Abstracts*)

The 3rd EFLM-UEMS Congress

Laboratory Medicine at the Clinical Interface
Liverpool, United Kingdom, 7th–10th October, 2014

Organisers
European Federation of Clinical Chemistry and Laboratory Medicine
Union Européenne Des Médecins Spécialistes
Association for Clinical Biochemistry & Laboratory Medicine

Under the auspices of
International Federation of Clinical Chemistry and Laboratory Medicine

Organizing Committee
Rebecca Allcock, Carrie Chadwick, Ian Godber, Joe O’Meara, Stephen Smith, Ana-Maria Simundic, Patrick Twomey, Ian Watson, Mark Weaver, Gilbert Wieringa (Chair)

Scientific Programme Committee
Chris Chaloner, Joris Delanghe, Danielle Freedman, Ilkka Mononen, Sverre Sandberg, Gilbert Wieringa, Gerhard Zlabinger, Ian Watson (Chair)

Secretariat
Meeting Makers, Block 4, Unit 4, Kelvin Campus,
West of Scotland Science Park, 2317 Maryhills Road, Glasgow, G20 0SP, UK
Association for Clinical Biochemistry & Laboratory Medicine
130-132 Tooley Street, London, SE1 2TU

Guest Editor
Elizabeth Hall, Publications Committee, Association for Clinical Biochemistry & Laboratory Medicine

*)These abstracts have been reproduced directly from the material supplied by the authors, without editorial alteration by the staff of this Journal. Insufficiencies of preparation, grammar, spelling, style, syntax and usage are the authors’ responsibility.
PL4

22 and you: the impact of genomics on patient care

Gi Tsongalis
Geisel School of Medicine at Dartmouth, Pathology, Lebanon, United States

The last several years have seen an explosion in the adoptability of clinical genomic testing by healthcare providers. Clinical laboratories have implemented various molecular biology testing platforms, including next generation sequencing, for the detection and quantification of numerous human and microbial gene targets associated with a variety of diseases. Constitutional and somatic mutation analysis for human diseases have come to be standard of care in several specialties and these will be discussed in more detail.

PL6

Tackling the alcohol epidemic

J Gilmore
United Kingdom

Europe is the highest continent for alcohol consumption in the world and within Europe there are marked variations. What European countries have in common is a huge health, crime and economic burden as a result. There is abundant evidence on what drives population consumption and harm, but few countries are applying evidence-based policies to tackle the issue. The problems and potential solutions will be discussed.

S1.1

Patient monitored renal function after transplantation

Pj van der Boog1, CL van Lint1, S van Dijk1, AJ Rovekamp2, TJ Rabelink1, CM Cobbaert3
1Leiden University Medical Center, Nephrology, Leiden, Netherlands, 2TNO, Quality of Life, Prevention and Care, Leiden, Netherlands, 3Leiden University Medical Center, Clinical Chemistry, Leiden, Netherlands

Mainly in the first year after renal transplantation, patients are at risk for acute rejection. Therefore, during the first year post transplantation, kidney transplant patients visit the hospital on average 20 times to have their serum creatinine checked. If patients were enabled to monitor kidney function at home, this would have important advantages. First, home monitoring is thought to improve early detection of rejection. Second, the high number of necessary outpatient visits could be reduced. Third, involving patients in monitoring renal function after transplantation corresponds to the idea of Predictive, Preventive, Personalized and Participatory medicine (P4). In line with the latter, patients who self-monitor usually experience higher levels of quality of life and more empowerment.

In a pilot-study, patients’ attitude towards and experience with self-monitoring after kidney transplantation was investigated. Thirty kidney recipients monitored creatinine and blood pressure at home during the first 12 weeks after transplantation. Creatinine was measured with a handheld point of care device which was suitable for monitoring creatinine trends over time but not for diagnostic purposes. Results showed that patients were motivated for participation and reported high levels of general satisfaction. The use of both the creatinine and blood pressure meter was considered pleasant and useful, but trust in the accuracy of the creatinine device was relatively low. Low trust in accuracy was related to high analytical variation in both repeated and subsequent measurements. Half of the patients considered telephonic consults to be a full-fledged alternative substitute for face to face appointments. Average adherence to the monitoring protocol was good, but large intra-individual differences were found that increased over time.

Although the accuracy of the creatinine device and the implementation of self-monitoring into regular care need to be improved, the current findings suggest that patients are positive regarding the possibilities of self-monitoring after kidney transplantation.
S1.2

Enabling patients to access their medical records and gain understanding too

A Hannan
Haughton Thornley Medical Centres, Hyde, United Kingdom

Real-time Digital Medicine. Since 2006, Haughton Thornley Medical Centres has offered patients full online access to the GP electronic health record. Currently over 25% of patients (over 2900 patients) have signed up for the service. Many patients are now able to view their test results online and via their smartphone supported by the practice-based web portal www.htmc.co.uk. Patients are encouraged to view their results and use Lab Tests Online to gain a better understanding of their test results. This has developed from a Partnership of Trust formed between patients and clinicians and the IT systems that support each other. Dr Hannan describes how the practice has enabled patients to view their records and gain a better understanding of their health. He describes Instant Medical History which enables patients to add a medical history to the record too as we go beyond just viewing records or sharing them. He describes what types of patients have signed up and some of the approaches taken. He also describes what difficulties there have been, how we have overcome them and what plans there are as commissioners to build on this success and can spread this further. He will bring a patient along as well so that there is the opportunity for the audience to hear first hand their views too and the opportunity to ask questions from your perspective on what this may mean for you.

S1.3

Patient focused laboratory medicine

WP Oosterhuis1,2
1Atrium Medical Center, Clinical Chemistry, Heerlen, Netherlands, 2EFLM WG-PFLM, Milan, Italy

The EFLM working group PFLM was recently established. One of the terms of reference is to evaluate methods, how laboratory specialists can communicate directly with patients. A survey was done among European countries to evaluate how laboratory results are reported. The results were, that in some countries reports are rarely sent to patients (in some countries this is not even allowed), in other countries it is quite normal that the patients receive the test results. Patients seem to be not very well informed by their doctors: not about the tests that were requested nor about the interpretation of the results. We expect that great need exists with patients for information, including information about laboratory results. With the development of web-based patient records, it might become a reality that laboratory results can be obtained freely by patients. But even with the free access of their test results, most patients will not understand these. Can the laboratory play a role in informing the patient about the test results including the interpretation? The laboratory could also inform the patient about guidelines applicable in their case. Patients can easily be informed about additional (verified) information available on the Internet. This could all help to improve the quality of the patient-doctor contact.

A study was done to confirm these ideas. 40 patients were asked by their general practitioner if they wanted to receive their laboratory results, including an interpretative report (average 1000 words). A reference was made to additional information on the internet. The patients were interviewed after receiving the report. A great majority of patients were positive about this information, and almost all wanted to receive these reports in the future. We conclude that there is a challenge for the laboratory to play a role in better informing patients about their laboratory results.

S2.1

Hepcidin in human iron disorders: analysis and diagnostic implications

DW Swinkels
Radboudumc, Nijmegen, Netherlands

The iron regulatory peptide hormone hepcidin is synthesized by the liver, secreted in the circulation and regulated by body iron status, inflammation, erythropoietic activity and oxygen tension. Moreover, serum hepcidin levels have been found to increase during the day. Circulating hepcidin lowers serum iron concentrations by counteracting the function of ferroportin, the cellular iron exporter present in the membrane of macrophages and enterocytes. The bioactive form of hepcidin comprises 25 amino-acids. However, amino-acid degradation leads to three smaller isoforms (hepcidin-24, 22 and 20) of unknown significance. These isoforms are present in the serum of certain diseases among which are chronic kidney disease and sepsis. Proof-of-principle studies highlight hepcidin as a promising diagnostic tool and therapeutic target in the management of iron disorders. However, development of hepcidin assays has proven to be challenging. Hepcidin is a small, evolutionary
conserved peptide and therefore it is difficult to generate antibodies against this peptide for laboratory assays. Moreover, hepcidin tends to aggregate and stick to laboratory plastics. There are two classes of hepcidin assays: mass-spectrometry (MS) and immunoassays (IA). MS has the advantage of distinguishing between the various hepcidin isoforms. IA assays generally lack specificity for bioactive hepcidin-25 and measure total hepcidin levels. The clinical relevance of specifically measuring hepcidin-25, however, is unclear. In addition, hepcidin levels differ widely between assays. This hinders the comparability of data collected by the use of the various assays and preclude the use of clinical decision limits. Since hepcidin is a hormone, hepcidin values should always be interpreted in the context of other indices of iron metabolism and inflammatory parameters. In conclusion, hepcidin is a promising diagnostic tool but before hepcidin can be fully included in clinical practice, more work should be done to harmonize assay outcomes, and to further substantiate clinical applications and related clinical decision limits.

S2.2

Iron overload assessment: an evolving field

P Brissot, E Bardou-Jacquet, T Cavey, O Loreal, Y Gandon, M Ropert
National Reference Center for Rare Genetic Iron Overload Diseases. University Hospital Pontchaillou, Rennes, France

Iron overload diseases correspond to a wide spectrum of conditions, either acquired (transfusion iron overload) or genetic (haemochromatosis). Iron overload assessment concerns three main targets.

1. Assessment of body iron stores: Classically, the gold standard method was the liver biopsy permitting to assert iron excess and to determine liver iron concentration (LIC) which is closely correlated with total iron stores. However, it is an invasive technique which cannot be repeated and is increasingly replaced by a non-invasive approach. This approach combines:
   i. determination of serum ferritin levels, after having excluded confounding factors such as inflammation, dysmetabolism, alcoholism and hepatic dysfunction, and
   ii. hepatic iron load assessment by magnetic resonance imaging (MRI). MRI has become a key investigation to show hepatic iron overload, to determine a reliable LIC, and to evaluate iron deposition in other organs such as the spleen, heart or pancreas.

2. Assessment of iron bioavailability: Plasma transferrin saturation (TS) is an essential indicator of iron availability, especially to bone marrow. In the domain of genetic iron overload, it helps differentiating forms that are related to hepcidin deficiency leading to increased serum iron concentration (such as the classical HFE-related haemochromatosis, and non-HFE haemochromatosis due to hemojuvelin, hepcidin or transferrin-receptor 2 mutations) and those due to cellular iron retention causing low serum iron concentration (such as the ferroportin disease).

3. Assessment of iron toxicity: Non-transferrin bound iron (NTBI) and especially labile plasma iron (LPI) represent toxic forms of circulating iron, which appear whenever TS is increased. TS can therefore be considered as an indirect marker of potential iron toxicity. In conclusion, the major improvement in iron overload assessment has been to resort to a non-invasive approach, valuable not only for the diagnosis but also for the therapeutic management of iron overloaded patients.

S2.4

Transferrin receptor and the diagnosis of iron deficiency

K Punnonen
Eastern Finland Laboratory Centre (ISLAB), Clinical Chemistry and Laboratory Medicine, Kuopio, Finland

Transferrin receptor (TfR) mediates the transport of iron-transferrin complexes from plasma into the cells. In the human body some 80% of the TfRs are localized on the surface of the RBC precursors in the bone marrow. In iron deficiency, cells express more TfR and also the plasma levels are increased as the extracellular parts of the TfR receptor are constantly shed into the plasma. Importantly, inflammatory conditions elevate plasma ferritin but they do not interfere with the sTfR concentrations. The sTfR measurements have been considered to be of valuable especially in the differential diagnosis of anemia of chronic disease and iron deficiency anemia and they have been included in a good number of preferred diagnostic algorithms. Nevertheless, as laboratory professionals we often see a difference between suggested diagnostic routines and the everyday clinical practice. In Finland there are five major laboratory organizations which cover approximately 70-80% of laboratory tests in the public sector and each year approximately 50 000 to 60 000 of both ferritin and TfR tests are being performed. In these organizations (n=5) the ratio of TfR and ferritin test requests varied from 0.67 to 1.07. In other words, in Finland the number of TfR test requests almost equals with that of ferritin. The diagnostic companies have an excellent overview of the global test usage and Roche Clinical Diagnostics kindly provided data on the sales of the anemia markers in comparison to ferritin tests. On the global market the ratio of TfR tests to ferritin tests is 0.015 and in Europe the corresponding ratio is 0.01. The ratios of these test requests (ranging from 0.01 to 1.07) reflect huge differences in the diagnostic practices in different countries. Is it tradition rather than science or published guidelines that determine the usage of laboratory tests?
S3.1

Failed implementation of IVD standards - rebuilding the system from scratch

LM Thienpont
Ghent University, Laboratory for Analytical Chemistry, Gent, Belgium

Laboratory medicine struggles for >40 years with reaching comparable data (= traceability), independent of the assays used. There has yet not been a real breakthrough, despite the expectations end of the nineties, when the IVD Directive was published. This European legislation proposed a “top-down” approach to traceability. However, it failed to fulfill the commitment, as can be shown from several examples. I will also discuss why I think top-down approaches are difficult to implement. To compensate for the failure, many initiatives popped up, among them: Calibration 2000 (NL), Pathology Harmony (UK), Harmonization of Laboratory Testing (AU&NZ), Patchwork Standardization Program (JP), and Harmonization.net (Global). We (UGent & STT-Consulting) also recently started a project to improve interchangeability of results, but decided to try it bottom-up. The distinct features why our approach should warrant more success, are: i) bottom up cooperation between laboratories and manufacturers, mediated by UGent/STT-Consulting; ii) consequent use of high quality samples in calibration and assessment; iii) building the system from scratch by addressing high volume clinical chemistry analytes first; iv) setting analytical performance specifications on the basis of current performance, but mirrored to goals from biological variation; v) implementing the project by the development and (possible sale) of products. We named “our child” the “Empower” project. It comprises 2 main products that aim at assessing the quality and stability of laboratory testing with emphasis on interchangeability of results across laboratories and manufacturers: the “Master Comparisons” and the “Percentiler”. The former refers to a point-estimate by dedicated external quality assessment, the latter to daily on-line monitoring of patient medians with user access. The latter is particularly attractive in view of the current trend to using “big data” for public health economy planning, implementation, and control of global healthcare policies. I will present some notable results from both project activities.

S3.3

Mandation of ISO 15189: why?

M Vaubourdolle
Hôtels Universitaires Est Parisien - AP-HP, Biology and Pathology Department, Paris, France

2010-2013 French reform of Medical Biology leads to an harmonization of private and public practices, choice of “medical” biology vs. “industrial” biology, reorganization of territorial distribution with multisite labs with proximity antennas, proven quality by mandatory accreditation using NF EN ISO 15189 and 22870 standards. This mandatory aspect has been chosen in order to increase the speed of labs restructuration and to propose a coherent global reform. The labs are thus faced to the circle quadrature: improve quality towards ISO accreditation and increase efficiency to compensate enhanced financial constraints. This equation is now accompanied by a marked decrease in the number of labs. In effect, the merge to multisite labs is an essential engine to simplify the accreditation process and to allow the respect of the new regulation requirements (complete accreditation before 2020). Given the mandatory context, the question is not now for us: “WHY should we work for an ISO 15189 lab accreditation?” but “HOW to engage quickly the lab in the ISO 15189 accreditation process?” We know that the mandatory accreditation of all French labs in the next 6 years is a big challenge since laboratory quality management is often heard as an heavy new constraint and an indirect restructuration tool. But, with the point of view of an accredited lab, we can say that it is also a very efficient lab management tool (management by quality), a federative project for all the laboratory team and a necessary way for our “medical” specialty to “survive” in the actual “industrial” context.

S4.1

Childhood diabetes as a worldwide epidemic - but why?

M Knip1,2
1University of Helsinki, Children’s Hospital, Helsinki, Finland, 2University of Helsinki, Diabetes and Obesity Research Programme, Helsinki, Finland

The rate of childhood type 1 diabetes has increased substantially over recent decades in most countries, and it has been estimated that the incidence has increased on an average by 3-5% per year during the last 20 year. This conspicuous increase cannot be due exclusively to genes, but must be related to changes in environment and life style and the interaction between such changes and predisposing genes. Currently there is no definite explanation to why the disease rate has increased so rapidly. A series of hypotheses have, however, been generated.
According to the hygiene hypothesis the decreased microbial exposure in early childhood being a consequence of improved standard of hygiene has skewed the programming of the immune system favouring allergies and autoimmune diseases. Accumulating evidence indicates that an antidiabetogenic enterovirus infection may trigger beta-cell autoimmunity preceding the presentation of clinical disease with months and years. On the other hand it is known that the circulation of enteroviruses in the background population has decreased at the same time as the diabetes rate has increased. This seeming contradiction can be explained by the so called polio hypothesis according to which the reduced circulation of enteroviruses results in a weaker maternal protection of the offspring against invasive enterovirus infections in infancy. According to the accelerator and overload hypotheses the environmental load on the insulin-secreting beta cells has increased as a consequence of accelerated linear growth and weight gain as well as the increasing imbalance between the energy intake and physical activity in childhood. The bovine insulin hypothesis is based on the observation that early exposure to bovine insulin through infant formulas results in an immune response to bovine insulin that escapes oral tolerance induction in those children who develop beta-cell autoimmunity. This immune response may later target human insulin and insulin-producing cells.

S4.2

How can we intervene to prevent or cure type 1 diabetes?

