaiting implementation.

18PW41: POCT et médecine de premier recours

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Le médecin de premier recours et le laboratoire du praticien

La qualité des prestations de laboratoire doit être identique dans tous les laboratoires, qu'ils soient du praticien, hospitaliers ou privés. Un tel but peut être atteint pour le laboratoire du praticien par une formation complémentaire spécifique, car la formation post-graduée ne prépare pas le futur médecin installé à une utilisation rationnelle et judicieuse de cet important outil diagnostic. En effet, les connaissances de la pré-analytique, des limites des appareils existant dans le domaine du POC et de l'interprétation des résultats nécessitent une formation spécifique. En Suisse, le KHM a été mandaté par la FMH pour mettre sur pied les Attestations de formation complémentaire du laboratoire du praticien. La collaboration entre les spécialistes des disciplines du laboratoire médical et les médecins de premier recours a permis de développer une formation adaptée à la pratique et à la réalité technologique.

Un tel travail d'amélioration de la qualité des prestations sous-entend tout de même que le partenaire étatique accepte de rémunérer correctement ce travail (puisqu'il s'agit d'un tarif d'Etat et non d'un tarif négocié) et une évaluation des prix basée sur une marge bénéficiaire de couverture unique des frais est totalement inadéquate. La direction prise actuellement par l'Office fédéral de la santé publique est proprement scandaleux et montre clairement que les instances politiques sont hypocrites et probablement incompétentes: on clame tout haut que la Suisse a besoin de médecins de premier recours pour assurer un suivi médical compétent et dans la même foulée, les mesures sont prises pour tuer l'attractivité de ce métier. Je pense que la Suisse a surtout besoin de fonctionnaires compétents et intelligents, capables enfin d'appréhender la réalité du système de santé de notre pays. Il y a encore beaucoup à faire …

Ainsi, je développerais dans ma présentation les efforts faits par le corps médical pour accepter des contraintes supplémentaires de formation et de frais, et ce malgré quelques oppositions, qui montraient surtout une méconnaissance complète du sujet. Les critères minimaux d'utilisation des laboratoires médicaux complètent les instructions pour les contrôles de qualité interne et la formation complémentaire des spécificités du laboratoire du praticien.

18PW42: Near Patient Testing: Die Sicht des Zentrallaboratoriums

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Neben der Qualität spielt die Turn Around Time (TAT) eine wesentliche Rolle bei medizinischen Laboranalysen. Dies gilt für sämtliche Analysen, obschon nur ein Teil davon tatsächlich sofort zur Verfügung stehen muss. Der Komfort der schnellen Verfügbarkeit von Laboranalysen hat zur Entwicklung von Systemen geführt, die eine Analytik in der Nähe des Patienten erlaubt, das sogenannte Near-Patient Testing (NPT) oder Point Of Care Testing (POCT). NPT bedeutet analytische Untersuchung einer Patientenprobe beim oder in der Nähe des Patienten. Es bedeutet weder möglichst viele Bluttests mit kleinen Apparaten, noch analytische Tests in kleinen dezentralisierten Labors ausserhalb des Zentrallabors durchzuführen. Es steht vielmehr für Laboranalytik mit einfach zu bedienenden Apparaten mit schneller Verfügbarkeit der Resultate auf klinisch sensiblen Gebieten mit Personal, welches gewöhnlich eine minimale Ausbildung und Erfahrung auf dem Gebiet der Labormedizin hat. Insbesondere an kritischen Orten wie den Intensivpflegestationen, Operations- und Aufwachräumen, Notfallstationen und Gebärsälen hat das NPT zunehmende Bedeutung erlangt und ist aus der täglichen Routine kaum mehr wegzudenken. Die zunehmende Verbreitung dieser Methodik birgt aber auch die Gefahr, dass diese Art der Analytik sich unkritisch und ungerechtfertigt ausbreitet. Kliniker in den Intensivpflege- und Notfallstationen wünschen immer schnellere Resultate; dies gilt auch für die zahlreichen Ambulatorien, in denen keine medizinische Notwendigkeit für sofort verfügbare Analysenresultate, aber ein grosser Druck auf die Wartezeiten der Patienten besteht. Die wachsende Nachfrage für diese Art der Analytik führt zu einer stetigen Ausweitung der angebotenen Tests durch die Industrie. Dies hat zu einigen Kontro-

versen in Bezug auf Vor- und Nachteile des NPT geführt. NPTesting kann zu Verbesserungen in der Patientenpflege führen. Es muss aber von einem qualifizierten Staff eines professionellen, klinischen Laboratoriums geführt und überwacht werden. Bei der Einführung in die Klinik sollte der tatsächliche Bedarf mit den Klinik-Verantwortlichen geklärt werden. Falls dieser Bedarf zutrifft sollten die folgenden Punkte im gegenseitigen Einverständnis zwischen Klinik(en) und Zentrallaboratorium beschlossen werden:

– Verantwortlichkeit für das Labor innerhalb der Klinik
– Zusammenarbeit mit dem Zentrallabor und Verantwortlichkeiten
– Ausbildung des Personals
– Qualitätsüberwachung (interne und externe Qualitätskontrolle) und Apparatewartung

Vorteile des Near-Patient Testing:

– schnellere Verfügbarkeit der Resultate und entsprechend schnellere therapeutische Intervention
– Kürzung und Vereinfachung der praeanalytischen Phase (Probenverarbeitung, Zugabe von besonderen Reagentien)
– Verminderung praeanalytischer Fehler
– kleinere Blutvolumina
– geringere Anforderungen an die Ausbildung
– Bequemlichkeit für Patienten (geringere Wartezeiten)

Nachteile:

– Vergleichbarkeit mit konventioneller Analytik
– Dokumentation der Resultate (Integration in das Laborinformationssystem)
– Belastung des Pflegepersonals
– Kosten
– Zuverlässigkeit bei Benützung durch ungeschultes Personal (unkritische Einführung neuer Tests)

NPT kann zur früheren Erkennung lebensbedrohender Zustände und zu einer früheren therapeutischen Intervention führen. Falls dies zu einer Verbesserung der Patientenversorgung, vermindertem Verbrauch von Blutprodukten, verkürzter Aufenthaltsdauer im Spital- oder in der Intensivpflege führt, ist die Einführung von NPT selbst bei höheren Kosten gerechtfertigt. Dies konnte allerdings in kontrollierten und randomisierten Studien nicht bestätigt werden. Verkürzte Turnaround-Zeiten und frühere therapeutische Interventionen gingen nicht mit einer verkürzten Spitalaufenthaltsdauer oder reduzierter Mortalität einher. NPT ist eine Ergänzung zum konventionellen Spitallaboratorium, kein Ersatz. Es sollte rigoros auf die Messung lebenswichtiger Funktionen, die eine unmittelbare therapeutische Intervention nach sich ziehen, beschränkt werden. Dafür ist die Blutglucose ein gutes Beispiel. Andere Parameter, die sich für das NPT anbieten sind die Blutgase, Hämatokrit und Hämoglobin, Natrium, Kalium, ionisiertes Kalzium, aktivierte Blutgerinnungszeit, und unter Umständen Troponin. Near-Patient Testing wird dort relevant, wo die Turnaround-Zeiten des Zentrallaboratoriums zu lange sind. Dies kann auf zu lange Transportwege, ineffizientes Probenhandling auf der Station oder im Zentrallaboratorium, verzögerte Resultatübermittlung oder weitere Gründe zurückzuführen sein. Diese sollten vor der Einführung genau abgeklärt und die entsprechenden Massnahmen getroffen werden. Trotzdem kann die Einführung von NPT notwendig werden, insbesondere um das Zentrallaboratorium vor einer zu grossen Zahl an unechten Notfällen zu entlasten.

18PW43: «Near Patient Testing» en Suisse; le point de vue de l'enseignant AFCLP (Attestation de Formation Complémentaire du Laboratoire du Praticien)

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Pour répondre aux exigences de la QUALAB concernant l'assurance qualité au sein du laboratoire du praticien, la FMH doit garantir une formation post graduée aux médecins détenteurs d'un laboratoire de cabinet médical. La FMH a confié au Collège de Médecine de Premier Recours (CMPR) la tâche de développer un programme permettant de garantir cette formation. Le programme AFCLP proposé par la commission AFCLP du CMPR (composée de représentants des sociétés scientifiques SSCC, SSH, SSM et de délégués de la FMH, SSP, SMI, SMG) a été accepté par la FMH en juin 2000 et mis en œuvre à partir de 2003. L'objectif global du cours est de permettre au médecin praticien de diriger un laboratoire de cabinet médical (analyses des soins de base). Les objectifs spécifiques du cours se focalisent sur les points suivants: Assurance qualité (contrôle de qualité interne et externe y compris); Sécurité et Hygiène; Préanalytique; Postanalytique; Analytique (Tests rapides Chimie, Microbiologie et Hémostase, Microscopie, Status urinaire (Stix, Sédiments, Uricult), Morphologie hématologique, Interférences); Interprétation des résultats (intervalle de référence, sensibilité, spécificité, valeurs prédictives, rapports de vraisemblance y compris). Depuis 2003, si 8250 attestations ont été distribuées (avec un script du cours) aux praticiens bénéficiant des conditions transitoires, seuls 743 médecins ont pour le moment suivi les cours obligatoires et payants permettant l'octroi de l'attestation AFCLP (source Rapport annuel 2007, Commission AFCLP de la CMPR). L'expérience des cours AFCLP démontre que les connaissances de base qui y sont enseignées sont indispensables à la culture médicale générale en plus d'être un minimum autorisant la direction d'un laboratoire de praticien effectuant des «Near patient testing» (7518 laboratoires du praticien en suisse, effectuant environ 80% des analyses demandées par les médecins de premier recours).

La formation AFCLP permet de mettre en lumière par des exemples concrets liés à la pratique du laboratoire du praticien (analyse des soins de base) ce que l'incertitude de mesure veut dire à la fois en terme d'inexactitude et d'imprécision. Ces deux dernières notions ne peuvent être appréhendées que par la pratique et l'interprétation avisés du contrôle de qualité interne (Cqi) et externe (Cqe). Au delà de leur rôle dans la détection des erreurs et de la simple obligation permettant le remboursement des analyses, le rôle des Cqi et Cqe se trouve dès lors éclairé par leur apport au savoir médical fondé sur les preuves. Ce savoir en médecine de laboratoire concerne notamment 1) le calcul de la différence critique, utile au suivi du patient et 2) l'évaluation du

biais de la mesure qui permet, entre autres, une plus juste appréciation des recommandations internationales basées sur des valeurs seuils.
Si l'on en croit les commentaires effectués par les médecins de premier recours à la fin des sessions de cours, l'enseignement de la bonne pratique du laboratoire d'analyse médical devrait avoir une incidence sur la façon de (moins) prescrire l'analyse de laboratoire. Cet enseignement post gradué devrait donc être généralisé et les nouvelles exigences des sociétés FMH en médecine générale et en médecine interne concernant la formation au laboratoire évoluent dans le sens de cette conclusion.
De mon point de vue d'enseignant AFCLP et de participant à la commission AFCLP du CMPR, il est indispensable de clarifier les débats autour de la pratique du laboratoire du praticien en séparant clairement les aspects liés à la formation continue en médecine de laboratoire, de ceux qui touchent à la politique de remboursement des analyses. La commission AFCLP de la CMPR devra, à mon sens, clarifier ce débat en proposant une réorganisation de la formation AFCLP. L'organisation de cette formation pourrait gagner à être faite en deux modules dissociés permettant une approche différenciée du savoir en médecine de laboratoire entre le praticien avec et sans laboratoire. L'enseignement de la pratique du laboratoire du praticien (module 2) devrait venir en complément de l'enseignement post-gradué à la prescription d'analyse médicale (module 1).
Dans le contexte des discussions tarifaires actuelles (liste des analyses), les médecins de premier recours seront-ils encore intéressés par cette problématique de l'enseignement en médecine de laboratoire sans cet incitatif que représente pour eux leur laboratoire de proximité, et que la démarche qualité engagée par la FMH au travers du CMPR (entre autres par le cours AFCLP) était censée leur garantir?

18PW44: Laboratoire d'hématologie de proximité?