J Ludvigsson
Linköping university, Linköping, Sweden

The cause of Type 1 diabetes is still unknown but primary prevention can be tried:
- The TRIGR trial studies whether it is possible to prevent T1D by avoiding exposure to cow’s milk during the first months of life.
- Gluten, in similar studies, had no effect.
- Probiotics so far only in pilot trials.
- Enterovirus vaccine is underway.
- The Beta cell stress hypothesis means that factors that cause T2D can contribute to T1D. T1D is supposed to be the result of an autoimmune destruction of the pancreatic beta cells.
- Immune suppression includes, cyclosporine, steroids, azathoprin and later specific monoclonal antibodies. AntiCD20 had some short effect, while antiCD3 seems to be the most promising.
- Immune modulation was tried with plasmapheresis. Recent years auto-antigen treatment has given exciting results. In Phase II GAD-treatment preserved C-peptide as well as antiCD-3, but easy without any treatment-related adverse events. Phase III trial failed. New studies are ongoing with GAD in combination with vitamin D and Ibuprofen. Further studies are planned in combination with Etanercept.. Auto-antigen treatment early has been tried to prevent T1D, with GAD, but also studies with insulin. Parenteral insulin had no effect (DPT), and oral insulin failed but subanalyses showed possible effect. Intranasal insulin failed. Diapep277 has had some effect in adults, however difficult to interprete, but no effect in children.
- Several other efforts have been made eg to protect the beta cells with large doses of Nicotinamide (failed in ENDIT), vitamin D (failed), antidioxidantia combinations (failed), diazoxide (transient effect), IL-1 inhibition (failed) etc

Conclusion: So far there is no method for clinical use neither to prevent nor cure or preserve residual beta cell function at onset of the disease. Combination therapies will be tried.

S5.1

Non-numerc data presentation

C Webster
Birmingham Heartlands Hospital, Birmingham, United Kingdom

The visual presentation of data can reveal relationships and context that may not be readily seen on simple tabulation or summary. Non-numeric or categorical data can present particular challenges in this area. There are two types of categorical data; nominal, where categories are equally valued and ordinal, where categories allow to rank order on some scale of measurement. Presentation and analysis of both types of data can require some conversion - or coding - to numerical data, for example categorical data can be presented via frequencies or their percentage in the sample. Other types of non-numerical data may be visualised with minimal manipulation for example geographical data. Powerful computational tools are now readily available that allow the visual representation of this data. A good visualization conveys key information to those who may have trouble interpreting numbers and/or statistics. This makes data accessible to a wider audience and therefore could be useful when presenting data to patients.
S5.2

Computer generated interpretation for professionals and patients: generating patient specific meaningful interpretations that might generate (semi-) automatic additional tests, alerts and advise for professionals and patients using all relevant available information (including past test results, clinical information and demographics)

P Trienekens
Pacific Knowledge Systems, Breda, Netherlands

The modern clinical laboratory is no longer a result factory but a purveyor of information. New diagnostic areas generate growing amounts of data and increasing complexity. The focus of improving the quality of laboratory operations is now shifting from the analytic phase to the pre-analytic and post analytic aspects of laboratory testing.

Current decision support tools, within clinical laboratories are mostly integrated within the Laboratory Information System. They generally use individual laboratory results and do not integrate with existing clinical information (e.g. relevant clinical, pharmaceutical and demographic data). More added value is needed: context specific clinical decision support (CDS) that uses all the relevant information available to direct workflow and to create efficient, intimate and unambiguous patient directed interpretation of laboratory results. CDS improves the quality of pathology reporting or may automate the clinical workflow by demand management, error detection, reflex testing, autovalidation and alerts.

Experience with the CDS “RippleDown” of Pacific Knowledge Systems will be discussed.

The current design of most pathology reports is limited in their usefulness. They are primarily a record of test results for the laboratory and the treating physician. Due to this technical emphasis, they are often incomprehensible and sometimes even confusing to physicians and/or patients. Embedding pathology data in a report that is personalised according to the end-user’s attitude will improve outcome for the physician, the patient and the patient’s family. It will lead to improved comprehension of results, better understanding of implications, greater trust in the provider and greater post-consultation compliance (e.g. taking medication, lifestyle changes).

S6.4

Education and training in laboratory medicine in the United States

G Herbek
College of American Pathologists, Northfield, United States

The Accreditation Council on Graduate Medical Education (ACGME) accredits residency programs in the US. The ACGME developed competency-based Milestones as a framework for determining resident performance resides within the six ACGME Core Competencies. The ACGME, along with American Board of Medical Specialties (ABMS), convened working groups for each specialty to develop these Milestones intended to help all training programs produce highly competent physicians. Pathology Milestones go into effect in June 2014. Performance on the Milestones will become a source of specialty-specific data for the specialty Review Committees to use in assessing the quality of training programs, facilitating improvements to program curricula and resident performance, and building accountability for the effectiveness of graduate medical education.

Physicians boarded after 2006 by the American Board of Pathology (ABP) are required to participate in the American Board of Medical Specialties Maintenance of Certification (MOC) process. This process includes demonstration of licensure, continuous learning and self-assessment, cognitive expertise, and a practical performance assessment. Physicians boarded by the ABP prior to 2006 may voluntarily participate in MOC. The CAP offers numerous Self-Assessment Modules which help fulfill MOC requirements. As an internationally recognized leader in laboratory and quality improvement, the CAP offers pathologists and laboratory professionals around the globe convenient, online learning opportunities. Online learning options include journal based and case based activities, as well as bundled learning series options that provide a focused program of study within a topic area. Most of the CAP’s online courses carry AMA PRA Category 1 credits™, and some of our courses carry credit from the Royal College of Physicians and Surgeons of Canada.

For more information about the College of American Pathologists, visit cap.org.

S7.2

Communication with clinicians

S Sandberg1,2
1NOKLUS, Bergen, Norway, 2Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway

What expectations do clinicians have to the laboratory? It is probably very simple. It is about timeliness and, of course, the clinicians definitely will expect the laboratory result to be “true” - and if they have their own instruments: what they read in the display, they trust.
The result must be present in the right time so that actions regarding the patient can be implemented soon. For emergency patients in a hospital this means that the result e.g. must be present during a surgical intervention e.g. PTH measurements. It is only rational to implement a POCT after timeliness, quality and costs compared to centralised laboratory testing has been evaluated. This should be discussed with the clinicians.

In the office laboratory, the expectations from the physicians are that the result should, in general, be available during consultation. If it not, clinicians can usually wait 1-2 days for the result from the central laboratory. Reimbursement is a powerful tool to decide which analytes should be analysed by at the GP office. The laboratory profession should therefore discuss with clinicians and the insurance companies/government what analyses should be reimbursed.

Clinicians will usually think that Specialists in laboratory medicine only have knowledge about “technical things”. It is therefore important to show that laboratory specialist have a knowledgebase that is important for the clinicians when they diagnose and monitor patients. Clinicians usually will not understand or be interested in theoretical questions about “what analytical quality is necessary”. To be able to talk about this, we have to introduce the theme by asking questions about e.g. what changes in the concentration of a constituent (critical difference - CD) they will react on. This is a good starting point for fruitful discussions about uncertainty in results and how to use and interpret laboratory results.

S7.3

Quality Assurance of Result Interpretation

W. Marshall
London, United Kingdom

Laboratory specialists operate extensive procedures to ensure that the results that they generate are of sufficient quality to support clinical decision making. These include measures to ensure secure identification of material through its journey from the patient to the production of a report; real time internal quality control schemes that provide information about precision, and external quality assurance schemes that provide a retrospective indication of accuracy and bias. Such schemes have a long history. More recently, attention has been focused on assessing the quality of interpretative comments that may be added to reports by laboratory personnel during result authorisation.

Although the UKNEQAS Interpretative Comments Scheme was developed primarily as an educational tool, it could provide the basis for a more rigorous assessment of the quality of such comments. The scheme will be described and its strengths and weaknesses for this purpose discussed. The final step in the quality assurance pathway involves the decisions made on the basis of laboratory results. This is a difficult area to assess formally but provides an excellent opportunity for laboratory and clinical specialists to work together to maximise the benefit of laboratory investigations for the patient.

S8.1

Monitoring immunosuppression

T. van Gelder
Erasmus MC, Rotterdam, Netherlands

Over the past 30 years important changes in the regimens used for immunosuppressive drug treatment following organ transplantation have taken place. In the use of immunosuppressive drugs the challenge is to find a balance between sufficient immunosuppression to prevent rejection of a transplanted organ, on the one hand, while at the same time leaving enough immune reactivity to prevent (serious) infectious complications or induction of malignancies. For a number of immunosuppressive drugs therapeutic drug monitoring (TDM) is routinely applied. Drug concentrations are measured in whole blood or plasma, and drug dose is adjusted to aim for certain predefined target concentrations. Immuno-assays, HPLC and mass spectrometry all are being used for this purpose. For this pharmacokinetic TDM strategy there is remarkably little evidence that outcome is truly improved. Target concentrations have been empirically derived from observational studies. For the most frequently used drugs an overview of current treatment monitoring is presented.

From a theoretical standpoint pharmacodynamic monitoring would make more sense, as the biological effect of a drug may correlate better with clinical outcome than a drug concentration. Several assays for pharmacodynamic monitoring have been developed, but none of them has gained much popularity.
S9.1

Vitamin D assays; what should be used?

A Heijboer
VU University Medical Center, Clinical Chemistry, Amsterdam, Netherlands

Vitamin D is a difficult analyte to measure and measurements should therefore be interpreted with care. Several techniques and methods to measure vitamin D are available, each with their own advantages and drawbacks. The Vitamin D Standardization Program aims to standardize the different techniques to measure Vitamin D. However, different samples can behave differently in the various assays, which is a challenge for accurate measurement and also for standardization. Chromatography based techniques separate Vitamin D2 from D3, where immunoassays measure both, although not always in an equimolar fashion. Epi-vitamin D can be measured separately or together with vitamin D using LC-MS/MS, but is not measured in most immunoassays. As nowadays total vitamin D is routinely measured, vitamin D binding protein (DBP) concentration is of importance in the measurement of vitamin D. Not only can DBP influence results of immunoassays and lead to falsely high or low results in individuals with divergent DBP concentrations, the DBP concentration also makes the interpretation of the vitamin D concentration difficult. The latter problem being independent of the method used but due to the principle of a ‘total vitamin D’ measurement. A high vitamin D concentration might be paralleled by a high DBP concentration, and the individual might actually have a low free or bioavailable vitamin D concentration. Calculation of free or bioavailable vitamin D by measuring DBP in addition to vitamin D could possibly help in the interpretation. However, to make it even more complicated: variants of DBP exist with different affinities for vitamin D. Measuring free or bioavailable vitamin D is a challenge as well, due to very low concentrations. These are only some of the difficulties which should be taken into account while measuring vitamin D and interpreting patient results or drawing conclusions from scientific studies.

S10.1

Impact of innovative practice in Europe - Swedish experiences

P Simonsson
Siemens Healthcare Diagnostics, Upplands Väsby, Sweden

A number of trends are now reshaping laboratory medicine in Sweden. As in other countries organisations are changing and large centers of laboratory medicine are created, spanning several different specialities and often covering a whole region of Sweden. This is based on technological developments, a need for cost reduction, and the development of cross-disciplinary diagnostic areas. At the same time, the advancement of knowledge and technology has resulted in a sub-specialisation and a centralisation of testing. In addition, the traditional connection between the clinical and the scientific laboratories and communities has weakened. The need for strengthened interfacing with clinical doctors is increased as the organisations grow in size and complexity. These trends are changing the work of the medical doctor in the laboratory to being a team member in regional networks of different professionals.

S10.2

Innovative practice in Europe: a French experience

GC Faure
Université Lorraine, Immunologie, Vandoeuvre les Nancy, France

Innovative Medical Biology, during the past decades, has been a major activity and mission of University Hospitals in France, as part of teaching duties, in initial and postgraduate training, and of clinical research activities in order to improve patients’ management. Based on translational research from bench to bedside, it allowed the development and validation of novel biological assays, for instance in autoimmunity and cellular immunology, with applications in daily medical practice for many clinical specialties from rheumatology to haematology and more recently neurology. Implementation of straight regulations in so-called clinical research and of a stringent application of quality policies is impairing and discouraging creativity. This would be especially detrimental in a field chronically facing lack of funding for diagnostic biology, since the increasing costs of new therapies take a heavy toll on hospital finances. In the mean time, innovative laboratory developed tests (LDTs) are badly needed for personalized medicine and rare diseases. That research which remains feasible in this field is also mandatory for the training of young MDs. In the rapidly evolving field of biological medicine. It is to fear that in a not so long future, automation and standardisation remove any opportunity to develop innovation where the patients are, and paradoxically in university settings.
S10.3

Impact of innovative practices in Europe - the Czech experience

T Zima
Charles University, 1st Faculty of Medicine, Inst. Medical Biochemistry and Laboratory Diagnostics, Prague, Czech Republic

Innovative practice influences large scale of different activities including the laboratories. We can introduce three innovative companies/organizations of the Czech Republic. CleverTech is a modern company specialized in innovative research and development. It Charles University spin off company. The mobile communication unit Senior Inspect-Basic allows crisis communication, fall and immobility detection, location tracking and other additional functions. The condition of monitored persons is under observation for 24 hours a day, to allow seniors an independent life in their own home and the freedom outside of the house. In case of more serious health problems, additional (wireless) medical sensors may be connected to the system to measure an extended set of physiological parameters.

Dynex specialization is the sale and production of laboratory devices and diagnostics, as well as the service and consultation. Dynex also realized many projects in innovation and education and cooperated with many academic, research organizations. Among outputs of these projects were new devices like Dynablot or Dynamic 3000 for laboratories.

Medical Data Centre (MDC) connected to the Charles University is focused on the economy of clinical procedure, development of benchmarking tools, helping hospital managers to identify signals of inefficiencies by indicators of clinical and economic parameters between provider - Clinical-economic profiles (KEP). The system analyses the most important cost items of a hospital care (incl. e.g LAB services), adjusts for patient's severity and compares with available indicators. The system was adopted by the largest health insurance company and by providers and regional governments.

The innovative practice and cooperation between academy and industry might be perceived as a comparative advantage nowadays, but it will soon become an essential prerequisite for a successful future in both worlds.

Studies were supported by project RVO-VFN 64165 and PRVOUK P25/LF1/2.

S11.3

Immunotherapy of neurological diseases

H-P Hartung
Heinrich-Heine-University, Department of Neurology, Düsseldorf, Germany

Many disabling acute and chronic diseases of the nervous system are thought to be autoimmune in nature or immune mediated. They range from Multiple Sclerosis, Neuromyelitis optica, antibody mediated encephalitides, Guillain-Barré-Syndrome, CIDP, monoclonal gammopathy associated neuropathies, paraneoplastic neuropathies to myasthenic syndrome and immune myopathies (IBM, poly-and dermatomyositis). A number of conditions, all antibody mediated, have been shown to respond to IVIG therapy or plasma exchange. Most controlled trials on immunotherapies have been conducted in Multiple Sclerosis where significant progress has been made and multiple immunomodulatory agents have been approved. These include interferon β, glatiramer acetate, natalizumab, an VLA4 adhesion molecule blocking monoclonal, the first-in-class sphingosine-1 -phosphate receptor modulator fingolimod, the CD52 directed T cell depeleting monoclonal alemtuzumab, the DHODH inhibiting blocker of T cell proliferation teriflunomide, and dimethylfumarate. A CD20 T-cell depleting monoclonal antibody, ocrelizumab, is in late stage development. Controlled trial evidence is also available for clinical efficacy of IVIG in Guillain-Barré-Syndrome and CIDP. In this presentation, an overview of the immunotherapies targeting different checkpoints in the pathogenesis of the above disorders will be given and the risks balanced against the benefits.

S12.1

Genetic screening in the 21st century

MC Cornel
VU University Medical Center, Clinical Genetics, Amsterdam, Netherlands

Population screening programs aim to identify disease in the presymptomatic phase, so that early treatment helps to avoid irreparable health damage. In reproductive choices, screening may furthermore inform couples about increased risks that might be avoided (embryoselection, prenatal diagnosis & selective abortion, using gamete donors, avoid pregnancy, etc). High throughput technology has improved the possibilities for genetic screening in the last decade, and next generation sequencing promises to create even more opportunities. There is a need to balance pros and cons in multidisciplinary development of recommendations and policy. Many countries are already improving their screening programs using new technologies, and commercial parties have started offering preconceptional screening as well as genetic
susceptibility testing. In all phases of life will genomic technologies have impact. In neonatal screening programs, many European countries have scaled up their programs from a few disorders up to 30 and more opportunities will come. Next generation sequencing has entered the field of prenatal screening for Rhesus factor, and some argue that neonatal screening will sooner or later be based on next generation sequencing. Both current neonatal screening and RhD screening are relatively straightforward in ethical terms: health promotion is sought, while Non Invasive Prenatal Testing for Down syndrome raises controversies. Even more than in the past, all separate steps in the screening process must be considered for an optimal result. Laboratory workers and clinicians need to attune the possibilities to be able to respond to the needs of citizens and patients and to integrate ethical and legal frameworks. Responsible innovation demands collaboration between all stakeholders involved and mutual learning. Life expectancy increases in many countries, leading to an aging population. Genetic screening can contribute to a longer and healthier life, but at the same time it is a real challenge.

S12.2

Handheld lab-on-a-chip

A Rios
University of Castilla - La Mancha, Analytical Chemistry, Ciudad Real, Spain

Miniaturization is rapidly growing, with novel ideas emerging in recent years. Micro total analysis (µTAS), also called ‘lab-on-a-chip (LOC)’ technology, promises solutions for high throughput and highly specific analysis for chemistry, biology and medicine, while consuming only tiny amounts of samples, reactants and space. Manz et al. proposed the µTAS concept, opening a new era in the field of the miniaturization of physic-chemical processes. The aim of this approach was to fabricate integrated systems ideally able to perform a number of functions in an automated way, in a miniaturized system.