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L'hématologie qu'elle soit cellulaire ou liée à la coagulation a été et reste l'une des pierres angulaires du laboratoire de proximité au cabinet médical. Face à un patient symptomatique, personne ne peut nier l'utilité de pouvoir diagnostiquer au plus vite une anémie, que ce soit par le biais d'un abaissement de l'hématocrite, du dosage de l'hémoglobine ou à l'aide d'un compteur hématologique. De même la suspicion clinique d'un trouble de l'hémostase ne peut que bénéficier d'une estimation rapide du compte plaquettaire et du temps de prothrombine. Ces simples exemples, évidents pour qui comprend la médecine de premier recours, permettent cependant d'évoquer les problèmes actuels auxquels fait face le laboratoire du praticien et d'envisager des solutions d'avenir.
Afin d'être à même d'effectuer ces examens d'hématologie le praticien doit posséder une équipement de base déjà relativement onéreux et un personnel à même de l'utiliser avec les compétences nécessaires. C'est par ailleurs au niveau des examens hématologiques de base que se retrouvent une partie importante des pièges de la préanalytique tels que la présence de microcoagulats ou d'agrégats plaquettaires dans les tubes de prélèvement, pour ne parler que des plus fréquents.
C'est par contre également dans ce domaine que le sens et les connaissances cliniques du médecin et sa connaissance des patients sont tout particulièrement importants pour placer les résultats des examens dans une perspective globale qu'un laboratoire externe ne peut en aucun cas appréhender.
Entre avantages et limitations se retrouve donc le laboratoire hématologique du praticien, qui plus est confronté à des problèmes financier liés à sa structure même: examens peu fréquents; personnel, appareils et réactifs onéreux; formation spécifique «mangeuse de temps» …
Face à ces divers problèmes, et au vu de l'importance du laboratoire du praticien de proximité, des solutions existent cependant. En premier lieu, cela va de soi, la priorité doit être donnée à la qualité, que ce soit par la formation du médecin et de son personnel, mais également par la pratique de contrôles de qualité interne aussi bien qu'externes. Ceci nécessite également une reconnaissance de l'importance de ce laboratoire de proximité et des efforts consentis par les médecins de la part des autorités et ce par un remboursement équitable des prestations ce qui n'est malheureusement pas le cas actuellement. Finalement il faut aussi envisager le médecin et son laboratoire dans le contexte d'une prise en charge globale des analyses hématologiques des patients incluant le soutien de laboratoires médicaux et d'hôpitaux possédant des consultants hématologues expérimentés. L'apport de ces derniers sera souvent prépondérant dans la recherche de solution aux problèmes hématologiques et permettra alors d'éviter des frais ou des hospitalisations inutiles.

18PW51: New developments in diagnostics of rheumatoid arthritis

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Early diagnosis of rheumatoid arthritis (RA) has become more important since early treatment strategies with new drugs not only effectively suppress inflammatory disease activity but also slow down or prevent the development of functional and structural damage.
Inflammatory musculoskeletal changes as well as joint damage can be detected early by sensitive imaging techniques like ultrasonography, magnetic resonance tomography or scintillography.
The early diagnosis of rheumatoid arthritis has been improved by techniques detecting autoantibodies against citrullinated peptide antigens (ACPA) in addition to traditional rheumatoid factors (RF). ELISA techniques with cyclic citrullinated peptides (CCP) and mutated citrullinated vimentin (MCV) proved to be sensitive and highly specific (>97%) for RA. Anti-CCP antibodies in RA are associated with HLA-DR4 shared epitope and tobacco smoking in contrast to RF-negative RA and undifferentiated arthritis and are strongly associated with persistent and erosive joint disease. Both RF and CCP antibodies can be detected in the preclinical and early clinical phase of the disease. Anti-CCP antibodies can be detected in 70% of patients with established RA, mostly in RF-positive patients (>90%) but also in RF-negative individuals (20–65%). In addition anti-MCV ELISA seems to detect additional ACPA-positive patients increas-

ing diagnostic sensitivity. Anti-CCP antibodies are also present in the majority of patients with RF-positive juvenile idiopathic arthritis, elderly onset RA and palindromic rheumatism documenting that these conditions a variants of RA. In contrast to RF anti-CCP antibodies don't increase in frequency in the population with age. Prevalence in other rheumatic, inflammatory or infectious diseases is low. The high predictive value of anti-CCP antibodies especially in combination with RF allows early diagnosis of RA even when only few joints are involved. Other autoantibodies or other biologic markers so far don't play an important role in the diagnosis of RA.

18PW52: Autoimmune hepatitis: update on diagnostic procedures

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Autoimmune hepatitis (AIH) is an inflammatory liver disease of a most variable nature, which usually presents a considerable diagnostic challenge. AIH can occur in all age groups, both sexes, and in all racial groups in any country. Preentations can vary from subclinical mild elevations of liver enzymes up to fulminant hepatic failure. Thus, in any patient with elevated liver enzymes, AIH has to be considered in the differential diagnosis. On the other hand, there is no single test to reliably exclude AIH. Diagnosis needs to be based on a combination of clinical, laboratory, serological and histological features, which need to be considered in a diagnostic algorithm. This should be based on the novel simplified diagnostic criteria of the International Autoimmune Hepatitis Group (Hepatology 2008; 48: 169):

1. Elevated IgG (or gamma globulins)
2. Significant Autoantibodies (ANA, SMA, SLA/LP, LKM)
3. Histology
4. Absence of viral hepatitis

ad 1. Selective IgG elevation is underestimated as a diagnostic cornerstone, and it is a very cheap test. IgG, or at least total gamma globulins, should be the first step in laboratory screening. Nonetheless, 5–10% of patients with AIH do not have this finding at first diagnosis.

ad 2. Standard autoantibody testing should use immunofluorescence both on Hep2 cells and on tissue sections. In addition to titre and number of autoantibody reactivities, the exact immunofluoresence testing may be helpful. SMA in AIH, for example, are characteristically directed against F-Actin. ANA may sometimes be directed against dsDNA, which lead to AIH originally being called lupoid hepatitis. The crithidium assay should be used for demonstration of anti-dsDNA. SLA/LP is the only antibody almost entirely specific for AIH, but only present in about 20–25% of patients. SLA/LP can not be measured by immunofluorescence, but requires ELISA and/or immunoblotting. Confirmation by two methods should be attempted in patients, in whom the diagnosis primarily rests on this test result.

ad 3. Histology should be considered a condition sine qua non, as otherwise other conditions such as non-alcoholic steatohepatitis (NASH) or drug-induced disease can not be reliably excluded.

ad 4. AIH and viral hepatitis can occasionally occur in the same patient, but usually, autoantibodies in a patient with viral hepatitis are secondary to viral infection. Only typical histology, high IgG and high autoantibody titres and/or SLA/LP make a diagnosis of AIH in a patient with positive viral markers.

Response to immunosuppression in AIH is universal and is life-saving. The good response may proof the diagnosis.

18PW61: Quality control in morphology and haematology

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Friday, September 19

19PL12: Management and Accreditation in Europe

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The management and accreditation of medical laboratories have a common language, which are the standards ISO/IEC 17025 and ISO 15189. It is recognised widely that in order to demonstrate its competence, a medical laboratory has to be managed according to one of the previous standards. Accreditation being, the evaluation an organisation's competence, also uses these standards as references for the accreditation of medical laboratories. Therefore if a laboratory wants to obtain a third-party recognition of its competence, through the establishment of a good man-agement system, then accreditation can provide this recognition.

In order to establish the status of the accreditation of medical laboratories in Europe for Swiss MedLab, a survey was prepared by the SAS (Swiss Accreditation Service) and supported by EA (European co-operation for Accreditation). The results of the survey will also be used by EA and it's relevant working group (EA Health care working group).

Around 60% of EA members responded to the survey, guaranteeing that the results are very representative of the status of the accreditation in Europe.

The results of the survey show that the accreditation of medical laboratories is well implemented as around 90% (22 out of 24) of the European accreditation bodies propose the accreditation of medical laboratories. A total of around 1000 laboratories are accredited to date and another 200 are in the pipeline. It is important to underline the fact that the majority of the accreditations granted follow a voluntary process.

The percentage of public and private laboratories varies a lot throughout the European countries and it can be estimated that the balance between the accreditation of public and private laboratories is fairly even.

The accreditation of medical laboratories started over 15 years ago and to date, most of the medical fields are being considered for accreditation. The main fields (Clinical Chemistry, Microbiology, Haematology, Immunology and Molecular Genetics) are being accredited by the majority of accreditation bodies, whereas for some specific fields (eg Andrology, Dermatology, POCT), only a limited number of

accreditation bodies have accredited them, until now.
The two standards mentioned before (ISO/IEC 17025 and ISO 15189), are generally used as reference standards. Two accreditation bodies are not yet using ISO 15189 and two accreditation bodies are only using ISO 15189 as the reference standard. These two accreditation bodies started accrediting medical laboratories after the publication of the first version (2003) of ISO 15189. So, as most of the accreditation bodies were accrediting medical laboratories before the initial publication of ISO 15189, it is not a surprise that the majority is still using both references.
We were also interested in highlighting the main areas of non-conformities raised during the assessments. It appears clearly that the activity generating the most non-conformities is the "Examination procedures – ISO 15189, Ch. 5.5", followed by "Assuring quality of examination procedures – ISO 15189, Ch 5.6". This is at the same time "expected", as these chapters are the most critical aspects and therefore highly focused by the assessment teams, and "worrying" as it should be expected that laboratories are more at ease with the technical aspects that the quality aspects. The main areas of non-conformities raised do not confirm this latter point. It could be interesting, in the future, to make a survey with a focus on the types of non-conformities, in order to highlight any point that may need guidance or additional education.
A number of working groups are established at the national (SAS sector committee) and international level (EA, ILAC (International Laboratory Accreditation co-operation) and ISO) in order to ensure the harmonised assessments of medical laboratories, and if necessary to provide guidance and education to the medical laboratories.

19PL13: Education in laboratory medicine

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World wide different terms are used for the term Laboratory Medicine: Clinical Chemistry, Clinical Biochemistry, Clinical Biology, Clinical Pathology, Clinical Laboratory Sciences, and Laboratory Diagnostics. Generally it is accepted that this discipline is an interdisciplinary and integrated part of clinical medicine. The objectives of this discipline are:
– Measurement of biochemical, immunological and molecular biological processes in man for diagnosis, risk assessment, treatment and follow-up in health and disease.
– Interpretation of laboratory results and the rationale use of laboratory data and tests are based on the knowledge of pathophysiological processes and the condition of the individual tested.

Within the frame of the European Federation of Clinical Chemistry and Laboratory Medicine (EFCC) a European syllabus for postgraduate training of medical doctors, biochemists, chemists and pharmacists has been developed and published. This is a 4–6 years training programme covering all sub-disciplines of Laboratory Medicine. However, this syllabus is not mandatory for Europe, therefore great differences in the national training programmes in Europe exist.
In some countries training in Laboratory Medicine is restricted by law only for physicians, in others for scientists; this is in contrast to the policy of the professional organisations (IFCC, EFCC). The Austrian training programme consists of 4 years training in licensed diagnostic hospital laboratories and 2 years training in a clinical (internal medicine, surgery, gynaecology, intensive care medicine) medicine and/or another theoretical medical discipline (pathology, hygiene, microbiology, pharmacology, biology). The contents of the 4 years postgraduate training covers the following fields:
– Laboratory Management
– Biostatistics
– Clinical Chemistry
– Haematology
– Coagulation
– Immune-Haematology
– Microbiology
– Molecular Biology
– Interpretation of laboratory data

In most European countries an official examination organized by Universities or professional organisations (chamber of physicians, scientists, pharmacists) has to be passed for obtaining the an official certificate and to be licensed as a specialist in Laboratory Diagnostics. In addition the EFCC runs a register of European Clinical Chemists for those countries satisfying the criteria of the European syllabus.
Technicians working in clinical laboratories are trained for 3 years in an Academy for medical technologists run by governments or Universities. In this programme most practical, analytical, and basic theoretical aspects of laboratory diagnostics and medicine are trained. The main content of these courses are:
– Physiology, Histology, Pathology
– General Chemistry, Clinical Chemistry, Biochemistry
– Haematology, Coagulation
– Immunology, Serology
– Laboratory Organisation, Automation, Statistics
– Laboratory Technologies

After passing the final examinations certificates (bachelor) are obtained. Generally the technicians are involved in the measurement of samples and are accountable for its accurate performance. In most countries technicians are supervised by the academic staff of the diagnostic laboratory.

19PW11: The concept of the regional laboratory network in Switzerland

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Incidents involving biological agents and posing a considerable threat to public health could occur in Switzerland at any time. To tackle such problems, appropriate laboratory diagnostic capacities allowing the rapid identification of the pathogens involved are paramount. The Regional Laboratory Network was created in order to build up decentralized capacity for primary diagnostics and analytical triage of the most important microorganisms related to bioterrorism. It mainly covers environmental analyses but may also take on a back up role for diagnostic needs in the field of human and veterinary medicine.
The Regional Laboratory Network has been established as an open platform thereby providing sufficient flexibility

to cope with the diverse organizational needs of the regions. Seven cantons: Bern (BE), Basel Town (BS), Geneva (GE) / Vaud (VD), Lucerne (LU), Ticino (TI) and Zurich (ZH) have made considerable investments and upgraded existing laboratories or local networks of laboratories to set up six regional laboratories. A catalogue of requirements defines the minimum standard that regional laboratories must meet in terms of infrastructure, preparedness and availability, diagnostic capacity, quality assurance and regional organization.

Besides the regional laboratories the network includes the National Reference Centre for Anthrax (NANT), the National Reference Centre for Emerging Viral Infections (NAVI), which was designated in June 2005, the Spiez Laboratory and the Institute of Virology and Immunoprophylaxis (IVI).

The regional laboratories ensure primary diagnostics for pathogens of biosafety level 3. The reference centres have to acquire the knowledge and know-how necessary to develop analytical methods, to make these methods available, and – as far as it is reasonable and possible – to pass them on to regional laboratories. Their tasks and duties also include confirming results of primary diagnostics, typisation of pathogens and participation in international networks of cooperation and quality control.