Undoubtedly, the development of LOC applications is clearly multidisciplinary, with research and engineering opportunities straddling across chemical engineering, biology, physics, materials, processing science, among other disciplines. Thus, in biomedical field, the first step in proper prevention and treatment of disease is accurate diagnosis, but diagnostic technologies that are successful in the economically developed world often are difficult to use in developing countries. By developing effective technologies, such as those based on LOC, in healthcare in areas without access to trained medical personnel may be possible. According to the World Health Organization, diagnostic devices for developing countries should be assured: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users. This philosophy clearly connects with LOC portable devices.

This presentation deals with the possibilities offered by LOC systems for carrying out analyses and processes of interest in biomedical field. Integrated procedures for manufacturing, fluid handling and signal detection in microfluidics into a single, easy-to-use point-of-care (POC) assays will be described. It will be discussed the main features of these miniaturized systems, as well as a critical evaluation of the potential advantages, the disadvantages and the present challenges of such approaches.

S12.3

Smartphone-based personal health diagnostics

S Sia
Columbia University, New York, United States

Lab-on-a-chip (LOC) devices have a tremendous potential for revolutionizing personal health. In the U.S., patients and consumers can have greater access to traditionally complex diagnostics, and in developing countries, mobile diagnostics provides immediate diagnosis in the field. We will discuss our lab’s current efforts in these areas, in conjunction with partners in industry, public health, and local governments. Our tests span a variety of technologies, and target HIV, sexually transmitted diseases, and chronic diseases.

S13.1

Clinical application of biomarkers of ethanol misuse

A Helander
Karolinska Institutet, Laboratory Medicine, Stockholm, Sweden

Because early signs of excessive ethanol (alcohol) use are often modest, and many people deny or underreport their drinking practices, underdiagnosis of alcohol-related problems is common. For this reason, a number of biochemical markers have been developed furnishing objective ways for detection and follow-up of acute and chronic drinking.
Biomarkers suitable for routine clinical use should involve a readily available sample and be sensitive and specific for the intended purpose, as reflected in low risk for false-negative and false-positive results. They should also involve a standard laboratory technique that is available in hospital laboratories.

Hand-held breath alcohol analyzers are convenient for on-site estimation of the blood-ethanol concentration but their limitations, such as calibration control and lack of selectivity, needs to be appreciated. A positive breath test should preferably be confirmed by laboratory analysis of ethanol in a blood sample, at least when used for legal purposes.

When ethanol is no longer present in the body, measurement of the conjugated ethanol metabolites ethyl glucuronide (EtG) and ethyl sulphate (EtS) is a useful way. EtG and EtS are detectable in urine for many hours up to several days after drinking, the time-window largely being dose-dependent. A positive urinary EtG and EtS test thus provides a strong indication that the person was recently drinking alcohol, even when ethanol is no longer detectable.

For detection of prolonged heavy and possibly risky drinking, measurement of carbohydrate-deficient transferrin (CDT) in serum and phosphatidylethanol (PEth) in whole blood are useful tests. The main asset of CDT and PEth compared with conventional “liver enzymes” (e.g. GGT) is the higher specificity for alcohol-related effects implying low risk of false-positive identifications.

Applying this panel of alcohol biomarkers, alone or in combination, has proven useful for detection and follow-up of acute and chronic alcohol consumption in many clinical and medical-legal situations.

S13.2

Which biomarkers matter in oncology?

U-H Stenman
Helsinki University, Clinical Chemistry, Helsinki, Finland

A considerable number of biomarkers are regularly used for diagnosis, monitoring and screening of various cancers. Biomarkers can be classified as diagnostic, prognostic and predictive. In order to be clinically useful, a marker should provide information that is additional to that obtained by clinical examination and imaging techniques. Sensitive diagnostic markers facilitate early diagnosis while prognostic markers can be used to identify patients with aggressive tumors that need additional therapy. Predictive markers can at an early stage help to identify tumors that respond to a certain therapy. Various expert groups have issued consensus statements regarding the use of biomarkers for various clinical purposes. Markers play an important role in the follow up of some cancers, especially in testicular cancer and trophoblastic tumors. Treatment decisions for patients with these tumors are often based on biomarker results alone. Quite useful markers are available for many other tumors, e.g., colorectal, ovarian, thyroid and hepatocellular cancers. Few biomarkers are useful for screening but assay of prostate specific antigen (PSA) is widely used for opportunistic screening although this practice has been criticized. PSA facilitates early detection on prostate cancer but also leads to extensive overdiagnosis and overtreatment. PSA is very reliable for monitoring of prostate cancer after and during therapy, but it has not yet been shown that this improves patient outcome. Many other markers are widely used for monitoring of the response to therapy but very few have actually been shown to improve outcome. Predictive markers have become important thanks to the increasing possibilities for personalized treatment. So far, these markers are virtually always determined on tumor tissue. This may change when better methods to isolate tumor cells from circulation become available.

S13.3

The latest in cardiac biomarkers

P Collinson
St George’s Hospital and Medical School, Clinical Blood Sciences, London, United Kingdom

The measurement of cardiac remains the mainstream test for the investigation of patients presenting with chest pain. The major development here is the shift from the contemporary generation, which only partially define the normal reference interval, to high sensitivity assays which can detect cardiac troponin in over 50% of the normal healthy population. The use of cut-offs for troponin closer to or at the 99th percentile has been shown to identify high-risk individuals who respond to conventional anti-ischaemic strategies. High sensitivity assays allow earlier rule in and rule out protocols. In addition, the measurement of troponin within the reference interval allows identification of high risk individuals in the normal population and may be a useful strategy for risk stratification for primary prevention. There remains considerable interest in the use of a combination of a high sensitivity troponin with a supplementary biomarker of circulatory stress to permit rule out of low risk individuals on the first presentation.
The measurement of B type natriuretic peptide (BNP) is now established as a test for rule out of heart failure in both primary and secondary care. Clinical and cost effectiveness evidence has led to positive recommendations from the National Institute for Health and Clinical Excellence for widespread adoption and inclusion into quality indicators. The role of BNP in treatment monitoring remains an area of study with the balance of probability suggesting targeted measurement is of value.

Finally, point of care testing strategies for assessment of chest pain patients have demonstrated that when incorporated in an appropriate process flow, significant impact on length of hospital stay can be achieved. The technology to deliver high sensitivity troponin measurements by point of care testing is currently in development and will be expected to impact on clinical care within the next 12 to 24 months.

**S15.1**

**Emerging resistances to antibiotics - 2014**

Pl Nordmann  
Univ Fribourg, Medicine/Microbiology, Fribourg, Switzerland

Emerging antibiotic resistance mechanisms are emerging now worldwide at a high rate both in community and nosocomial infections. Although being observed in Gram-positive and Gram-negative bacteria, multidrug resistance observed in Gram negative in the most important source of concern in 2014. Multidrug resistance and pandrug resistant strains are increasingly identified in species such as Klebsiella pneumoniae; Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii. The most important resistant traits are the extended-spectrum β-lactamases and the carbapenemases that hydrolyse at least the expanded-spectrum cephalosporins and the carbapenems, respectively. Those resistance traits are commonly associated to resistance markers to fluoroquinolones and aminoglycosides which are the two other main antibiotic classes of broad spectrum activity. Those emerging resistance traits are mostly the results of overuse and misuse of antibiotics in developing countries in human medicine and transfer of multidrug resistant bacteria by humans themselves (travel, immigration.). Veterinary medicine play a minor role for selection of those multidrug resistant bacteria identified in human infections. The perspective of impossible-to-treat infections is raising due to lack of perspective of the development of effective novel antibiotics. This perspective may cancel the developments of the modern medicine since 1950’s such as important surgery, transplantation or intensive care.

**PC2.1**

**The role of HbA1c in the diagnosis of diabetes mellitus. Pro**

JP Berg1,2  
1Oslo University Hospital, Dept of Medical Biochemistry, Ullevål, Oslo, Norway, 2Institute of Clinical Medicine, University of Oslo, Oslo, Norway

Diabetes mellitus is characterized by chronic hyperglycemia which is associated with microvascular and macrovascular complications. The diagnostic cut points for diabetes were derived from the risk of developing retinopathy using a range of glycemic measures such as fasting plasma glucose (FPG), 2-h plasma glucose after oral glucose load (2-h PG) and HbA1c. Early studies defined an HbA1c threshold above which the likelihood of having microvascular disease rises sharply, but the test was not recommended for diagnosis of diabetes due to lack of standardization of the assay. The HbA1c analysis is now standardized by reference measurement procedures and traceable to a reference material. WHO included HbA1c ≥ 6.5 % (48 mmol/mol) as a diagnostic test for diabetes in 2011. HbA1c has several advantages compared with FPG and 2-h PG in the diagnosis of diabetes. It is associated with long-term complications and used to guide diabetes therapy to prevent the same complications. HbA1c reflects long-term (2-3 months) glycemic exposure, has minimal biologic variation with no need for fasting or timed samples and is relatively unaffected by acute stress or other illness related fluctuations in glucose metabolism. The preanalytical handling of the HbA1c sample is easy, and there is no need for ice-water, cold centrifuge or special tubes that inhibit glycolysis. The analysis is standardized and traceable to a common reference material. Even some HbA1c POC instruments deliver diagnostic quality so that the diagnosis to be made in outpatient clinic laboratories. The introduction of HbA1c as a diagnostic criterion should make it easier to detect diabetes at an early stage, provided knowledge of its limitations. Early identification of patients with diabetes is fundamental for successful prevention of the debilitating late complications, which, after all, is the main reason for treating type 2 diabetes.
**Oral Abstracts**

**O1**

**Soluble transferrin receptors versus traditional iron studies as markers of iron status in the post-operative inflammatory response**

*AU Kelly¹, K Tober², A Duncan¹, P Galloway¹, D Talwar¹*

¹Glasgow Royal Infirmary, Department of Biochemistry, Glasgow, United Kingdom, ²Glasgow Royal Infirmary, Department of Anaesthetics, Glasgow, United Kingdom

**Introduction:** Non-invasive assessment of iron stores is desirable in patients with chronic inflammatory conditions to diagnose subclinical iron deficiency. Iron is also implicated in mediating oxidative stress, and a suitable marker of stores would aid research in this area.

However, traditional biochemical markers of iron status (ferritin, iron, transferrin and transferrin saturation) are affected by the acute phase response.

There is evidence that soluble transferrin receptors (sTfR- the truncated form of membrane-bound transferrin receptors) are not affected by inflammation, and may be a more robust marker of intracellular iron content. This hypothesis does not appear to have been tested in severe inflammation, eg major surgery.

**Aim:** To assess whether sTfR values change in response to post-operative inflammation, and compare utility of sTfR to traditional markers of iron status.

**Methods:** Serum samples were collected pre-operatively and on day 6 post-operatively from 17 patients undergoing elective oesophagectomy at Glasgow Royal Infirmary, and analysed for sTfR, iron studies, CRP, albumin and haemoglobin. A reference range for soluble transferrin receptors was determined on samples from healthy volunteers.

**Results:**

- There was a CRP rise (median 1.2mg/L pre-op, 147.5mg/L day 6) and albumin fall (median 39g/L pre-op, 19g/L day 6) in response to inflammation.
- Significant changes in iron parameters were noted, with rise in ferritin (median 179ng/ml pre-op, 568ng/ml day 6) and fall in calculated transferrin saturation (median 26.2% pre-op, 10.5% day 6). Most day 6 values were outwith reference intervals.
- An sTfR fall was observed (median 18.73nmol/L pre-op, 15.19nmol/L day 6), which was not statistically significant (Friedman test p value 0.09). No post-operative results exceeded the upper reference interval.

**Conclusion:** We demonstrated no clinically significant changes in sTfR values during post-operative inflammation, compared to traditional markers of iron status. sTfR may be a more accurate marker of iron stores in the presence of inflammation.

**O2**

**From monovalency to polyvalency: the Slovak experience**

*G Kovac, A Porubenova*

Slovak Medical School, Institute of Laboratory Medicine, Bratislava, Slovakia

**Aim:** Change description from monovalent to polyvalent environment in laboratory diagnostics in Slovakia during 1994-2014.

**Method:** Listing and commenting outcomes reached in the process of change.

**Results:** In 1994 Slovak Society for Laboratory Medicine (SSLM) started studying financing systems and organisational structure of laboratory diagnostic in EU and USA. In 1997 costing based system for financing laboratory diagnostics was implemented. Since 1997 SSLM organizes annual conferences with invited speakers from EU. In 1999 the process of accreditation was introduced. In 2000 SSLM and Slovak Medical School (SMS) established new polyvalent discipline: laboratory medicine based on UEMS Blue Book (BB), EC4 syllabus (EC4S) and McClatchey’s Clinical Laboratory Medicine textbook. Since 2004 Institute of Laboratory Medicine (ILM) at the Slovak Medical School is accountable for postgraduate education in polyvalent laboratory medicine. In 2005 SSLM in cooperation with University hospital established the first integrated and consolidated polyvalent department of laboratory medicine in Slovakia. Since 2008 SSLM in cooperation with SMS, Slovak Health Insurance Institution (SHII) and Alphamedical Ltd run case studies focused on quality indicators (2010), personal benchmarks (2012), quality systems (2013) and quality monitoring (2014).

**Conclusions:** SSLM in cooperation with SMS, SHII, Alphamedical Ltd, UEMS and EC4 changed the shape of laboratory diagnostics in Slovakia: system of financing, integration and consolidation, accreditation and laboratory medicine (as a polyvalent discipline with accredited
curricula harmonized with BB and EC4S) represent the main tools. 30 accredited clinical laboratories, more than 10 polyvalent departments and about 40 polyvalent specialists in laboratory medicine with postgradual state exam document that change.

Acknowledgment: To Huib Storm, Gilbert Wieringa and all SSLM members and stakeholders for their support and cooperation.

03

Patient percentile monitoring - a valuable quality indicator for the examination phase

K Van Uytvanque¹, D Stöckl², LM Thienpont¹
¹Laboratory for Analytical Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Gent, Belgium, ²STT-Consulting, Horebeke, Belgium

Quality indicators (QI) are tools to evaluate the quality and effectiveness of laboratory testing. QI’s are necessary for monitoring the performance through the different examination phases. The latter consists of the testing of patient samples, hence, is an important part of the medical diagnosis, treatment and patient monitoring process. From this perspective, we propose the ‘patient percentile monitoring’ project as QI for the examination phase.

The project consists of the daily monitoring of medians of twenty commonly measured analytes in outpatients. All type and sizes of laboratories can participate. They are expected to calculate and send their medians to us in an automated way. We collect all information in our database, but after exclusion of weekends. We then monitor the data by plotting of the moving median, but also the laboratories themselves can do it with help of a user-interface. We proposed preliminary limits for the assessment of the stability of performance. They are oriented on the biological variation, but, at the same time, respect the analytical reality. The laboratories are grouped by peer to allow instrument-specific comparison.

We have 85 participating laboratories with 153 different devices. We observe mid- to long term differences between different instruments, but also within-laboratory differences, sometimes accompanied by shifts or drifts. Another observation is that the moving median for certain analytes, e.g., C-reactive protein and gamma-GT, has higher variation in hospital laboratories. Focusing on outpatients seems promising for the assessment of laboratory bias.

The patient percentile monitoring tool gives laboratories a direct, real-time QI to monitor the examination phase in compliance with ISO 15189:2012 accreditation requirements. We believe it will allow laboratories to better evaluate the mid- to long term stability of performance. Observed stability issues may be an incentive for root cause analysis, so that finally the tool may contribute to improved performance.

05

Vitamin D status in chronic liver disease

C Marinova, L Mateva, D Svinarov
Medical University of Sofia, Sofia, Bulgaria

Background: Vitamin D (VD) status is crucial for improving health, and for prevention of disease. The main objective of this study was to determine the prevalence of VD deficiency and insufficiency in patients with chronic liver disease (CLD), and to assess its relationship to the severity of disease.

Methods: Study encompassed 36 healthy control subjects (HCs) and 288 patients with proven CLD who consented to participate: 117 with nonalcoholic liver disease (NALD), 32 with alcoholic liver disease (ALD), 32 with chronic HCV infection (CHCV), 36 with chronic HBV (CHBV) infection, and 71 with liver cirrhosis (LC). Determination of 25-hydroxyvitamin D (25OHD, sum of 25OHD3 and 25OHD2) was performed by a validated, DEQAS certified ID-LC-MS/MS method with accuracy and precision within 7.5% and linearity range 3.0-300.0 nmol/L.

Results: (mean ±SD): Total 25OHD for all patients was 34.4±22.0 nmol/L (range 3.6-113.9), and for HCs = 64.0±18.5 (range 20.6-105.6); 39% of patients (3% of HCs) had 25OHD below 25 nmol/L (deficiency); profound insufficiency (25-50 nmol/L) was found in 41% of patients (17% in HCs); another 17% were in the range 50-80nmol/L, assessed as mild insufficiency (58% of HCs), and only 3% of patients (22% for HCs) were in sufficiency, 25OHD>80 nmol/L. Seasonal difference in VD status was significant with nadir in march and twice higher zenith in august. Lowest 25OHD levels were registered in LC patients (17.6±13.6 nmol/L), with predominance of deficiency and severe insufficiency, without effect of season or cause for LC; VD status was lower in de-compensated vs compensated LC, p< 0.005. ALD patients had lower 25OHD levels compared to NALD, p< 0.005; VD status in CNCV and CHBV was comparable to NALD.

Conclusion: Most of our CLD patients were with vitamin D deficiency and insufficiency and there was an inverse relationship between 25OHD levels and severity of disease.
06

Relationships of vitamin D and pediatric inflammatory bowel disease with bone tissue

D Rodríguez-Cano1, V Moreno-Moral1, C Caballero-Villarroya1, M Aguilar-Quintero1, A Romero-Urrutia1, R Cañete-Estrada1, F Rodríguez-Cantalejo1
1IMIBIC/Reina Sofia Hospita/Cordoba University, Clinical Analysis Service, Cordoba, Spain, 2IMIBIC/Reina Sofia Hospita/Cordoba University, Pediatrics, Cordoba, Spain

Introduction: Described harmfulness to the activity of the inflammatory bowel disease (IBD) is for bone tissue, promoting osteoclast activity mediated by proinflammatory cytokines. In addition, active IBD may involve malabsorption of micronutrients, including calcium and vitamin D (essential in the mineral metabolism).