A national committee was established to facilitate coordination and decision-making. It is currently chaired by the Federal Office of Public Health and comprises representatives of the regional laboratories, the two National Reference Centres, the Spiez Laboratory, the Institute of Virology and Immunoprophylaxis and the Federal Office for the Environment. The Federal Expert Commission for Biosafety (FECB) and the National NBC Protection and Coordination Office also serve on this committee.

Thanks to efforts made by all the institutions and partners involved in the Regional Laboratory Network, it has been possible to regionalize the diagnostic activities through practically the whole of Switzerland. The network is characterized by a mixture of federal and central components, which are complementary. The advantages of this system and the factors that make it suitable to deal successfully with crises are its flexibility, proximity to the forefront of problems and an appropriate amount of redundancy.

Future challenges for the Regional Laboratory Network will be to ensure the surge capacity in the long term, the participation of partner cantons and their sharing of the costs, integration into the national CBRN strategy, regular updating of the catalogue of requirements and continuous adjustment of the tasks and services provided to adapt to new threats.

19PW12: Maximum containment laboratory in Spiez: facility for special pathogens

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On the premises of Spiez Laboratory a new maximum containment laboratory will be built. All constructional precautions are based on the best available technology and guarantee for the safety and security inside and outside of the facility, during normal operations as well as in case of an extraordinary event, such as fire, earthquake or storm. The laboratory is constructed according to the principle "box-in-the-box": The entire laboratory area on the ground floor is surrounded by an inner, aerosol-thight construction made of concrete with an epoxy coating. Personnel working in the BSL4 units will wear protective suits with external air supply. The Entrance and exit of personnel and material is managed through air lock systems. The inner concrete construction is surrounded by a security corridor. A second building shell made of a glass-steel construction will seal the building off towards the outside. The ventilation system creates different pressure zones, with the most negative pressure zone in the BSL4 units. Airflow from the outside to the inside is guaranteed, even in case of a leakage of the glass-steel or the concrete shell.

The maximum containment laboratory will be in charge of the following key tasks:

– Routine diagnostics of special pathogens (potential biological warfare agents) for military and civilian needs
– National reference function for human pathogens of risk group 4 (hemorrhagic fever viruses)
– Analysis of suspicious bioterrorist samples
– Education and training in biosafety and biosecurity on BSL3 and BSL4
– Research and development

19PW13: Question: bioterrorism and other events involving bio-agents? Answer: The Regional Laboratory Network

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The Regional Laboratory (RL) Zentrum Ost is located in Luzern and serves the cantons of Luzern, Nidwalden, Obwalden, Schwyz, Uri, and Zug for the processing of samples drawn under the suspicion of intentional release of dangerous biological agents. RL Zentrum Ost is operational since 1. July 2007. It is integrated into the Department of Medical Microbiology at Luzerner Kantonsspital Luzern, which runs an approved BL-3 facility for this purpose as well as for routine diagnostic mycobacteriology.

The methodology for rapid diagnosis by PCR of a given set of bacterial agents known for their bio-terroristic potential is developed by several national centres such as the National Centre for Anthrax (NANT). The resulting laboratory protocols are then dispatched to the RLs, which adopt them and integrate them into their routine procedures. Quality is assured by ring trials organised by the NANT.

The performance of RL Zentrum Ost in the two ring trials so far executed will be presented. Moreover, several recent cases of infection with Francisella tularensis in central Switzerland will be reported. These cases exemplify the value of the RLN in rapid diagnosis of very rare and difficult-to-cultivate pathogens, even in situations with no suspicion of abuse. In that respect, the RLN – with time – might reveal a certain degree of under-diagnosis of very rare and unexpected pathogens for the reason of little chance to gain expert experience by clinical laboratory personnel.

19PW14: Management of suspect viral haemorrhagic fever patient in Geneva

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The goal of the Swiss National Centre for Emerging Viruses (CRIVE) is the detection of viruses of the biosecurity level class 3 and 4 in humans. This centre is part of the Laboratory of Virology which also houses the National centre for Influenza. It is localized at the University Hospital of Geneva and functions in close collaboration with the Federal Office of Public Health (FOPH).

The analysis of clinical specimens at risk to contain dangerous viruses has to be done in appropriate laboratory. To fulfil to this task, we set up the first laboratory of the P4 type in Switzerland to manage and analyse samples suspected of containing hemorrhagic fever viruses or other dangerous viruses, like avian influenza H5N1 or SARS. This is an improved P3 level laboratory which contains an isolator "glove box" type (within which the biosecurity level 4 is reached) under regulations that follow strict rules according to the reference laws. No amplification of class 4 viruses by cell culture or in animals is performed in this laboratory. The basic principle is a cascade of negative pressures increasing step by step from the first SAS to the isolator, in order to guaranty strict pathogens confinement.

In this room, virus inactivation is the main work performed on the clinical samples. Once this step is accomplished in high security conditions, the following analysis are serology and viruses. It is noteworthy that we can also carry out some complementary blood analysis (blood chemistry or coagulation tests for instance) by using several devices located in the isolator, for patients hospitalized in Geneva. Because hemorrhagic fever symptoms are not specific at the beginning of the disease, we also perform differential diagnostics for malaria, Dengue, HIV and acute hepatitis A, B and C and other agents.

The patient suspected of hemorrhagic fevers or other dangerous pathogens must be managed and treated in an appropriate manner, and these need harmonised coordination and case evaluation between the clinicians, infectious disease specialists and the microbiologists. One of the first priorities, for instance, is to estimate whether the patient should be isolated or not in special unit wards. The isolation leads to a heavy work load for the hospital staff. In order to limit the risks of pathogen transmissions, specific precautions have to be applied during any patient care and there is a need to avoid any direct contact with body fluids, particularly when the viral load is very high. This new laboratory provides a diagnostic facility in Switzerland and the samples don't have to be sent in another special laboratory abroad anymore.

Concerning the packaging and the shipping terms of human samples by road in Switzerland, we clarified and normalized these points, according to national and international regulations, by working in collaboration with the Federal Office of Public Health and other concerned federal offices. This work has led to a document soon published in the "FOPH bulletin". It indicates how to precede with samples from patients suspected of hemorrhagic fevers, Chikungunya or Tick-borne encephalitis for instance, taking and management and how to send them to Geneva. All the informations cited in this paragraph as well as our timetable and the list of analysis are also available on our web site (http://virologie.hug-ge.ch).

Some viruses are genetically very variable and evolve quickly. Consequently, the CRIVE has to adapt regularly the diagnostic tests for new variants viruses. Moreover, new detection tests have to be set up in case of outbreaks due to emerging or re-emerging viruses. Since 2005 the centre is also a member of the European Network for Imported Viral Diseases (ENIVD).

19PW21: Pathophisiology of metabolic syndrome

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The metabolic syndrome is a constellation of interrelated abnormalities (obesity, dyslipidaemia, hyperglycaemia, and hypertension) that increase the risk for cardiovascular disease and type 2 diabetes. This is a common metabolic disorder which increases in prevalence as the population becomes more obese. The most accepted hypothesis to describe the pathophysiology of the metabolic syndrome is insulin resistance. Insulin resistance has been defined as a defect in insulin action that results in hyperinsulinaemia. To compensate for defects in insulin action, insulin secretion must be increased to sustain euglycaemia. If this compensation fails, defects in insulin secretion predominate and hyperglycaemia occurs. A major contributor to the development of insulin resistance is an overabundance of circulating fatty acids, released from an expanded adipose tissue mass. FFA reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Increased level of circulating glucose increases pancreatic insulin secretion resulting in hyperinsulinaemia. Although free fatty acids can stimulate insulin secretion, prolonged exposure to excessive concentrations of FFA results in falls in insulin secretion. The mechanism for this alteration has been attributed to lipotoxicity. In the liver, FFA increase the production of glucose, triglycerides and secretion of very low density lipoproteins (VLDL). The consequence is the reduction in glucose transformation to glycogen and increased lipid accumulation in triglyceride (TG). Insulin is an important antilipolytic hormone. In the case of insulin resistance, the increased amount of lipolysis of stored triacylglycerol molecules in adipose tissue produces more fatty acids, which could further inhibit the antilipolytic effect of insulin, creating additional lipolysis. Thus, hypertriglyceridaemia is an excellent reflection of the insulin resistant condition and is one of the important criteria for diagnosis of the metabolic syndrome. The other major lipoprotein disturbance in the metabolic syndrome is a reduction in HDL cholesterol. This reduction is a consequence of changes in HDL composition and metabolism. In the presence of hypertriglyceridaemia, a decrease in

the cholesterol content of HDL results from decreases in the cholesteryl ester content of the lipoprotein core with variable increases in triglyceride.

The WHO and ATP III definitions of metabolic syndrome both include abdominal obesity, but it is a necessary requirement in the IDF definition. That reflects the IDF position – though the pathogenesis of the metabolic syndrome and its components is complex, abdominal obesity is a key causative factor. According to some theories, with increases in visceral adipose tissue, a higher rate of flux of adipose tissue-derived free fatty acids to the liver through the splanchnic circulation would be expected. The relation between insulin resistance and hypertension is well established. Several different mechanisms are proposed. First, insulin is a vasodilator when given intravenously to people of normal weight, with secondary effects on sodium reabsorption in the kidney. In the setting of insulin resistance, the vasodilatory effect of insulin can be lost, but the renal effect on sodium reabsorption preserved. Fatty acids themselves can mediate relative vasoconstriction. Hyperinsulinaemia may result in increased sympathetic nervous system (SNS) activity and contribute to the development of hypertension.

19PW22: Genetic risk factors for metabolic syndrome

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Metabolic syndrome (MetS) is defined as clustering of different conditions that increase the risk of developing cardiovascular disease and diabetes. According to definition used, components of MetS are insulin resistance, hypertension, dyslipidaemia, obesity, microalbuminuria and glucose concentration. Pathogenesis of each of these components is multifactorial and cumulative action between large number of genes and environment is required for its development.

Heritability, as a measure of assessing genetic components in the disease, has not yet been determined for the MetS, although the heritability for its components is substantial and well recognized. Family and twin studies estimated heritability for hypertension to be from 20–60%. Genetic contribution in type 2 diabetes ranges from 37% in dizygotic to 50% in monozygotic twin pairs. Insulin resistance also strongly clusters within families, thus 45% of first relatives with insulin resistance are also insulin resistant. Genetic background can explain 40% of variation in body fat and 70% in abdominal obesity.

Approaches on identifying genes involved in metabolic syndrome development include animal models on the levels of the basic research, genome scan models and candidate gene approach.

Genome scan models include search of entire genome in order to localize loci associated with studied phenotype. As a result, chromosomal regions associated with disease are identified, but nothing is known about biological function of genes involved. Several genetic loci have been identified in association with metabolic syndrome: 1p36.13, 3p25, 10p11.2, 19q13.4. After location in genome is identified, a search of candidate genes localized in the identified region begins.

The most commonly used way to study candidate genes of polygenic traits is genetic association study. However, this approach has some serious shortcomings that can influence the result. Depending on the definition used, different components and cut-off values are used in the recruitment criteria. As a result, MetS study population is very heterogeneous which frequently leads to failure to replicate results in independent studies. An important source of difficulties in these studies is small gene effect of single gene in polygenic traits. Due to that, a very large number of participants are needed to detect an influence of genetic variants. A meta-analysis is a useful tool to asses the results of genetic-association studies.

Based on the thrifty genotype hypothesis, genes involved in energy storage could be predisposing to MetS. This large group of candidate genes involves genes associated with different phenotypes: obesity (leptin, leptin receptor, melanocortin receptor); free fatty acid metabolism (adiponectin, β-adrenergic receptor, lipases); insulin sensitivity (PPARγ, insulin receptor substrates); lipid metabolism (ApoE, Apo ApoA5, ApoC3); hypertension (ACE) and inflammation (TNFα, CRP, IL-6). These genes and their polymorphic variants have been extensively studied in association with both, metabolic syndrome in general and individual components of metabolic syndrome.

PPARγ controls expression of many genes involved in lipid and glucose metabolism. It is localized on chromosome 3p25, a locus that has been identified in several diabetes and metabolic syndrome genome scans. Pro12Ala polymorphic variant has been associated with BMI, insulin sensitivity, HDL cholesterol, type 2 diabetes, hypertension, triglycerides and glucose concentration. Adiponectin is secreted by adipocytes, regulates homeostasis, glucose and lipid metabolism and is inversely correlated with BMI and body fat. Several polymorphic variants (–11,377C>G, 276G>T) have been associated with plasma levels of adiponectin and/or metabolic syndrome and associated phenotypes. β-adrenergic receptors are involved in lipid metabolism via catecholamine-induced lipolysis. β3AR is a candidate gene for obesity, and its Trp64Arg variant has been associated with hyperinsulinemia, obesity and hypertension. Interleukin 6 (Il-6) is a proinflammatory cytokine secreted from leukocytes and adipocytes. Polymorphic variants in IL-6 gene (–579G>A, 572G>C, –174G>C) have been associated with metabolic syndrome and this finding has been independently replicated.