In IBD patients has been a prevalent reduction in bone mineralization, can result in osteopenia (40-50 %) and even osteoporosis (5-30 %), with its inherent risk of fracture. This is a significant extraintestinal comorbidity of IB, which has been linked to steroid therapy, the activity of IBD, its development time and lifestyle, among others.

Material and methods: Case-control study of 85 children aged 3 to 17 years (39 cases and 46 controls IBD). History, bone densitometry and determination were performed on blood count, glucose, urea, creatinine, calcium, phosphorus, magnesium, alkaline phosphatase (ALP), urate, PTH, osteocalcina, T4, TSH, cortisol, insulin, albumin, prealbumin, protein, lipid and iron metabolism, PCR, orosomucoid, beta2 crosslap, 25(OH)vitamin D and its soluble leptin receptor, IL-6, FGF23, osteoprotegerin, sclerostin and RANK-L.

An inter-group study comparing different parameters (Student’s t test or Mann Whitney) and intra-group study using a multiple linear regression.

Results: The bone-mineral biochemical profile is different in IBD population, showing lower concentrations of calcium and FA, but over RANKL. The 25(OH)vitamin D was negatively correlated with markers of IBD activity, suggesting its anti-inflammatory and immunomodulatory action. The deleterious effect on bone tissue involves increased activity of IBD and/or increased corticosteroid dose is confirmed.

Conclusion: IBD in children has distinctive features in metabolic bone area with implications for other aspects. For better control of IBD, instead of climbing the corticosteroid dose, the use of calcidiol advocated by its dual impact on bone: direct (ossifying action) and indirectly (by reducing the inflammatory status, resulting osteopenia).

08

Homocysteine metabolism and the associations of global DNA methylation with selected gene polymorphisms and nutritional factors in patients with dementia

M Bednarska-Makaruk1, A Graban2, A Sobczyńska-Malefora3, M Mitchell4, DJ Harrington4, K Voong3, L Dai4, W Lojkowska2, A Bochynska2, D Ryglewicz2, A Wiśniewska1, H Wehr1
1Institute of Psychiatry and Neurology, Department of Genetics, Warsaw, Poland, 2Institute of Psychiatry and Neurology, First Department of Neurology, Warsaw, Poland, 3Viapath, Nutristasis Unit, Haemostasis & Thrombosis, London, United Kingdom, 4Viapath, Molecular Genetics, Haemostasis & Thrombosis, London, United Kingdom

Epigenetics (i.e. DNA methylation) together with the environmental and genetic factors are the key to understanding the pathogenesis of many diseases including dementia. Disturbances in DNA methylation have been already implicated in dementia. Homocysteine metabolism, with folate and vitamin B12 as essential cofactors, is integral to methylation processes. We evaluated the association of global DNA methylation, homocysteine, folate and B12 status with dementia. Selected polymorphisms of genes previously associated with dementia development were determined by standard methods. Plasma and erythrocyte 5-methyltetrahydrofolate (markers of folate and B12 status) were measured by HPLC. APOE, PON1 p.Q192R, MTHFR c.677C>T and IL1B-511C>T polymorphisms were identified using PCR-RFLP.

Subjects with dementia had significantly higher homocysteine (p=0.012) and methylmalonic acid (p=0.016) and lower folate (p=0.002) and plasma 5-methyltetrahydrofolate (p=0.005) than controls. There was no difference in DNA methylation between patients and controls. A tendency to higher DNA methylation in patients with vascular dementia (p=0.061) was noted. Multivariate regression analysis of the whole group of investigated individuals demonstrated significant associations between DNA methylation and folate (p=0.003), erythrocyte 5-methyltetrahydrofolate (p=0.036), creatinine (p=0.003) and glucose (p=0.007) concentrations and IL1B-511C>T (p=0.002) and PON1 p.Q192R (p=0.044) genotypes. The association with MTHFR c.677C>T was significant only in controls (p=0.017).
The biochemical results showed significantly lower folate and B<sub>12</sub> status in demented patients than controls. Global DNA methylation was associated with markers of folate status, creatinine, glucose and PON1 and IL1B polymorphisms.

**O9**

**The measurement of placental biomarkers in the detection of compromised pregnancies**

*DB Nice<sup>1</sup>, K Hayden<sup>1</sup>, L Higgins<sup>2,3</sup>, E Johnstone<sup>2,3</sup>, AE Heazell<sup>2,3</sup>*

<sup>1</sup>Central Manchester University Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, Manchester, United Kingdom, <sup>2</sup>Maternal and Fetal Health Research Centre, Institute of Human Development, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, United Kingdom, <sup>3</sup>St. Mary’s Hospital, Central Manchester University Hospitals NHS Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom

Placental dysfunction is thought to be responsible for a significant portion of stillbirths that occur in the third trimester of pregnancy. Current measurements of fetal growth cannot be used to directly monitor the functionality of the placenta. Biochemical tests that can detect placental dysfunction offer a novel means to identify high-risk pregnancies.

This study aimed to measure and evaluate the use of placental products: human placental lactogen (hPL), placental growth factor (PIGF) and progesterone in maternal plasma, serum and urine using commercially available assays to determine the most appropriate biofluid. The analytes were compared to assess whether biomarker concentration could differentiate infants of normal and small for gestational age (SGA) birthweights.

**Methods:** Three cohorts of 25 participants presenting after 28 weeks gestation with reduced fetal movements or SGA pregnancies were recruited alongside healthy controls, and serum, EDTA, lithium heparin and urine samples collected. hPL was measured by DRG ELISA kit, and progesterone by Roche immunoassay. PIGF was measured using three methods: Alere Triage, R&D ELISA and Roche PIGF and sFlt-1.

**Results:** All analytes could be measured reliably in serum, EDTA and lithium heparin plasma. Precision, accuracy, linearity and stability were assessed. There was no relationship between hPL levels in urine and serum or plasma. Serum PIGF measured by R&D ELISA was the most effective in the differentiating SGA infants (AUROC 0.85; \( p = 0.002 \)) and if sampling occurred before 35 weeks. Serum hPL had an AUROC 0.72 for prediction of SGA (\( p = 0.004 \)). Progesterone was not effective in discriminating between normal and SGA pregnancies (AUROC 0.59; \( p = 0.25 \)).

**Conclusion:** hPL and progesterone could be reliably measured in serum, EDTA and lithium heparin. Pregnancies with a fetus that was SGA were more likely to have lower concentrations of hPL and PIGF compared to appropriately grown infants.

**O10**

**Cardiac troponins in skeletal muscle disorders: a retrospective study to assess their utility in excluding myocardial injury in Pompe disease**

*I Wootton<sup>1</sup>, T Hiwot<sup>2</sup>, C Dawson<sup>1,2</sup>*

<sup>1</sup>Queen Elizabeth Hospital Birmingham, Clinical Laboratory Services - Biochemistry, Birmingham, United Kingdom, <sup>2</sup>Queen Elizabeth Hospital Birmingham, Endocrinology / Inherited Metabolic Disorders, Birmingham, United Kingdom

High sensitivity cardiac troponins (hsTnT or hsTnI) are sensitive markers of myocardial injury. Rapid changes in serum hsTnT or hsTnI levels are indicative of an acute coronary syndrome, whilst modest stably elevated levels are common in patients with cardiomyopathy and other causes of myocardial injury. Although conventionally assumed to be highly specific for cardiac muscle, recently the specificity of hsTnT has been questioned in patients with disorders of skeletal muscle, including muscular dystrophies and inflammatory myopathies. The aim of this retrospective study was to test the utility of cardiac troponins in assessing for potential myocardial damage in adult patients with Pompe disease. Pompe disease is a lysosomal storage disorder caused by deficiency of the enzyme, acid glucosidase alpha, which results in accumulation of glycogen in skeletal muscle, and in the most severe forms, cardiac muscle. We measured serum hsTnT levels in thirteen adult patients with Pompe disease seen in a routine outpatient clinic appointment in a national centre for adult inherited metabolic disorders. hsTnT was greater than the 99th percentile (14 ng/L) in twelve, with results ranging from from 11 - 59 ng/L. In eleven of these, creatine kinase (CK) was also elevated (range 70-950 U/L, reference range 24-195 U/L). In five patients with elevated hsTnT, hsTnI was measured on the same sample, and found to be below the 99th percentile (10ng/L). Additionally, all of these five had normal NT-proBNP levels and transthoracic echocardiograms indicating normal myocardial function. There are no other published reports of the utility of cardiac troponin assays in assessing for myocardial damage in patients with Pompe disease. Our results show that elevated hsTnT levels in Pompe disease are likely to be of skeletal muscle origin and hsTnI not hsTnT should be used to exclude myocardial injury in this disorder.
A novel host-immune protein signature for diagnosing bacterial infections and guiding antibiotic treatment

K Oved1, O Boico1, A Cohen1, R Navon1, T Friedman1,2, I Etshtein1,3, O Kriger4, R Yacobov4, R Wolchinsky4, I Kulbatski5, G Denkberg8, Y Dotan3,9, Y Fonar3, R Dagan5, A Klein4, I Potasman3,10, P Feigin11, I Chistyakov3,5, R Dagan12, A Klein4, I Potasman3,10, E Eden1

1MeMed Diagnostics, Tirat Carmel, Israel, 2Rambam Medical Center, Haifa, Israel, 3Technion-Israel Institute of Technology, Rappaport Faculty of Medicine, Haifa, Israel, 4Hillel Yaffe Medical Center, Department of Pediatrics, Hadera, Israel, 5Bnai Zion Medical Center, Department of Pediatrics, Haifa, Israel, 6Technion-Israel Institute of Technology, Faculty of Biology, Haifa, Israel, 7University Health Network, Toronto, Canada, 8Applied Immune Technologies, Haifa, Israel, 9Bnai-Zion Medical Center, Department of Internal Medicine, Haifa, Israel, 10Bnai-Zion Medical Center, Infectious Diseases Unit, Haifa, Israel, 11Technion-Israel Institute of Technology, Department of Industrial Engineering and Management, Haifa, Israel, 12Soroka Medical Center, Pediatric Infectious Disease Unit and Clinical Microbiology Laboratory, Beer-Sheva, Israel

Objectives: Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Traditional host-proteins such as procalcitonin, C-reactive protein, and interleukin-6 can help determine infection etiology, but their performance is negatively affected by inter-patient variability. Our goal was to develop and validate a host-immune signature that measures both novel and traditional viral- and bacterial-induced proteins, and computationally combines them into a predictive score that distinguishes between bacterial and viral etiologies.

Methods: We prospectively recruited 1002 hospitalized and emergency department patients with acute infection, and controls with no apparent infection. Patients underwent comprehensive clinical and laboratory assessment, and the final diagnosis was determined by a panel of three independent experts. We quantitatively screened 600 circulating host-proteins and developed a multi-parametric signature using logistic-regression on half of the patients, and validated it on the remaining half.

Results: The cohort included 319 bacterial, 334 viral, 112 control and 98 indeterminate patients (139 were excluded). The best performing signature had an area under the curve (AUC) of 0.94 ± 0.02. It consisted of the following novel viral-induced and traditional bacterial-induced soluble proteins: TNF-related apoptosis-inducing ligand, Interferon gamma-induced protein-10, and C-reactive protein. The signature was superior to any of the individual proteins (P < 0.001), as well as routinely used clinical parameters and their combinations (P < 0.001). The signature was robust across different physiological systems (respiratory, urinary and systemic), times from symptom onset (0-12 days), and pathogens (56 species), with AUCs between 0.87 and 1.0.

Conclusions: The present host-signature based assay provides valuable information over routinely used clinical variables and is readily usable on blood samples drawn as part of routine care. It has the potential to improve the management of patients with acute infections and reduce antibiotic misuse.

EuroLabFocus2014

Wednesday Posters

Bone Disease & Calcium Metabolism

W1

Beware of microorganisms during vitamin D replacement after bariatric surgery

D Chandrajay1, D Narayanan1, SG Pollard1, JH Barth1

1Leeds General Infirmary, Chemical Pathology and Metabolic Medicine, Leeds, United Kingdom, 2Leeds General Infirmary, Hepatobiliary Surgery, Leeds, United Kingdom

Introduction: Vitamin and micronutrient deficiency is well recognised after Bariatric surgery. Underlying surgical complications after bariatric surgery can lead to impaired bioavailability of orally vitamin supplements.

Case history: 28 year old Asian lady underwent Roux en Y gastric by-pass in Dec 2002 for morbid obesity (BMI 41). Biochemical measurements prior to surgery were normal and vitamin D concentration was not measured. She was commenced on ferrous sulphate, multivitamins and calcium/vitamin D3 at 8 weeks postoperation. She moved from liquid to solid diet at 4-6 months. 12 months post-op, she developed watery diarrhoea with bloating and flatulence. Symptoms subsided initially with dietary changes; however diarrhoea recurred within 3 months. Colonoscopy and biopsy was normal. She became increasingly tired with muscle aches and pains. In Oct 2006, cyanocobalamin injections were commenced for B12 deficiency. Vitamin D was
< 10 nmol/L with hypocalcaemia (2.09 mmol/L) and secondary hyperparathyroidism (PTH 84.1 pmol/L). She was commenced on oral ergocalciferol 500ug per day but Vitamin D rose to just 16 nmol/L in 6 months. Subsequently she had a trial of 3 monthly IM Ergocalciferol injections for 4 years and later 300000 units of Colecalciferol as an oral bolus dose followed by 40000 units per week. Her Vitamin D concentrations fluctuated between 25-37 nmol/L. She continued to have diarrhoea and muscle aches and pains. A clinical diagnosis of blind loop syndrome with bacterial overgrowth was made and treated with cyclical antibiotics (3 weeks Tetracycline). The frequency of diarrhoea decreased to 12 per day. The Vitamin D concentration increased into sufficient range (61 nmol/L).

**Conclusion:** Bacterial overgrowth is a recognised side effect after bariatric surgery. It can present with fat malabsorption and diarrhoea leading to impairment of micronutrient and vitamin absorption. The usual regimen of vitamin D replacement may be insufficient and an individualised approach may be required.

### W2

**Hypercalcaemia referrals from primary care: a retrospective audit**

N Maghsoodi\(^1\), RP Vincent\(^2\)

\(^1\)King’s College Hospital, Clinical Biochemistry, London, United Kingdom, \(^2\)King’s College Hospital, London, United Kingdom

**Background:** Hypercalcaemia is a common clinical condition with Primary hyperparathyroidism (PHP) being the commonest cause. The UK annual incidence rate (AIR) of hypercalcaemia is 30 /100,000 and peak age incidence is 50-60 years and mainly in females. Our hospital serves a population with age ≥ 18 of around 500,000. Between July 2010 and November 2012, 81,575 bone profiles were requested by the primary care setting covered by KCH. A corrected calcium (cCa) ≥ 3.00 mmol/L is a critical phoning limit in our biochemistry laboratory.

**Aim:** To assess the incidence of hypercalcaemia in the community, the referral pattern of hypercalcaemia in the primary care and the laboratory practice on phoning out results.

**Methods:** This retrospective audit included bone profiles [cCa, phosphate & alkaline phosphatase] from primary care between July 2010 and November 2012. Those with cCa < 2.8 mmol/L, aged < 18 years and known to have hypercalcaemia previously were excluded.

**Results:** Overall 22 patients had cCa ≥ 2.8 mmol/L (19F) aged 59 (36-82) years. 68% (n = 15) were referred specifically for hypercalcaemia, 23% (n = 5) for other reasons and 9% (n = 2) were not referred. 32% had PHP, 18% were on Ca/vitamin D supplements, 14% had malignancies and 36% had other causes (including tertiary hyperparathyroidism) or no known cause. Vitamin D was requested in 50% and parathyroid hormone in 60% but none had a urine calcium request. All cCa ≥ 3.00 mmol/L were phoned by the laboratory. Our study led to referral and diagnosis of a patient with PHP who was not investigated since 2010.

**Conclusions:** The AIR of hypercalcaemia in our population is 6.8% per 100000. There is a wide variation in the referral practice for hypercalcaemia in the community. A guideline on hypercalcaemia management for the primary care may improve patient outcome.

### W3

**An audit of bone turnover marker requesting in the Ulster Hospital**

P Hamilton, E Duly, T Trinick

South Eastern Health and Social Care Trust, Belfast, United Kingdom

Analysis of markers of bone turnover may be helpful in the diagnosis and management of patients with metabolic bone disease. Results of samples received for analysis of bone turnover markers in the clinical biochemistry laboratory of the Ulster Hospital between April and December 2013 were reviewed. Total N-terminal propeptide of type I collagen (T1PNP) and collagen type 1 c-telopeptide (BCT) were analysed on a Roche Cobas platform. Bone alkaline phosphatase (BAP) was analysed on a DiaSorin Liaison platform. 34 samples were requested, 64% from patients with osteoporosis. Other indications for testing included osteopenia, proven or suspected Paget’s disease of bone, or investigation of an abnormal alkaline phosphatase level. 55% of requests from patients with osteoporosis were before the commencement of treatment. 23% of patients with osteoporosis were taking bisphosphonates, 14% teriparatide, 4% strontium and 4% denosumab. Patients taking any pharmacological therapy for osteoporosis had significantly lower TP1NP levels than those on no treatment (median 33.38 μg/l (quartiles 24.07, 42.03) vs 60.23 (50.03, 69.83), p < 0.01). A similar reduction was seen in BCT levels (0.29 ng/ml (0.15, 0.38) vs 0.53 (0.44, 0.57), p = 0.02), and with BAP (7.8 (7.48, 10.23) vs 16.5 (12.93, 20.15, p < 0.01). Significant differences in all three markers were noted when treatment with bisphosphonates was considered in isolation. However, no differences were noted when results were compared between patients taking teriparatide and those on bisphosphonates or no therapy. In conclusion, in our large district general hospital, requests for bone turnover markers were received approximately once per week. For patients with osteoporosis, treatment significantly lowered the three markers tested.
W4

Thames audit on the laboratory investigation of hypercalcaemia

E Ashley
Kingston Hospital NHS Foundation Trust, Clinical Biochemistry, Kingston-upon-Thames, United Kingdom

Clinical biochemistry departments provide varying levels of interpretative and clinical advice on the presence of hypercalcaemia in their respective hospitals. The aim of the questionnaire audit was to identify different practices across the region, provide local recommendations for the investigation of hypercalcaemia and to establish an appropriate algorithm to assist the laboratory.

The questionnaire was sent out to hospitals in London and the Thames region in October 2013 with a response rate of 62%. It primarily focused on the investigation of hypercalcaemia in the primary care setting, with particular reference to primary hyperparathyroidism (PHPT).

Demographic data collected on hypercalcaemia between March and September 2013 highlighted that it is a more frequent finding in inpatients (61%), it presents more commonly in females (58%) and in patients greater than 70 years of age (40%). Only 43% of laboratories append written clinical comments to reports including appropriate follow-up investigations such as confirming the calcium measurement and requesting a paired sample for PTH measurement. Of those that do not provide written advice, 39% of laboratories discuss results with the clinician and/or add on appropriate tests. For patients where PHPT is queried, 39% offer the urine calcium:creatinine clearance calculation to exclude familial hypocalciuric hypercalcaemia (FHH) from the differential diagnosis.

At the follow-up audit group meeting, recommendations were suggested and an algorithm has been agreed for patients who present with hypercalcaemia in the first instance. The aim of the laboratory in the investigation of patients with query PHPT is to provide calcium, PTH and vitamin D status before their first Endocrinology appointment to prevent unnecessary referrals. PHPT should be biochemically confirmed, FHH should be excluded by using the calcium:creatinine clearance measurement, and other potential causes of hypercalcaemia should be removed (eg. thiazide diuretics) or treated (eg. vitamin D deficiency) prior to radiological investigations.

W5

Long-term outcome of bisphosphonate therapy in patients with primary hyperparathyroidism

D Segula, S Nikolova, E Marks, LR Ranganath, V Mishra
Royal Liverpool & Broadgreen University Hospitals NHS Trust, Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

Aim: Primary hyperparathyroidism (PHPT) is commonly associated with reduced bone mineral density (BMD) presenting with osteoporosis, increasing the risk of bone fragility fractures in these patients. Bisphosphonates due to their anti-resorptive action are known to improve the BMD and reduce the risk of bone fragility fractures. Therefore bisphosphonates are considered as an alternative to surgical treatment in managing osteoporosis in PHPT patients. The aim of this observational study was to assess the effect of long-term bisphosphonate therapy on BMD, bone fragility fracture and biochemical markers of bone metabolism in patients with PHPT.

Methods: Fifty patients (mean age 74 years) with PHPT who were treated with long-term bisphosphonate therapy were studied retrospectively. The mean baseline (before commencing bisphosphonate therapy) BMD T-scores for lumbar spine (L2-L4) and left femoral neck were -2.5 and -2.1, respectively. Fourteen patients had bone fragility fractures before initiation of bisphosphonate therapy.

Results: After a mean 5 years of bisphosphonate treatment, there was a significant increase in lumbar BMD T-score (-2.5 to -2.1, p=0.013) and a non-significant change in left femoral neck BMD T-score (-2.1 to -2.2, p=0.497). There was no increase in bone fragility fracture rate (p=0.167). Serum corrected calcium reduced from 2.74mmol/L to 2.60mmol/L (p<0.001) and urine calcium to creatinine ratio from 0.70 to 0.55 (p<0.0001), both within the reference range.

Conclusions: Our study suggests that long-term bisphosphonate therapy improves lumbar BMD and prevent further increase in bone fragility fracture rate. Additionally it improves hypercalcaemia in PHPT.

W6

Local adjusted calcium equation - review following a change in the Roche calcium assay

SL Davies, C Hill, LM Bailey, AM Milan
Royal Liverpool & Broadgreen University Hospitals NHS Trust, Department of Clinical Biochemistry & Metabolic Medicine, Liverpool, United Kingdom

Introduction: Serum calcium concentration is adjusted for albumin to more closely reflect physiologically active free calcium which is not bound to proteins or anions. Locally-derived adjusted calcium (ACa) equations normalised to mean calcium of 2.4mmol/L are advocated by
Pathology Harmony for harmonised ACa reference intervals of 2.2-2.6 mmol/L. Review of the local ACa equation was prompted by a change in the Roche calcium assay (cresolphthalein complexone to NM-BAPTA).

**Methods:** Primary care data (n = 13,482) were examined between May and July 2013. Data were excluded if urea, creatinine, or alkaline phosphatase were above the upper limit of normal, total calcium > 3.00 mmol/L and albumin < 20 g/L or > 50 g/L. Least squares regression coefficients of total calcium on albumin were used to calculate the ACa equation according to literature protocols. The new and old ACa equations were compared to assess the impact of the calcium method change.

**Results:** Total calcium and albumin results were normally distributed (mean concentrations of 2.32 mmol/L and 43.2 g/L, respectively). Linear regression of total calcium on albumin (y = 0.0152x + 1.6671; R² = 0.2155) gave a new ACa equation of: ACa = Total Ca + 0.0152 (43.2-[Alb]). 95% population reference intervals for ACa were 2.15-2.49 mmol/L (new equation) and 2.16-2.51 mmol/L (old equation); old ACa equation = Total Ca + 0.0133 (44.1-[Alb]). Correlation was described by y = 0.9955x - 0.0016; R² = 0.9953. Mean absolute and percentage differences in ACa were -0.002 mmol/L and -0.7%, respectively.

**Conclusions:** Changes in the Roche calcium assay (cresolphthalein complexone to NM-BAPTA) minimally affected the local ACa equation. However, this work illustrates the importance of locally-derived ACa equations and the necessity for regularly revalidating these equations, particularly following method changes. This aligns with Pathology Harmony recommendations and ensures ACa results remain appropriate for local analytical methods and patient populations.

**W7**

**How accurate is your sclerostin measurement? Comparison between two commercially available sclerostin ELISA kits**

**L Piec, C Washbourne, JCY Tang, E Fisher, WD Fraser**

**University of East Anglia, Medicine/BioAnalytical Facility, Norwich, United Kingdom**

**Introduction:** Circulating sclerostin (SOST) levels have been measured in a plethora of disorders such as ankylosing spondylitis, chronic kidney disease, diabetes, fractures, hypercortisolism, multiple myeloma and spinal cord injury. SOST is a crucial regulator of the skeletal anabolic action of PTH and as so, anti-sclerostin antibodies are being investigated as potential therapeutic molecules for metabolic bone diseases. Accurate measurement of SOST is therefore of utmost importance for the diagnosis and therapy effectiveness of bone disorders. However, reports so far suggests further study is needed before SOST measurements are introduced into routine clinical practice.

**Objective:** To compare two commercially available assays for measurement of circulating SOST.

**Method:** EDTA-plasma samples from 36 anonymised healthy individuals were analyzed using ELISA kit from Biomedica and TecoMedical. Both assays are based on immuno-capture using two antibodies which have been raised against human recombinant SOST and are highly specific for this molecule.

**Results:** Circulating SOST levels were found to be significantly different between TECO and Biomedica assays with discrepancies of up to 32 pmol/L. The TECO assay demonstrated less variability between duplicates while the Biomedica kit over-recovered diluted samples by up to 60%. When samples containing various concentrations of endogenous sclerostin were spiked with a known amount of SOST, recovery was 88.5% and 104% respectively.

**Conclusion:** The variability in values generated from Biomedica and TECO assays has raised questions regarding the specificity of antibodies used by the two manufactures, and whether there is possible interference affecting one of the assays remains unclear. Until such issues are resolved, measurement of sclerostin remains invaluable for understanding the mechanism by which osteocytes regulate bone turnover but should be used in discretion and interpretation should be carried out with guided clinical evidence.

**W8**

**Assessment of C3-Epi-25-Hydroxyvitamin D concentration in adult serum: LC-MS/MS determination using [2H3] 3-epi-25OHD3 internal standard and NIST traceable commercial 3-epi-25OHD calibrators**

**JT Tang, C Washbourne, I Piec, W Fraser**

**University of East Anglia, Norwich Medical School, Norwich, United Kingdom**

**Background:** The C-3 Epimer of 25 Hydroxyvitamin D3 (3-Epi-25OHD3) is produced in the liver by the epimerisation pathway of 25-hydroxy vitamin D3. It differs from 25OHD3 in configuration of the hydroxyl group at the third carbon (C-3) position. Concerns have been raised that isobaric interference may result in over-estimation of total 25OHD when measured by liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Objective:** The aim of the study was to assess the occurrence of 3-Epi-25OHD, in adult serum samples. A LC-MS/MS technique was developed to resolve and quantify 3-Epi-25OHD, from 25OHD3. The newly available NIST (SRM972a) traceable 3-Epi-25OHD commercial standards were used to ensure assay accuracy.
Method: Serum was extracted by isotopic dilution protein precipitation using \( [2H_3] \) -3-epi-25OHD3 as internal standard. The extract was chromatographed using a PFP column. Mass detection and quantification were performed by ESI-LC-MS/MS in positive mode.

Results: The method was able to fully resolved 3-Epi-25OHD3 from 25OHD3. The intraassay CVs for the epimer were 6.3% and 4.1% at 25.4 and 62.1 nmol/L respectively; and interassay CVs were 8.3% and 6.5% at 27.6 and 63.2 nmol/L respectively. In our sample cohort with 25OHD3 ranged between 3.4 - 165 nmol/L, 3-Epi-25OHD3 was detected in 91.9% of samples (mean = 3.8 nmol/L). No detectable 3-Epi-25OHD2 was found in our sample study. A patient on high dose vitamin D supplement was found with 141 nmol/L of 25OHD3 and 44 nmol/L of 3-Epi-25OHD3.

Conclusion: Using \([2H_3]\) -3-epi-25OHD3 as internal standard and NIST aligned calibrators enabled us to obtain an accurate assessment of 3-epi-25OHD concentration in adult serum. Although the concentration of serum 3-epi-25OHD3 was found to be low the presence was observed in the majority of our samples. The findings in this study showed that 3-epi-25OHD3 contributed to the overestimation of 25OHD3, that could potentially resulted in misinterpretation of total vitamin D status.

W9

Development of an ELISA for the measurement of free 25OH vitamin D

N Heureux1, M Anciaux1, F Mathieu1, M Poncelet1, L Swinkels2, T Huijs2, S Boerrigter2, E Lindhout2, M Martens2, G Mayer2
1DIAsource Immunoassays, Louvain-la-Neuve, Belgium, 2Future Diagnostics, Wijchen, Netherlands

The objective of this project was to develop and characterize a simple immunoassay for the quantification of free 25-hydroxyvitamin D (25OH Vit D). Recent studies suggest that the concentration and genotype of Vitamin D binding protein (DBP) are important factors that determine the bioavailability of 25OH Vit D in blood. It has been suggested that measurement of free, non-protein bound 25OH Vit D in serum, may provide more relevant diagnostic information than total 25OH Vit D, for instance in chronic kidney disease, bladder cancer and pancreatic cancer, or in hemodialysis patients. A two-step enzyme-linked immunosorbent assay (ELISA) was developed for the quantification of free 25OH Vit D assay, using a highly specific anti-25OH Vit D monoclonal antibody. The assay was calibrated against a symmetric dialysis method. The calibrator range was 0-35 pg/ml. The LoB was 1.1 pg/ml; the LoD was 1.7 pg/ml. Total assay precision was 5.7% at 6.3 pg/ml, 3.8% at 15.9 pg/ml and 4.8% at 24.8 pg/ml. The assay was recently used to determine the variability in free 25OH Vit D levels in clinical populations (D. Bikle J. Steroid Biochem. Mol. Biol. 2013). An assay was developed that reproducibly determines the level of free 25OH Vit D in serum. The assay can be used as a valuable tool in studies to establish the clinical relevance of free 25OH Vit D.

W10

PPIs may be a common cause of hypomagnesaemia

M Waterson1, T Lane2, D Grimstead2, N Benjamin3
1South Devon Healthcare NHS Foundation Trust, Clinical Biochemistry, Torquay, United Kingdom, 2South Devon Healthcare NHS Foundation Trust, Torquay, United Kingdom, 3South Devon Healthcare NHS Foundation Trust, Medicine, Torquay, United Kingdom

Aim: The MHRA issued a warning in 2012 stating that “severe hypomagnesaemia has been reported infrequently in patients treated with Protein Pump Inhibitors (PPIs), although the exact incidence is unknown”. After several local cases suggested that this may be more common than previously reported, a review of the incidence of hypomagnesaemia and the association with PPIs at Torbay Hospital was undertaken.

Methods: A list of patients in A/E or in-patient wards with Mg < 0.5 mmol/L over a six month period was extracted from the Pathology IT system. The incidence of PPI use, in conjunction with diuretic use and concurrent hypocalcaemia, was determined using discharge summaries and drug charts.

Results: 7223 magnesium measurements were made in these patients, 99 had magnesium < 0.5 mmol/L. 65 (66%) patients were on a PPI; 20 (31%) were also on a diuretic. 12 had significant hypocalcaemia (< 1.8 mmol/L). In only 9 (14%) patients was the PPI stopped. There was no difference in the average age of patients with low magnesium on a PPI or not (67.8 v 67.5 years).

Discussion: PPIs cause magnesium malabsorption. Symptoms of hypomagnesaemia include seizures, arrhythmias, hypotension and tetany, but they may begin insidiously and be overlooked. Significant hypocalcaemia can occur Hypomagnesaemia is potentially fatal. Hypomagnesaemia is known to improve after replacement and discontinuation of the PPI. In our study there was a strong association between hypomagnesaemia and PPI use, but no difference in age between the PPI group and others, whereas the literature indicates a greater preponderance in elderly patients. There appeared to be only minor consideration of PPIs as a cause of hypomagnesaemia as only 9 patients had the PPI stopped.

To increase awareness the laboratory is now adding an automatic comment to all low magnesium results asking requestors to assess PPI use and consider alternative therapy.
Quantification of total 25-hydroxyvitamin D: a comparison between the Elecsys® vitamin D total assay and LC-MS/MS

CI Washbourne, D Green, I Piec, J Tang, WD Fraser
University of East Anglia, Norwich Medical School - BioAnalytical Facility, Norwich, United Kingdom

Background: Increasing demand for vitamin D testing has led to many semi- and fully-automated methodologies for quantification of serum 25-hydroxyvitamin D (25OHD) concentrations. The performance of an assay to measure 25OHD with precision and accuracy is essential in assessing vitamin D status of an individual. Liquid chromatography-mass spectrometry (LC-MS/MS) has been identified as the gold-standard method for determination of 25OHD due to its capacity to distinguish 25OHD3 and D2.

Objectives: To evaluate the Roche Diagnostics Elecsys Vitamin D total assay and compare it with a NIST-aligned (LC-MS/MS) method.

Method: 293 patient samples with serum 25OHD determined by LC-MS/MS were selected for comparison. Of those samples, 153 contained both 25OHD3 and D2, ranging between 2.6-128.7 and 5.7-166.9nmol/L, respectively, and 140 exhibited 25OHD3 ranging between 7.6-234.8nmol/L with undetectable 25OHD2. The samples were analysed on a COBAS 6000 using the Elecsys method according to the manufacturer instructions.

Results: Intra-assay imprecision of the Elecsys method was 3.9% (514±19nmol/L) and 2.4% (89.3±2.2nmol/L). Inter-assay imprecision was 7.5% (51.2±3.8nmol/L) and 5.1% (84.1±4.3nmol/L). An overall negative bias of 11.6% in total 25OHD concentrations was observed between the Elecsys and LC-MS/MS methods. The sample group consisting solely of 25OHD3 showed a negative bias of 2.2%; the sample group containing both 25OHD3 and D2 were found to have a significantly higher negative bias of 20.2%.

Conclusions: Our findings showed the Elecsys method is precise at the concentrations of 25OHD tested. However, when compared with a fully validated LC-MS/MS method, the Elecsys method was found to underestimate total 25OHD. This is exacerbated by the presence of 25OHD2 in the sample, suggesting there is a lower binding affinity for 25OHD2, in the Elecsys method resulting in lower recovery. Our findings were in accordance with current publications on comparison studies between other commercially available IM, CBP assays and LC-MS/MS.

Outcomes of one year follow up post zoledronate therapy for osteoporosis: automatic pause or continue treatment?

MV Patel, R Senanayake, S Hope, C Jess, K Rance, B Shine, MK Javaid
1Oxford University Hospitals, Department of Clinical Biochemistry, Oxford, United Kingdom, 2Oxford University Hospitals, Department of Rheumatology, Oxford, United Kingdom, 3Oxford University, Nuffield Department of Orthopaedics, Rheumatology and MSK Sciences, Oxford, United Kingdom

Introduction: Clinical trial evidence supports the use of 6 years of therapy with zoledronate but in practice many centres use three annual infusions. It is unclear whether to continue treatment one year after the final infusion or automatically pause. We wished to determine the suppression of bone formation, measured by P1NP, in patients with a diagnosis of osteoporosis, one year after their final infusion in the real world setting.