Besides candidate genes for metabolic syndrome, some genes have been identified to have a protective role in MetS development. One of such genes is ghrelin, a protein that regulates energy balance, insulin signalling and plasma glucose. R51Q variant has been associated with significant risk reduction in MetS development.

19PW23: Pharmacogenetic approach in metabolic syndrome therapy

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Metabolic syndrome as a group of disorders poses a higher risk of coronary

heart disease and type 2 diabetes mellitus. The basic therapeutic objectives are overweight reduction, physical activity and treatment of risk factors such as hypertension, coagulation tendency and dyslipidaemia. If physical activity and diet regulation fail, medicamentous therapy is introduced. Drug efficacy depends on patient lifestyle, biological factors and genetic factors. However, it is known that adverse drug effects occur in 7% of hospitalized patients, 0.3% of these with lethal outcome. Genetics is the key risk factor and the only one that does not change with lifetime and pharmacogenetics investigates the connection between the individual's genetic predisposition and drug metabolizing ability. Polymorphisms of genes responsible for interindividual variability may be a consequence of alterations on genes of drug metabolizing enzymes, transporters, receptors or signal molecules. An individual can be a homozygous or heterozygous carrier of polymorphic alterations on one or more genes contributing to lower or higher variability in the absorption, distribution and metabolism, or in the receptor and ion channel interaction resulting in different drug response.

Polymorphism of a drug metabolizing gene is connected with three major phenotypes: extensive, poor and ultra-extensive, while that of drug transporters and receptors results in a phenotype with slow and rapid drug absorption, and efficient or poor interaction with receptors. The consequence of pharmacogenetic polymorphism can lead to drug substrate accumulation and consequently to toxicity or inadequate drug dosage.

The guidelines on pharmacogenetic testing for pharmacogenetic profiles (the most important alleles) of the CYP2D6, CYP2C19, CYP2C9, NAT2 and TPMT genes and B2–AR receptors in clinical setting recommend the genes clinically proven to be useful in therapy optimization. Many drugs used in therapy for metabolic syndrome disorders are substrates of these highly polymorphic genes, and some of them will be discussed here.

In dyslipidaemia, statins that are frequently used in primary and secondary prevention of coronary heart diseases decrease cholesterol by reversible and competitive inhibition of HMG-CoA reductase resulting in LDL cholesterol and triglyceride decrease and HDL cholesterol increase.

Except for pravastatin, statins are metabolized by the CYP3A4, CYP2D6, CYP2C9 and CYP2C8 enzymes expressing variable effect. In homozygous carriers of variant alleles, the standard simvastatin dose has a stronger cholesterol lowering effect than in wild-type carriers. In contrast, carriers of CYP2D6 gene duplication or multiplication require higher simvastatin dose to lower cholesterol.

In therapy for type 2 diabetes, oral hypoglycemics (sulphonylurea, meglitinide, biguanide, alfa-glucosidase inhibitors) show great interindividual differences in the efficacy and side effects (hypoglycemia). Most of them are metabolized by the polymorphic CYP2C9 enzyme. Two important CYP2C9 allele variants, *2 and *3, that encode proteins with enzyme activity of only 5% to 12% may lead to poor metabolizer phenotype. It is recommended to adjust the standard tolbutamide dose to 50% in homozygous and to 20% in heterozygous CYP2C9*3 allele carriers. Beta blockers used for cardiovascular diseases are also mostly metabolized by CYP2C9 enzyme, thus pharmacogenetic profile testing is recommended to prevent the possible adverse effects and to determine the right dose.

In the poor metabolizer phenotype, RS-propafenone, an antiarrhythmic is metabolized by CYP2D6 into its active metabolite 5-hydroxypropafenone, leads to stronger beta blocking action and severe side effects. In hypertension therapy, sartans as prodrugs are metabolized into active form also by polymorphic CYP2C9 and CYP3A4 enzymes.

In therapy for thromboembolic complications, the most extensively used drug is the anticoagulant warfarin with a narrow therapeutic range and high rate of complications ranging from occult to severe bleeding. The most important genes in its action are CYP2C9 and VKORC1, explaining almost half of warfarin dose variability. Special algorithms (www.warfarindosing.com) calculating warfarin dose involve CYP2C9 and VKORC1 genotypes, and some biological characteristics offer safer choice of therapeutic dose and avoid related complications. The knowledge of genotype alone does not always allow for the pharmacologic phenotype to recognize because pharmacotherapy requires a multifactor approach. However, finding of more reliable concordance between the genotype and the phenotype and their connection with other factors that influence therapeutic success will ensure an optimal approach to therapy while reducing adverse effects and lowering the cost of treatment.

19PW31: Harmonization of post-graduate education of medical doctors in laboratory medicine

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The European Union has listed the specialities recognized within its member states in Directive (2006/100/EC). The diversity of different specialities demands a harmonization. Since 1958 the European Union of Medical Specialists (UEMS) has taken responsibility as a non-profit organisation aiming to promote the professional interests of medical specialists. It acts as a lobby organisation working within the European Parliament, and is actively working to harmonize training and continuous medical education (CME) of its specialities and to promote the free movement of medical professionals within Europe.

Today delegates from 27 EU states and observers of non-EU countries have been appointed officially by their National Bodies to work within UEMS

UEMS operates a Secretariat in Brussels which is governed by a Board and Council in which all National Medical Bodies are represented by two delegates per country: UEMS Sections and Boards are cornerstones of UEMS and professional development in EU.

UEMS Section of Medical Biopathology was founded in 1962 as Section of Laboratory Medicine which was considered an umbrella for all diagnostic- and laboratory-related specialities including Pathology. In 1988 Pathologist Societies decided to form their own section. The Section Laboratory