Methods: All patients who received their final zoledronate infusion (5 mg annually for 3 years) in 2012 were included for osteoporosis. Pre-treatment serum calcium, vitamin D and eGFR were measured and vitamin D was corrected if necessary. Serum P1NP was measured one year post-treatment. The mean ± SD P1NP level for this group, one-year post final infusion was 21.0 ± 9.2 μg/L indicating reduced bone formation.%BMD change compared to previous for the cohort was +6.0% (n=58) for spine and -0.4% (n=53) for hip. Stratification based on P1NP level showed that %BMD were significantly greater in patients with P1NP ≤25 μg/L, +7.4%, p=0.015, (spine); +0.9%, p=0.021 (hip) versus ≥25 ug/L group +2.4% (spine); 3.9% (hip), respectively.

Conclusion: Bone formation suppression occurred in the majority of patients one year after receiving the final zoledronate infusion suggesting that it would be plausible to pause treatment automatically for at least 12 months.

LC-MS/MS measurement of urine free collagen crosslinks pyridinoline and deoxypyridinoline: urine markers of bone resorption

J Tang, J Dutton, D Green, I Piec, E Fisher, C Washbourne, W Fraser
1University of East Anglia, Norwich Medical School, Norwich, United Kingdom, 2Royal Liverpool & Broadgreen University Hospitals NHS Trust, Department of Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

Background: The pyridinium cross-links Pyridinoline (PYD) and Deoxypyridinoline (DPD) are established markers of bone resorption. Breakdown of cross-links are a result of osteoclast resorption, normally excreted in the urine, and in larger quantities when bone resorption is
increased. In contrast, when bone resorption is inhibited by bisphosphonate, oestrogen or calcitonin therapy, the excretion of PYD and DPD is decreased. The main application for DPD and PYD is in assessing and monitoring response to osteoclast inhibitory treatment (mainly bisphosphonates) in osteoporosis. Normalisation of DPD and PYD is the ultimate goal when treating Paget’s disease of bone.

**Objective:** To develop a sample clean up protocol to extract PYD and DPD from urine and quantify by LC-MS/MS. The method was used to assay urine samples from healthy individuals and patients samples requested for bone turnover markers.

**Method:** PYD and DPD were extracted by solid phase extraction. Mass detection and quantification were performed by a triple quadrupole tandem mass spectrometer.

**Results:** The method was able to fully resolved PYD and DPD. The intra assay and inter assay CVs for PYD and DPD were <10%. PYD was linear up to 2000 nmol/L, DPD up to 1000 nmol/L. Typical standard curve fit r² ≥ 0.998. Spiked recovery was determined by adding a known quantity of PYD/DPD urine samples containing different levels of endogenous PYD/DPD, percentage recovery were ranged between 98.4-116.5%. The method was compared with two commercial immunoassays for urine total PYD and DPD, with correlation coefficients r² of 0.862 and 0.832 respectively.

**Conclusion:** The presented LC-MS/MS method have been fully validated and showed excellent assay characteristics that is suitable for routine clinical hospital laboratory. Ratio of PYD:DPD is also a valuable diagnostic tool for Ehlers-Danlos Syndrome (EDS), where a reverse ratio is specific and diagnostic for the kyphoscoliotic type of EDS.

**W14**

**Trends in 25-OH vitamin D testing - have the National Osteoporosis Society guidelines had any influence?**

F Tidy1, B Shine1, K Javaid2, A Morovat1

1Oxford University Hospitals NHS Trust, Department of Clinical Biochemistry, Oxford, United Kingdom, 2University of Oxford, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford, United Kingdom

**Background:** Requests for 25-OH vitamin D (25VitD) tests have been increasing nationally, but there is wide variability in 25VitD requesting in the UK. We looked at 25VitD request patterns, and assessed the impact of the National Osteoporosis Society (NOS) Guidelines on Vitamin D and Bone Health, published in April 2013.

**Method:** OUH laboratory data were used to assess trends in 25VitD requesting between 2007 and 2013. An audit was conducted to compare the proportion of 25VitD requests meeting NOS Guidelines in June 2012 and June 2013.

**Results:** 25VitD requests increased over ten-fold in six years, with the percentage of requests from Oxford GPs rising from 13% to 44% over this period. Requests received from GP surgeries ranged from 0 to 223 (median 14) requests/1000 patients. The proportion of samples with sufficient 25VitD increased from <25% to >50% over six years. 89 samples were audited from both June 2012 and 2013. These were selected in proportion to the number of requests from each location in the 2012-2013 period. The two groups were of a similar age (55.3 vs. 54.3 years, respectively), but there were more men in the June 2013 cohort (34.8% vs. 24.7%). The Guidelines had no discernible impact on requesting, with similar increases in requests when 25VitD test was both indicated (30% to 37%) and not indicated (18% to 24%).

**Conclusions:** The international trend for increasing 25VitD requests is also seen in Oxfordshire, and is primarily driven by the primary care. However, there is wide variation between surgeries’ 25VitD requesting. This and the lack of impact of the NOS Guidelines suggest uncertainty amongst GPs regarding the indications for 25VitD testing. A further evaluation of the trends in the light of better clinical information may be required before attempting to address the one-quarter of non-indicated request from GPs.

**W15**

**Determination of reference values for serum total 1,25-dihydroxyvitamin D₃ using an extensively validated 2D ID-UPLC-MS/MS method**

NF Dirks1, F Martens1, D Vanderschueren1, J Billen1, MT Ackermans1, E Endert3, MA Blankenstein1, AC Heijboer1

1VU University Medical Center, Clinical Chemistry, Amsterdam, Netherlands, 2KU Leuven, Laboratory for Experimental Medicine and Endocrinology and Diagnostic Medicine, Leuven, Belgium, 3Academic Medical Center, Clinical Chemistry, Amsterdam, Netherlands

1,25-dihydroxyvitamin D₃, the hormonally active metabolite of vitamin D₃, tightly controls calcium blood levels. An increase of 1,25-dihydroxyvitamin D₃ leads to an increase in calcium concentration with calcium originating from various resources, including bone tissue. To assess a patient’s vitamin D status the precursor metabolite 25-hydroxyvitamin D₃ is determined. However, measurement of 1,25-dihydroxyvitamin D₃ is required when disorders of 1α-hydroxylation, extrarenal 1α-hydroxylation, or vitamin D receptor defects are suspected. The aim of this study was to determine reference values for 1,25-dihydroxyvitamin D₃, using a 2D ID-UPLC-MS/MS method.
The 2D ID-UPLC-MS/MS method (Xevo TQ-S tandem quadrupole mass spectrometer and an Acquity UPLC system (Waters)), able to measure picomolar concentrations of 1,25-dihydroxyvitamin D3 in human serum, was extensively validated with regard to sensitivity, specificity and robustness. Intra-assay variation was < 5% and inter-assay variation was < 7.5% over the whole dynamic range. Limit of quantitation was 3.4 pmol/L. Our method correlated well with a published 1,25-dihydroxyvitamin D3 LC-MS/MS method (Vanderschueren et al, JCEM, 2013) (r=0.98) and with the average 1,25-dihydroxyvitamin D results of the vitamin D External Quality Assessment Scheme (DEQAS) determined with LC-MS/MS (r=0.93).

Reference values determined in 96 plasma samples of healthy volunteers (46 women and 50 men, aged 20-70 year) were 59 to 159 pmol/L (non-parametric 95% confidence interval). The female part of the reference group showed a statistically significant decrease of 1,25-dihydroxyvitamin D3 concentration with age. The presence of significantly higher average 1,25-dihydroxyvitamin D levels in premenopausal women taking oral contraceptive pills compared to postmenopausal women suggests that this effect is estrogen-related.

In conclusion, we described a 2D ID-UPLC-MS/MS method able to measure 1,25-dihydroxyvitamin D with a high sensitivity and precision. In addition, reference values for 1,25-dihydroxyvitamin D were established using this method.

W16

Measurement of autoantibodies against osteoprotegerin in adult human serum: development of a novel ELISA assay

I Piec1, JCY Tang1, C Washbourne1, J Greeves1, S Jackson1, S Ralston1, P Riches1, WD Fraser1
1University of East Anglia, Medicine/BioAnalytical Facility, Norwich, United Kingdom, 2HQ Army Recruiting and Training Division, Upvon, United Kingdom, 3Rheumatoid Disease Unit, Institute of Genetics and Molecular Medicine. University of Edinburgh, Edinburgh, United Kingdom

Introduction: Neutralizing autoantibodies against Osteoprotegerin (α-OPGAb) blocking the inhibitory effect of OPG on RANK signaling pathway were identified in a man with celiac disease associated with severe osteoporosis (2009) and more recently in patients presenting Rheumatoid Arthritis, Systemic Lupus Erythematosus, Spondyloarthritis and Osteoporosis. These findings suggest a role for α-OPGAb as primary cause of high bone turnover.

Objective: To develop an enzyme linked immunosorbent assay (ELISA) for detection and quantification of α-OPGAb in patient serum samples.

Method: A full-length human recombinant OPG is immobilized on a plate to allow capture of the antibodies from the sera. In a two-step reaction, the αOPGAb is detected using a biotinylated antibody and a horseradish peroxidase-labelled streptavidin. Substrate is incubated in a timed reaction and color development measured. The concentration of human α-OPGAb in the samples is determined directly from a 4PL-fit standard curve.

Results: Intra-assay imprecision was < 5% at 274.4 ± 18.8 and 98.5 ± 2.9 ng/mL. Inter-assay imprecision was < 20% at 324.2 ± 53.3 and 166.8 ± 30.6 ng/mL. Linear range was 0-500 ng/mL. Lower and upper limit of quantification were 3.9 and 500 ng/mL. Cross reactivity was assessed against human sera containing raised thyroid antibody and RANKL to ensure assay specificity. Using the method presented, we established that the adult population would be considered positive with a titer above the cut-off limit (95%) of 100 ng/mL. Our preliminary data suggested that 7% of our sample population (n=136) presented elevated α-OPGAb.

Conclusion: We presented a novel ELISA assay for the detection and measurement of anti-OPG autoantibodies in human serum. The validated method showed excellent assay characteristics and is suitable for use in research and clinical hospital laboratories. In patients with severe form of osteoporosis, measurement of OPG autoantibodies could help clinicians identify appropriate treatment options for this particular subgroup of patients.

W17

Calcium measurement – an assessment of adjusted calcium and ionised calcium

P McGing1, C Galvin1, B Gillman1, Y O’Meara1, M Fitzgibbon1
1Mater Misericordiae University Hospital, Department of Clinical Chemistry and Diagnostic Endocrinology, Dublin, Ireland, 2Mater Misericordiae University Hospital, Department of Nutrition and Dietetics, Dublin, Ireland, 3Mater Misericordiae University Hospital, Nephrology Department, Dublin, Ireland

Introduction: The laboratory measurement of blood calcium is of vital clinical importance. However, there are many associated problems, particularly in hypoalbuminaemic patients.

This study was undertaken to derive and assess formulae for adjusting calcium concentrations with the two commonly used albumin methods, Bromocresol Purple (BCP) and Bromocresol Green (BCG). Because of concerns regarding the available reference ranges for ionised calcium, considered the ‘gold standard’, we also derived a new reference range for that test.

Materials and methods: Plasma samples (Lithium Heparin; n=1674) were analysed on the Abbott Architect c16000 for Calcium (Arsenazo III) and Albumin (BCP and BCG). Whole blood and plasma samples were analysed for ionised calcium (iCa) using an ABL90 Flex (Radiometer).
Results: Formulae for calculating adjusted calcium on our analysers were derived from regression analysis of albumin and calcium results using standard published method:

\[
\text{[Adjusted Calcium]} = \text{[Total Calcium]} + (0.012 \times (37 - \text{[BCP Albumin]}))
\]

\[
\text{[Adjusted Calcium]} = \text{[Total Calcium]} + (0.017 \times (39 - \text{[BCG Albumin]})).
\]

Application of these formulae to a validation cohort of patients gave similar adjusted calcium values with BCP formula on average 0.02 mmol/L higher than BCG formula (p = NS).

The reference range for ionised calcium from this study was 1.08 to 1.21 mmol/L (n = 120 ‘normal’ volunteers). Mean iCa was 1.14 mmol/L; 50.5% were below manufacturer’s recommended lower reference limit.

Discussion: These adjusted calcium formulae are quite different from the ‘standard’ calculation of \[\text{[Adjusted Ca]} = \text{[Total Ca]} + (0.02 \times (40 - \text{[Alb]}))\] used by many laboratories and underline the need for each laboratory to derive its own formula. Our results also illustrate the need for different formulae for BCP albumin versus BCG albumin.

The derived range for ionised Calcium of 1.08-1.21 mmol/L is lower than both the manufacturer’s range (1.15-1.29 mmol/L) and similar ranges quoted by the manufacturers of other instruments we have used.

W18

Relationships of vitamin D and pediatric inflammatory bowel disease with bone tissue

D Rodriguez-Cano, V Moreno-Moral, Caballero-Villarraso, M Aguilar-Quintero, A Romero-Urrutia, R Cañete-Estrada, F Rodriguez-Cantalejo

IMIBIC/Reina Sofia Hospita/Cordoba University, Clinical Analysis Service, Cordoba, Spain, IMIBIC/Reina Sofia Hospita/Cordoba University, Pediatrics, Cordoba, Spain

Introduction: Described harmfulness to the activity of the inflammatory bowel disease (IBD) is for bone tissue, promoting osteoclast activity mediated by proinflammatory cytokines. In addition, active IBD may involve malabsorption of micronutrients, including calcium and vitamin D (essential in the mineral metabolism).

In IBD patients has been a prevalent reduction in bone mineralization, can result in osteopenia (40-50 %) and even osteoporosis (5-30 %), with its inherent risk of fracture. This is a significant extraintestinal comorbidity of IB, which has been linked to steroid therapy, the activity of IBD, its development time and lifestyle, among others.

Material and methods: Case-control study of 85 children aged 3 to 17 years (39 cases and 46 controls IBD). History, bone densitometry and determination were performed on blood count, glucose, urea, creatinine, calcium, phosphorus, magnesium, alkaline phosphatase (ALP), urate, PTH, osteocalcina, T4, TSH, cortisol, insulin, albumin, prealbumin, protein, lipid and iron metabolism, PCR, orosomucoid, betacrosslap, 25(OH)vitamin D and its soluble leptin receptor, IL-6, FGF23, osteoprotegerin, sclerostin and RANK-L.

An inter-group study comparing different parameters (Student’s t test or Mann Whitney) and intra-group study using a multiple linear regression.

Results: The bone-mineral biochemical profile is different in IBD population, showing lower concentrations of calcium and FA, but over RANKL. The 25(OH)vitamin D was negatively correlated with markers of IBD activity, suggesting its anti-inflammatory and immunomodulatory action. The deleterious effect on bone tissue involves increased activity of IBD and/or increased corticosteroid dose is confirmed.

Conclusion: IBD in children has distinctive features in metabolic bone area with implications for other aspects. For better control of IBD, instead of climbing the corticosteroid dose, the use of calcidiol advocated by its dual impact on bone: direct (ossifying action) and indirectly (by reducing the inflammatory status, resulting osteopenia).

W19

Serum vitamin D status in pediatric obesity and relations with paradoxical ‘turnover’ and bone mineralisation

V Moreno-Moral, D Rodriguez-Cano, Caballero-Villarraso, F Prada-Blanco, L Gonzalez-Rivas, R Cañete-Estrada, F Rodriguez-Cantalejo

IMIBIC/Reina Sofia Hospita/Cordoba University, Clinical Analysis Service, Cordoba, Spain, IMIBIC/Reina Sofia Hospita/Cordoba University, Pediatrics, Cordoba, Spain

Introduction: There have been numerous studies on the relationship between obesity and osteomineral metabolism, especially in adults. In children, the literature is sparse and contradictory despite its special relevance to be their growing bones.

Methods and objectives: Case-control study of 60 pediatric patients with obesity and 53 controls (normal weight). Medical history and anthropometric measurements, blood pressure, bioimpedance and bone densitometry was performed. Samples for blood count and biochemistry including glucose, urea, creatinine, ions, calcium and phosphorus, magnesium, alkaline phosphatase, urate, PTH, osteocalcine, T4, TSH, cortisol, insulin, albumin, prealbumin, total proteins, PCR, betacrosslap, vitamin D, soluble leptin receptor, IL-6, growth factor FGF23,
osteoprotegerin, RANK-L and sclerostin. An inter-group study was conducted comparing the means of the different parameters (Student’s t test or Mann-Whitney) and intra-group study using multiple linear regression analysis.

**Results:** In the intergroup study, biomarkers osteomineral profile showed differences between the two groups showed obese children globally by ‘turnover’ accelerated, with increased levels of markers of bone resorption (PTH and RANK-L). However, 25(OH) vitamin-D, calcium, FGF-23 and sclerostin were lowered with respect to those of control group. However, the bone mineral density (BMD) was higher in obese. Interestingly, in the intragroup study found that obese had a direct relationship between the levels of 25 (OH vitamin-D and BMD. The same relationship was observed between serum leptin receptor levels and BMD in both groups.

In the obese group, it was observed that the concentration of 25(OH) vitamin-D was inversely associated with systolic blood pressure.

**Conclusions:** Reciprocal implications of pediatric obesity and metabolism are observed osteomineral. Pediatric obesity is associated with accelerated bone resorption without impact on BMD, as well as a 25(OH) vitamin D lowered compared to normal weight. This decrease was associated with higher systolic blood pressure.