Medicine renamed itself to Medical Biopathology including various specialities and covering the interests of monovalent and polyvalent laboratory specialists.
Delegates to the section elect a Board of Medical Biopathology and Divisions according to specialities represented in the section. At present these are six divisions: Polyvalent Medical Biopathology, Clinical Chemistry, Haematology and Transfusion Medicine, Clinical Immunology, Clinical Microbiology and Genetics.
Main activities of divisions focus on training in its speciality, evaluation of continuous medical education programmes and conducting visitations.
Core Curricula for training in polyvalent and monovalent specialty have been laid down by the divisions and the section and published in a Blue Book. All Curricula will be rewritten and republished in 2009.
The Board has created a Fellowship in Medical Biopathology the role of which is complementary to national examinations where they exist. European Board Examinations are regarded as a quality mark for safe independent practice at the end of specialist training. Candidates who pass a European Board Examination in Medical Pathology and who are certified specialists in an EU/UEMS member state may call themselves "Fellow of the European Board of Medical Biopathology" (F.E.B.M.B.).
European Accreditation Council for Continuing Medical Education (EACCME) is another cornerstone of UEMS. Organizers of medical Congresses can by internet send an application for approval to EACCME. If approved by the national medical society and by the appropriate section, board and division the congress will be approved by EACCME and participants will receive CME credits accordingly.
UEMS has created a Visitation program with "Guidelines for the Recognition of Training Centres" and "Standards for training of Medical Biopathologists" in Europe.
Criteria to assure quality of training incorporate submission of information and agreement to site visits. The visitation has to find proof of regular discussions of indications for laboratory tests and a weekly programme of teaching.
Ready access to an adequate library with international journals and recent books
and facilities for clinical and experimental research must be presented.
Program of training must give graded and progressive responsibility to trainee under the supervision of the responsible Medical Biopathologists and the training must be recorded in a detailed log book.
Harmonization of polyvalent and monovalent specialists in Medical Biopathology will lead to comprehensively specialized Medical Biopathologists who safeguard high quality and safety of Medical Biopathology for outpatients and in small and medium sized hospitals as well as highly monovalent specialized Medical Biopathologists in large hospitals, university hospitals and in research.

19PW33: Laboratory medicine: is there a place for scientists?

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On principle, scientists active in Laboratory Medicine have a special status: coming from scientific or technological education, they are given the possibility to apply their knowledge to the human context and to diagnostic and therapeutic problems in particular. In our Country, their role is perhaps even more relevant than that of the Colleagues working elsewhere in Europe: today, Switzerland has many private or hospital laboratories led by people with sound backgrounds in chemistry, biochemistry, pharmaceutics or biology who have passed in addition a federal, usually pluriannual mono- or pluri-disciplinary competence diploma (the so-called F.A.M.H. title).
From another point of view, it is not unusual that clinicians still look at scientists as a sort of "strange" (if not "dangerous") people, too much prone to show their scientific skill – or cleverness! – but unable in practice to understand what Medicine really is and needs. From a pluri-annual perspective it is however my opinion that MDs and PhDs do often not realize that they can be seen as "the two banks of a same river", the patients constituting the water flowing between them. In such a context, the will of the patients has to come into consideration: patients simply expect that the clinicians and their collaborators (and therefore also the scientists) work together with one objective: that to improve their health.
This view of the things has strong ethical, cultural and practical consequences: it asks for a continuous improvement of the paradigm that clinicians and scientists have not only to do well their respective jobs, but also that they continuously have to meet in a sort of "hot pot" allowing the exchange of the mutual competences nourished by mutual respect as well as – if possible – of human warm-heartedness. In other words, "the river of patients" asks for a continuous and untiring "crossing the bridge" between Medicine and Science.
This issue assumes a particular relevance in the context of the education of the future generations of clinicians and scientists. The Author will discuss about the various possibilities to create "bridges" between Clinic and Science.

19PL21: Diagnostics of leukaemia

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Haematological diagnostics, especially of leukaemias and lymphomas, has become more and more complex and specific during the last 10 years. A combination of cytomorphology, cytochemistry, histology, metaphase cytogenetics, fluorescence in situ hybridisation, immunophenotyping and molecular techniques is reasonable and necessary in many cases. This way, characterisation of a single disease as well as a specific subentity can be achieved, which is not only of diagnostic value but in many cases provides elementary, partly the most significant findings for prognosis. Also, with help of the above-mentioned methods, control of therapy is possible from the beginning as well as during course of the disease (minimal residual disease). Also gene expression profiling may add a lot more information in the near future and already achieves very high accuracies. Therefore, it is

important to determine the right combination for each patient by using disease specific algorithms. This allows a quick diagnosis and a prognostic rating as well as the control of therapy.

19PL23: Early diagnosis of bacterial infections

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We review the advantages and drawbacks of biomarkers in the diagnostic and prognostic assessment of systemic infections. Since the signs and symptoms of severe infections can be ambiguous, biomarkers provide a more reliable tool in ascertaining the presence of a relevant bacterial infection, its severity and treatment response. Procalcitonin and, to a lesser extent, C-reactive protein and interleukin-8 can improve the diagnostic assessment of infections and guide antibiotic therapy. Promising prognostic biomarkers include cortisol, proadrenomedullin, copeptin and natriuretic peptides. The strengths and weaknesses of biomarkers must be recognized in order to use them rationally and safely. Cut-off ranges of biomarkers must be chosen according to the specific clinical context and they should be used as a complementary tool, to reinforce the clinical diagnostic workup. Biomarkers cannot determine the causative organisms and associated patterns of antibiotic susceptibility. If used in the proper setting, serial measurements of diagnostic biomarkers may allow treatments to be adjusted at an early stage in patients with severe infections. This may involve either intensifying treatment when infection levels stay high or avoiding unnecessary prolonged courses of antibiotics when levels rapidly decrease, thereby improving the allocation of healthcare resources.

19PL24: Brain damage biomarkers: from discovery to clinics

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Cerebrovascular diseases (CVD) are a significant cause of mortality and severe neurologic disability in industrialized countries. Typically, diagnosis of these pathologies is based only on physician evaluation and brain imaging and no predictive tools exist for the long-term neurological outcome of patients. Therefore, CVD pose clinical but also social and economics problems, highlighting challenging questions: 1/ is it possible to identify brain injury related biomarkers in blood, sufficiently early, sensitive and specific enough to be used as clinical diagnostic tools? and 2/ could biomarkers be used, individually or in combination to follow-up the effect of the treatment or to predict patient outcome?

Following any form of brain insult, proteins are released from damaged tissues into the cerebrospinal fluid (CSF). Therefore, we used different proteomic strategies to compare the protein content of human post-mortem cerebrospinal fluid (CSF) and ante-mortem CSF. Thus, we identified proteins found as differentially expressed in post-mortem CSF ($p < 0.05$). Of these 250 proteins, more than 75% have been described as intracellular proteins suggesting that they were released from damaged cells. From these proteins, five have been further validated as biomarkers in age/sex matched European and American cohorts for the early diagnosis of stroke (UFD1, NDKA and PARK7), the therapeutic follow-up (GSTP) and the prediction of poor clinical outcome after stroke (NDKA) and Aneurysmal Subarachnoid Hemorrhage (a panel of biomarkers).

In conclusion, this study further established the utility of proteomic based-strategies combined to ELISA validation as first steps toward the discovery of blood markers of brain injury. Moreover, the results highlight the potential utility of these proteins individually or combined with other biomarkers to improve the referral to treatment and management of CVD patients.