---

**W20**

**Bone mineral profile and GH effects in hypophosphatemic rickets**

**J Caballero-Villarraso, J Gonzalez-Doblas, V Decena-Gamero, ME Roldan-Lopez, MD Cañete-Vazquez, F Rodriguez-Cantalejo**

**IMIBIC/Reina Sofia Hospital/Cordoba University, Clinical Analysis Service, Cordoba, Spain**

**Introduction:** There are several forms of hypophosphatemic rickets (RHF), characterized by a deficit in inorganic phosphate reabsorption in the proximal tubule of the kidney and commitment in bone mineralization. The RHF can cause bone deformities and short stature, and may even paradoxically accompanied by normal levels of vitamin D within a hypophosphatemia. The most common way is hypophosphatemic rickets X-linked (RHF-X).

**Material and methods:** Two separate cases of RHF-X are presented in two homozygous twin sisters who consult first 3 years and 9 months, stunting and genu varus in both tibias. The laboratory study showed in both sisters hyperphosphaaturia and hypophosphatemia. Molecular studies using mRNA amplification of the PHEX gene confirmed heterozygous mutation at both sisters.

**Results and discussion:** In both patients treatment with calcitriol with phosphorus starts. This normalization of the PTH was achieved a decrease and moderate elevation of phosphatemia and less fosfaturia but not reach normal ranges. Disappearance of radiological signs of rickets was also achieved. However, after menarche (at age 14), being in a percentile 1 size requested surgical bone lengthening by distraction bone (osteotomy+dynamic axial placement of fasteners [DAFs]). However, 14 months after surgery no callus formed, which prevented DAFs remove. It was decided to start treatment with growth hormone (GH), generating callus at 6 months. The absence of consolidation or callus is produced in less than 5% of cases, due to intrinsic factors such as patient malnutrition, infection, metabolic disorders secondary dysplasia, kidney, heart or liver. The presence of a RHF-X could justify the inability of bone healing (no callus).

**Conclusions:** GH treatment in patients with RHF-X phosphoremia and seems to improve bone mineral density, which may contribute to callus formation. It could be useful in future studies evaluating the administration and monitoring of GH, assayed in non-surgical and/or other fracture patients without metabolic bone disease.

---

**W21**

**How do GP patients respond to a vitamin D result?**

**RL Shea, J Berg**

**City Hospital, SWBH NHS Trust, Clinical Biochemistry, Birmingham, United Kingdom**

Serum Vitamin D workload at SWBH has been growing exponentially with 46,000 GP requests received in 2013/2014 for our inner-city Birmingham population. Request intervention was introduced in 2010 to limit testing to one test on each patient a year. With no NICE guidelines on who, why and when to test and retest for vitamin D, we were interested to see how GP patients’ vitamin D levels change on repeat testing. Data was gathered from 01/05/2011 until 31/03/13 for vitamin D results from GP patients. Duplicate records were identified by first name, surname and date of birth. If parameters did not agree then the record was ignored. Age, ethnicity, sex, date samples were received in the laboratory and results were recorded. Vitamin D status was defined in the following way:

- Severely Deficient=10.3-14.9nmol/L
- Deficient=15-30nmol/L
- Insufficient=30.1-50nmol/L
- Adequate=50.1-220nmol/L
- High-to-Toxic=220.1-500nmol/L
In total, 48,538 samples were received with 5,053 repeat samples identified and included in this study. 2,519 people had one repeat sample and 15 people had two repeat samples. Median time to repeat testing was 409 days (range 0-687). Repeat tests showed an overall improvement in concentration and status. 19% were severely deficient on initial test and only 8.9% were severely deficient on repeat testing. Median concentration went from 25.9nmol/L to 39.5nmol/L. The proportion of adequate patients went from 15.6% to 35.1%, however 64.5% remained less than adequate on repeat. 22.1% remained severely deficient and 54.8% went from adequate to less than adequate.

On repeat testing, patients generally improve, however there is great variation with a significant proportion of patients showing no improvement or even deteriorating. Further information on how GPs treat patients found to be vitamin D deficient and rate of compliance are needed in order to show that testing for vitamin D is worth the considerable NHS expense.

**Cardiovascular**

**W22**

Cardiac troponins in skeletal muscle disorders: a retrospective study to assess their utility in excluding myocardial injury in Pompe disease

I Wootton1, T Hiwot2, C Dawson1,2

1Queen Elizabeth Hospital Birmingham, Clinical Laboratory Services - Biochemistry, Birmingham, United Kingdom, 2Queen Elizabeth Hospital Birmingham, Endocrinology / Inherited Metabolic Disorders, Birmingham, United Kingdom

High sensitivity cardiac troponins (hsTnT or hsTnI) are sensitive markers of myocardial injury. Rapid changes in serum hsTnT or hsTnI levels are indicative of an acute coronary syndrome, whilst modest stably elevated levels are common in patients with cardiomyopathy and other causes of myocardial injury. Although conventionally assumed to be highly specific for cardiac muscle, recently the specificity of hsTnT has been questioned in patients with disorders of skeletal muscle, including muscular dystrophies and inflammatory myopathies. The aim of this retrospective study was to test the utility of cardiac troponins in assessing for potential myocardial damage in adult patients with Pompe disease. Pompe disease is a lysosomal storage disorder caused by deficiency of the enzyme, acid glucosidase alpha, which results in accumulation of glycogen in skeletal muscle, and in the most severe forms, cardiac muscle. We measured serum hsTnT levels in thirteen adult patients with Pompe disease seen in a routine outpatient clinic appointment in a national centre for adult inherited metabolic disorders. hsTnT was greater than the 99th percentile (14 ng/L) in twelve, with results ranging from from 11-59 ng/L. In eleven of these, creatine kinase (CK) was also elevated (range 70-950 U/L, reference range 24-195 U/L). In five patients with elevated hsTnT, hsTnI was measured on the same sample, and found to be below the 99th percentile (10ng/L). Additionally, all of these five had normal NT-proBNP levels and transthoracic echocardiograms indicating normal myocardial function. There are no other published reports of the utility of cardiac troponin assays in assessing for myocardial damage in patients with Pompe disease. Our results show that elevated hsTnT levels in Pompe disease are likely to be of skeletal muscle origin and hsTnI not hsTnT should be used to exclude myocardial injury in this disorder.

**W23**

The LDLR variant c.1426C>T; p.P476S as a novel cause of familial hypercholesterolaemia

L Tooth, P Collinson

St George's Hospital, London, United Kingdom

Mr KS is a 26 year old man who presented to the lipid clinic at St George’s Hospital with a baseline cholesterol of 8.8 mmol/L. He did not have any evidence of tendon xanthomas nor was there a family history of hypercholesterolaemia or premature death from vascular disease. At this point Mr KS did not meet the Simon Broome criteria for familial hypercholesterolaemia (FH). The patient had a BMI of 31.5 and lifestyle modification was suggested. The following year a younger brother was found to have a cholesterol of 9.6 mmol/L and an LDL receptor mutation was identified. LDL receptor mutations are the most common genetic defect and the prevalence of heterozygous FH in the UK population is estimated to be 1 in 500. Mr KS, together with his mother, father and a sister were screened by fluorescent DNA sequence analysis for the brother’s LDLR variant c.1426C>T; p.P476S. The mutation was identified in the mother and all three siblings. The mother had a cholesterol of 7.8 mmol/L and the sister a cholesterol of 9.1 mmol/L. The variant was absent in the father who had a cholesterol of 6.6 mmol/L. The LDLR variant c.1426C>T; p.P476S is a novel mutation not previously described and is likely the cause of the family’s raised cholesterol. Statins were prescribed to lower the cholesterol and as the siblings have a 50% risk of transmitting the variant to any offspring they have been referred to genetic counselling.
W24

Diagnostic uncertainty caused by a suspected macrotroponin I

SJ Oddy1, DJ Halsall1, MR Bennett2
1Cambridge University Hospitals NHS Trust, Clinical Biochemistry, Cambridge, United Kingdom, 2Cambridge University Hospitals NHS Trust, Cardiology, Cambridge, United Kingdom

A 72 year old male with a history of STEMI presented with chest pain two days post insertion of stent. The troponin I analysed by Siemens EXL 200 was 5230ng/L on admission, rising to 11419ng/L after 28hrs and falling to 6293 and 3903ng/L after 49 and 75hrs respectively. An ECG was performed and found to be normal, and an angiography found that previous stents and an 80% non stentable lesion were unlikely to be the source of the troponin I. Due to the mismatch between raised troponin I and normal ECG, positive antibody interference was suspected.

Dilution studies, an alternative troponin I assay, polyethylene glycol (PEG) precipitation and gel filtration chromatography (GFC) were all used to investigate possible interference.

Positive heterophilic antibody interference was not suspected because dilution studies showed no difference between controls and patient’s troponin I results. In addition, patient samples analysed by an alternative assay (Siemens Stratus Point of Care) were not significantly lower. However, the presence of a higher molecular weight immunoreactive protein in the patient’s samples was indicated by PEG precipitation. Two samples from the patient showed a low recovery (35% and 22%) compared with two controls (113% and 91%). GFC of a patients sample also showed the presence of a high molecular weight protein with troponin I immunoreactivity which was not present in a control sample. Finally, removal of IgG from the patient’s sample by addition of protein A also removed the majority of troponin I reactivity (19% recovery) compared to controls (91 and 88% recovery) suggesting that this higher molecular weight protein contains IgG.

We conclude that a macrocomplex containing troponin I and IgG forms the main circulating pool of detectable troponin I in this patient. However, genuine pathological release of troponin I on this high background of macrotroponin I cannot be excluded.

W25

Quality improvements in the diagnosis of heart failure - NT-proBNP testing in primary care in accordance with NICE guidelines

HM Barbour1, B Thwaites2, C Dickens1, H Sinclair2, G White1, M Saas1, D Kelly2
1Hampshire Hospitals NHS Trust, Clinical Biochemistry, Winchester, United Kingdom, 2Hampshire Hospitals NHS Trust, Cardiology Department, Winchester, United Kingdom

Introduction: A pilot study was set up following a successful application for regional funding to introduce NT-proBNP testing in accordance with the NICE chronic heart failure (CG108) guideline. Comprehensive data was collected for the patients and the complete patient pathway in order to assess quality improvements.

Methods: The programme was funded for one year with NT-proBNP testing on a Roche CobasSE411 analyser. At the outset a meeting was arranged with local GP practice representatives to introduce this pilot study.

325 patients, 152 male and 173 female, were studied. In addition to NT-proBNP we recorded basic demographics, renal function, cardiovascular risk factors and where applicable, time to clinic and echocardiogram appointment and basic echocardiogram results.

Results: Using the cut-offs recommended in the NICE guidelines, 190(58.5%) patients had an NT-proBNP less than 400ng/L heart failure unlikely, although 4 proceeded to echocardiogram with other indications.

Of the 82 patients (25.2%) with NT-proBNP 400-2000ng/L considered raised, and 53 patients (16.3%) with an NT-proBNP >2000ng/L considered high, the majority of patients proceeded to echocardiograms apart from 18 who were lost to clinical follow up. There were 7 patients with false positive results, NT-proBNP ranging from 404 to 3266ng/L, with no evidence of diastolic or systolic dysfunction on echocardiogram. The clinical risk factors in this group included atrial fibrillation, renal dysfunction, hypertension, smoking, diabetes and ischaemic heart disease.

NT-proBNP correlated with left ventricular function, increasing age and creatinine but not with diastolic dysfunction.

Conclusion: This study has demonstrated that 94.8% NT-proBNP results above 400ng/L recommended by NICE were associated with abnormal left ventricular function. The aim was also to reduce echocardiogram referrals and this was achieved for 190 patients with levels below 400. This has provided valuable local evidence to support future commissioning decisions allowing for further implementation of the NICE guidelines.
W27

Relationship of total bilirubin and C-reactive protein with traditional and novel risk factors for cardiovascular disease in apparently healthy subjects

K Bergmann1, K Olender1, J Siodmiak1, M Kretowicz2, J Manitius2, G Sypniewska
1Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Department of Laboratory Medicine, Bydgoszcz, Poland, 2Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Department of Nephrology, Hypertension and Internal Diseases, Bydgoszcz, Poland

Background: C-reactive protein (CRP), a marker of inflammation, is a well-known risk factor for cardiovascular disease (CVD). The aim of our study was to compare CRP and total bilirubin, a potential antioxidant and anti-atherogenic agent, with selected risk factors for CVD in apparently healthy subjects.

Materials and methods: Study included 145 normoglycemic, non-obese (BMI < 30 kg/m²), non-smoking subjects aged 25-40 years (73 women, 72 men). Blood pressure and basic anthropometric measurements were performed. Fasting plasma glucose, glycated hemoglobin (HbA1c), lipid profile, total bilirubin, insulin, C-reactive protein, apolipoproteins AI and B (apoAI, apoB), 25-hydroxyvitamin D (25(OH)D) and troponin T (hs-TnT) were measured on Abbott ARCHITECT ci8200 and Roche Cobas e411 analyzers. Serum adiponectin, visfatin and fibulin-1 (FBLN1) were assayed using commercially available ELISA kits. Carotid intima-media thickness (IMT) was measured by an ultrasound method.

Results: Total bilirubin and CRP ranged 0.221-1.98 mg/dL and 0.15-13.5 mg/L, respectively. Statistically significant difference between women and men was found for bilirubin (median 0.66 vs. 0.86; p=0.003). CRP correlated positively with anthropometric measurements, triglycerides (R=0.34; p=0.001), apoB (R=0.19; p=0.02), atherogenic indexes: TC:HDL-C (R=-0.22; p=0.008) and apoB:apoAI (R=-0.17; p=0.04) and inversely with HDL-C (R= -0.24; p=0.003) and apoAI (R= -0.17; p=0.04). Total bilirubin was related negatively with blood pressure, total cholesterol, LDL-C (R=-0.25; p=0.004), non-HDL-C (R=-0.23; p=0.005), apoB (R=-0.28; p=0.004) and atherogenic indexes. In contrast to CRP, total bilirubin was associated significantly with novel CVD risk markers: hs-TnT (R= -0.18; p=0.04), FBLN1 (R= -0.22; p=0.01), visfatin (R=0.20; p=0.04) and with IMT (R= -0.30; p=0.003), a subclinical indicator of atherosclerosis.

Conclusions: Both, total bilirubin and CRP are related with lipid profile and apolipoproteins, however bilirubin significantly correlates with novel cardiovascular risk factors, therefore it might be included in routine laboratory tests for evaluation of the CVD risk in apparently healthy individuals.
A multi-center analytical evaluation of the ARCHITECT STAT high sensitive troponin-I assay

M Krintus1, P Boudry2, N Estan Capell3, U Koller4, G Lefevre5, I Lennartz6, J Lodz7, M Nybo8, M Plebani9, J Shih10, O Skadberg11
1Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Department of Laboratory Medicine, Bydgoszcz, Poland, 2CHR Mons-Hainaut, Department of Clinical Biology, Mons, Benin, 3Dr. Peset University Hospital, Department of Clinical Biochemistry, Valencia, Spain, 4Vienna Hospital Association, Hospital Hietzing, Institute for Laboratory Diagnostics, Vienna, Austria, 5Hospital Tenon, Department of Biochemistry and Hormonology, Paris, France, 6Abbott Laboratories, Wiesbaden, Germany, 7University Medical Center, Johannes Gutenberg University Mainz, Laboratory Medicine, Mainz, Germany, 8Odense University Hospital, Department of Clinical Biochemistry and Pharmacology, Odense, Denmark, 9University Hospital of Padova, Department of Laboratory Medicine, Padova, Italy, 10Abbott Laboratories, Global Scientific Affairs, Abbott Park, United States, 11Stavanger University Hospital, Laboratory of Clinical Biochemistry, Stavanger, Norway

Introduction: Troponin is the preferred biomarker for the diagnosis of acute myocardial infarction. New high sensitive troponin assays have been developed with increased precision at the 99th percentile of the upper reference limit.

Objective: This study evaluated the analytical performance of a new high sensitivity troponin-I assay on the ARCHITECT instrument.

Methods: The ARCHITECT STAT high sensitive Troponin-I (hsTnI) assay is a double monoclonal, sandwich assay with chemiluminescent detection. Nine laboratories in Europe participated in this study using ARCHITECT i2000 STAT or i1000 STAT instruments. Total precision, LoB, LoD, LoQ, linearity and interference were determined. Method comparison was performed using the ARCHITECT STAT Troponin-I assay as the referent method and 2598 samples that spanned the dynamic range of the assays. The 99th percentile URL was determined using 1769 samples from healthy populations or blood donors from seven countries. Ethnic approval/ waiver was received for specimens collected for the reference interval and method comparison studies.

Results: Precision ranged from 1.5 to 8.9% using the manufacturer's controls. The LoB, LoD and LoQ results confirmed the package insert data. Common interferences did not affect the hsTnI results. The overall 99th percentile URL was determined to be 19.3 ng/L and was higher in men (27.0 ng/L) than in women (11.4 ng/L). Troponin was detectable in between 52.1 and 87.8% of the apparently healthy population depending on the LoD value used. Concordance between the investigated hsTnI assay and the contemporary TnI assay at the 99th percentile cutoff was found to be 95%.

Conclusions: These results demonstrate that the ARCHITECT STAT high sensitive troponin-I assay is a precise and highly sensitive method for measuring troponin I on a high throughput analyzer. This new assay meets the criteria of a high sensitivity troponin test with the 10% CV concentration below the 99th percentile URL.

Ethnic differences in serum homocysteine concentrations in Singapore

R Hawkins
Tan Tock Seng Hospital, Laboratory Medicine, Singapore, Singapore

Aim: Moderate hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular disease and for recurrent venous thrombembolism. In Singapore, Indians have more coronary heart disease than Malays, who in turn have more than Chinese. This study examined whether differences in serum homocysteine concentrations exist between the ethnic groups in Singapore.

Methods: Anonymised details of all initial serum homocysteine measurements performed at Tan Tock Seng Hospital laboratory from May 2012-April 2014 were extracted from the laboratory information system for analysis in Microsoft Access and Excel. Repeat samples on the same patient were excluded. Homocysteine was measured on a Beckman Coulter DxC-800 analyser using the 3-reagent Axis-Shield enzymatic assay. Linear regression analysis was performed using SPSS v16 for serum homocysteine as the output parameter and race (Chinese, Indian, Malay, Other), sex, age and inpatient/outpatient status as predictor variables.

Results: There were 628 samples (69% Chinese, 13% Indian, 8% Malay, 9% Other) with average age 48y (range 12-92), 79% inpatient and 63% male. Median homocysteine concentrations (umol/L) were: Chinese 11, Indian 12, Malay 12, Other 9. Beta regression coefficients (and p-values) were: Age (y) 0.079 (< 0.001), Male (vs. female) 2.4 (< 0.001), Indian (vs. Chinese) 2.311 (0.002), Malay (vs Chinese) 1.744 (0.076), Other (vs. Chinese) 1.283 (0.22), Inpatient (vs. Outpatient) 1.085 (0.075). % results with moderate (15-30) / severe (30-100) hyperhomocysteinemia: Chinese: 23 / 2, Indian: 24 / 6, Malay 26 / 2, Other 12 / 0.

Conclusions: Serum homocysteine concentrations are slightly higher in Indians and Malavs than in Chinese when controlled for age, sex and inpatient/outpatient status. The increase is small in magnitude and translates into only marginally higher rates of hyperhomocysteinemia in Indians and Malavs. It is thus unlikely to be a significant contributor to the increased coronary heart disease risk of these ethnic groups.
W30

National audit of high sensitivity troponin

GC McKeeman, PW Auld, the National Clinical Biochemistry Audit Group (NCBAG)
Belfast Health & Social Care Trust, Clinical Biochemistry, Belfast, United Kingdom

**Background:** Troponin analysis is integral to the diagnosis of myocardial infarction (MI). The development of assays with enhanced analytical performance (high sensitivity (HS) Troponins) allows quantification of Troponin in a significant proportion of the healthy population and a CV of 10% or better at the 99th percentile. This has created new challenges for both laboratory and clinician in the optimal investigation of patients with cardiovascular disease.

**Aim:** Some years into their use, we aimed to obtain an insight into how the laboratory community have adapted to the introduction of “HS Troponin” assays and assess the level of consensus that exists regarding their application.

**Methods:** A questionnaire based on a review of the published evidence and current opinion was circulated under the auspices of the NCBAG to individual Clinical Biochemistry Services. The survey asked about important aspects of Troponin analysis and clinical application.

**Results:** Responses were received from laboratories throughout the British Isles (n = 94) with the majority (80%) stating that they were using a “HS assay” for Troponin from one of the major suppliers: Roche Troponin T hs (n = 45), Siemens Troponin I-Ultra (n = 16), Beckman Troponin I (n = 9), Abbott hs-Troponin I (n = 5). Considerable variation in practice was evident, even within groups using equivalent assays, and as a result we are making a series of recommendations. These cover: scope, strategy, units, QC, interferences, reporting, timeliness, reference limits, delta criteria and clinical liaison.

**Conclusions:** This national audit has highlighted significant variation in approach to the clinical utilisation HS Troponin assays. This audit advocates a need for greater harmonisation of approach to the provision of Troponin testing services. The adoption of the recommendations and proposals arising from this audit will improve the application of Troponin testing in supporting clinical practice.

W31

Plasma cotinine is associated with social deprivation and subclinical atherosclerosis

AJ Dunlop, HE Turner, JJ Allison, I Clunie, BL Croal, DW Stephen, KA Deans, on behalf of the pSoBid Study Investigators
NHS Grampian, Clinical Biochemistry, Aberdeen, United Kingdom

**Background:** Cotinine is the primary metabolite of nicotine and is the preferred biomarker for verification of self-reported history of cigarette smoking. Cigarette smoking is a well-recognised classical risk factor for cardiovascular disease. The aims of this work were: to use plasma cotinine to verify self-reported smoking history; to study the association between plasma cotinine and social deprivation, and to examine the association between plasma cotinine and subclinical atherosclerosis (ultrasound evidence of carotid plaque).

**Methods:** Plasma cotinine was measured by an in-house liquid chromatography-tandem mass spectrometry method. Intra-assay Coefficient of Variation (CV) was 4.7% at a cotinine concentration of 1ng/mL; 0.9% at a concentration of 10ng/mL and 1.4% at 100ng/mL. Inter-assay CV was 6.7% at a mean cotinine concentration of 1.8ng/mL and 5.2% at 372.3ng/mL. EDTA plasma samples (n=572) from the Psychological, Social and Biological Determinants of ill-health (pSoBid) study were analysed for cotinine.

**Results:** Current smokers had higher cotinine concentrations (250 (171.1 to 385.8)ng/mL) than ex-smokers (0.1 (0.0 to 1.7)ng/mL; p < 0.0001) and non-smokers (0.0 (0.0 to 0.6)ng/mL; p < 0.0001). Cotinine was higher in the most deprived group (4.4 (95% CI 0.2 to 283.2)ng/mL) compared to the least deprived (0.0 (0.0 to 0.5)ng/mL), p < 0.001. Cotinine was associated with carotid plaque presence (OR of 1.46 (1.19 to 1.79) per tertile increase in cotinine, p < 0.001. After adjusting for self-reported smoking history and area-level social deprivation, this association persisted (adjusted OR 1.40 (1.04 to 1.87), p = 0.025.

**Conclusions:** Cotinine independently predicts subclinical atherosclerosis, even after adjustment for self-reported smoking history and social deprivation.

W32

Factor analysis of association of lipid, inflammatory, cardiac, and renal biomarkers with global Framingham Risk Score cardiovascular risk estimation

S Jovičić, S Ignjatović, R Kangrga, M Dajak, N Majkić-Singh
1Center for Medical Biochemistry, Clinical Center of Serbia and Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, 2Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

**Background:** Risk score algorithms for cardiovascular risk assessment are based on multivariable regression equations including traditional risk factors. However, other factors might identify cardiovascular risk contribution not originated from traditional risk factors. The aim of this
study was to analyze the nature of influence of examined biomarkers on cardiovascular risk and their clustering, as well as relations of identified factors with 10-year risk categorization based on global FRS, using factor analysis.

**Methods:** Electronic calculator „CVD Risk Check” was used for global FRS calculation. Principal component analysis was used to investigate clustering of markers of inflammation [high sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), fibrinogen, α1-acid glycoprotein (A1AGP), haptoglobin, C3 and C4 complement components], lipid metabolism [non-HDL and LDL cholesterol, triglycerides, apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), lipoprotein (a) (Lp(a))], renal [creatinine, uric acid, cystatin C (Cys-C)] and cardiac function [N-terminal pro-natriuretic peptide type B (NT-proBNP), high sensitivity cardiac troponin T (hs-cTnT)], obtained from 242 apparently healthy individuals.

**Results:** Factor analysis identified five clusters, which explained 67.4% of the total variance:
1. „systemic inflammation” (hsCRP, fibrinogen, SAA, A1AGP, haptoglobin, C3, C4);
2. „atherogenic dyslipidemia”,(LDL and non-HDL cholesterol, apo B, triglycerides);
3. „cardiorenal factor” (creatinine, uric acid, Cys-C, hs-cTnT);
4. „hemodynamic factor” (NT-proBNP); and
5. „lipoprotein factor” [apo A-I, Lp(a)].

In predicting increased risk (>10%) according to global FRS, predictive values were significant for four factors (OR 1.693-3.265, \(P<0.0001\)), and insignificant (\(P>0.05\)) for „lipoprotein factor”. The area under the receiver operating characteristic curve (AUC) of the five factor model was 0.862 in predicting global FRS >10%. It was not statistically significantly different from AUC of the multivariable logistic model of 18 original parameters (0.889, \(P>0.05\)).

**Conclusion:** Systemic inflammation, atherogenic dyslipidemia, cardiorenal function and hemodynamic status independently contribute to increased 10-year risk estimated with global FRS.

**W33**

Heart-type fatty acid binding protein in combination with high-sensitivity troponin I for early diagnosis of acute myocardial infarction

**IM Hersey, PO Collinson**
St George’s Hospital, Blood Sciences, London, United Kingdom

Patients presenting to the emergency department (ED) with chest pain constitute the largest single category of patients admitted to hospitals. Current practice requires serial troponin measurements to diagnose or exclude presence of an acute myocardial infarction (AMI). This can result in delayed treatment and increased admissions as well as increasing the patient’s anxiety. A cardiac biomarker that could diagnose, or exclude AMI earlier after the onset of chest pain, would reduce these existing problems.

To determine if a novel marker, heart type fatty acid binding protein (H-FABP), could be used alone or in combination with high-sensitivity troponin I at time of admission so that a diagnosis of AMI can be ruled out on a single sample.

Method verification for the H-FABP Randox Ltd immunoturbidimetric assay was carried out on the Siemens Advia 2400 chemistry analyzer. This confirmed that the assay precision, LoB, LoQ and linearity performed as stated by the manufacturer. Following this, serial samples from 217 patients presenting to the ED were collected and assayed for H-FABP to assess diagnostic performance.

Although the combined sensitivity of H-FABP and troponin at admission was superior to either marker alone, this was not sufficient for rule out AMI on a single sample as up to 10.7% of AMIs could potentially be missed. The highest sensitivity and negative predictive value was obtained by measuring troponin alone at the second timepoint (median of 4h 3m).

This study has shown that H-FABP is not a suitable marker for the rapid diagnosis or exclusion of AMI, the literature has shown that it may however have utility as a marker of prognosis however this requires further investigation.

**W34**

The cardiovascular biomarker GDF-15 is related to waist circumference in non-obese coronary artery disease patients

**E Vianello1, E Dazio1, E Galliera2, MG Marazzi2, R Rigolini3, F Bandera3, M Guazzi4, MM Corsi Romanelli4**

1University of Milan, Department of Biomedical Sciences for Health, Milan, Italy, 2University of Milan, Department of Biomedical, Surgical and Oral Sciences, Milan, Italy, 3I.R.C.C.S. Galeazzi Orthopedic Institute, Milan, Italy, 4I.R.C.C.S. Policlinico San Donato, U.O.C SMEL-1 Clinical Pathology, San Donato Milanese, Milan, Italy, 5I.R.C.C.S. Policlinico San Donato, Heart Failure Unit, Milan, Italy

**Aim:** Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor-β cytokine superfamily. Increased plasma level of GDF-15 is associated with increased risk of cardiovascular events in patients with cardiovascular diseases, like coronary artery disease (CAD) patients. Recent reports also suggested an association between GDF-15 and metabolic complication in obese subjects. Our aim was to evaluate whether circulating GDF-15 level in non-obese CAD patients may be related to indices of adipose tissue distribution.
**Methods:** We enrolled at I.R.C.C.S. Policlinico San Donato 53 non-obese male CAD patients and 20 healthy subjects, as a control group. Patients were measured for height, weight, waist and hip circumferences; body mass index (BMI) and waist to hip ratio (WHR) were calculated. GDF-15 circulating level was measured by ELISA assay.

**Results:** GDF-15 circulating level was higher in CAD patients than control subjects ($p < 0.001$). After patients stratification into two groups according to waist circumference cut-off of 94 cm (IDF 2005), GDF-15 level was higher in CAD patients with a waist circumference $\geq$ 94 cm ($p < 0.05$). No association has been observed with BMI and WHR.

**Conclusion:** Our data suggested that GDF-15 may provide a link between visceral adiposity and possible progression of cardiac stress linked to CAD.

---

**W35**

The utilisation of natriuretic peptide testing for the diagnosis of heart failure - a United Kingdom laboratory survey

*CJ Duff1,2, D Holland3, AA Fryer1,2*

1University Hospital of North Staffordshire, Department of Clinical Biochemistry, Stoke-on-Trent, United Kingdom, 2Keele University, Institute for Science and Technology in Medicine, Stoke-on-Trent, United Kingdom, 3Keele University, Benchmarking Service, Stoke-on-Trent, United Kingdom

**Background:** The measurement of serum natriuretic peptides is a key component national guidelines for the diagnosis of heart failure. However, it is apparent that practice is variable, both in terms of whether the test is available and if it is, the manner in which it is used.

**Aims:** To assess national variation in the adoption and use of natriuretic peptides for the diagnosis of heart failure and to explore the underlying reasons for any variation.

**Methods:** Electronic questionnaires were sent to 50 laboratories as part of the annual Keele University Benchmarking Service data collection 2012/13. Forty-six laboratories responded, representing hospitals across England, Scotland and Northern Ireland.

**Results:** Twenty-seven laboratories (59%) performed in-house analysis (BNP or NT-proBNP). In England, this figure was 81% while in Scotland it was 22%. Of the laboratories that did not perform the test, only 1 routinely referred specimens away for analysis at another centre. Funding constraints was the most common reason cited for not offering the test (79% of responses). The annual number of tests performed by each laboratory for primary care varied 6.5-fold (1.65 to 10.77 tests per 1000 patients). Variation was greater in secondary care, where the number of tests performed ranged from 0.03 to 9.35 per 1000 patients. Twenty-two laboratories (79%) specified clinical decision limits. Of these, 10 (45%) used age-related limits. Laboratories using NT-proBNP were more likely to specify age-related limits than those using BNP (56% vs. 9%, $P=0.043$, Fisher Exact Test).

**Conclusion:** A large proportion of hospital laboratories did not offer testing. In those that did, the large variation in the number of tests performed at each laboratory suggests inappropriate use at certain sites (i.e. over and under requesting). There was also major variation in how test results are interpreted, as indicated by the different decision limits quoted.

---

**W36**

Interplay HDL particles and inflammation markers in coronary stenosis

*I Korita1, A Bulo1, MR Langlois2, E Verhoye2, N Alliu1, E Refatllari1, V Blaton2*

1Medical University of Tirana, Department of Laboratory, University Hospital Center ‘Mother Teresa’, Tirana, Albania, 2UGent and KU, Department of Laboratory Medicine AZ St Jan Av Brugge, Leuven, Belgium

**Aim of the study:** The clinical value and the interrelationship of HDL was followed with acute phase proteins hsCRP, fibrinogen and serum amyloid A (SAA), with apo-lipoproteins, A-I and B and serum levels of cytokines in 198 patients with cardiovascular disease.

**Methods:** On exclusion criteria (MI, heart failure, CHD > 2 years, anticoagulant therapy, 198 patients were recruited and were subdivided with stenosis < 50% and $\geq$ 50% in accordance with coronary artery surgery study (CASS) guidelines. Lipids were measured on OLYMPUS AU640 analysers. LDL-ox was determined by immunosorbent assay and serum amyloid A (SAA) by immunonephelometry. Serum levels of cytokines and hsCRP were analysed by solid-phase chemiluminescent immunomodular assay on DPC Immulite 1.000.

**Results:** Highest ox-LDL were associated with highest percent of stenosis and HDL is highly inversely related to the degree of stenosis. The HDL data were confirmed with a similar significant change of apo A(I) concentration from 134 mg% with normal vessels and 123 mg% with $\geq$ 50% stenosis. HDL-C and apo A(I) are directly inversely related to the degree of stenosis and directly to the acute phase proteins, TNFα ($p< 0.1$) and IL6 are related to the degree of stenosis.

**Conclusions:** HDL-C is highly inversely related to the degree of stenosis, directly related to the acute phase proteins (APP) and inversely to pro-inflammatory cytokines. Serum amyloid A is responsible for the reassembly and dysfunction of HDL. Cytokines are mainly related to the dysfunction of HDL.
W37

The cardiac markers and oxidative stress parameters in advanced non-small cell lung cancer patients receiving cisplatin-based chemotherapy

U Demkow1, A Stelmaszczyk-Emmel1, B Bialas-Chromies2, M Szturmowicz3
1Medical University of Warsaw, Department of Laboratory Diagnostics and Clinical Immunology, Warsaw, Poland, 2Alab Medical Laboratories, Warsaw, Poland, 3Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Introduction: Cardiotoxicity is a well-known long-term consequence of lung cancer chemotherapy, however little is known about early subclinical changes in cardiac function.

Aim: The goal of the study was to assess early cardiotoxic effects of cisplatin-based chemotherapy in stage III and IV lung cancer patients, measuring serum levels of selected cardiac markers i.e. cardiac troponin T (TnT), creatine kinase-myocardial band (CK-MB) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in relation to oxidant effects.

Methods: We quantified the immediate impact of chemotherapy on examined markers in blood samples obtained from 12 non-small cell lung cancer (NSCLC) patients. All markers were measured using commercially available immunoassays. To investigate the oxidant effects of cisplatin-containing chemotherapy, we evaluated reduced glutathione (GSH), nitrite (NO2), derivatives of reactive oxygen metabolites (d-ROMs) and thiols (SH). Samples were collected prior to chemotherapy and 1 day after the first cycle of cisplatin administration.

Results: Chemotherapy did not cause statistically significant elevations in serum CK-MB. Serum TnT levels were undetectable at both time points in 11 out of 12 patients with a threshold of 0.01 ng/ml. In the single patient with undetectable TnT at the baseline, after the first infusion TnT level reversibly rose to 0.03 ng/ml. The pre-treatment value of NT-proBNP was slightly elevated in 7 out of 12 lung cancer patients. In 1 case NT-proBNP level significantly increased after chemotherapy (from 221.8 to 1489.0 pg/ml p < 0.001), in the remaining 11 patients it was stable. Cisplatin based combination chemotherapy induced significant nitroxidative stress in 5 patients (p < 0.05). The other measured oxidative stress parameters remained unchanged after the first infusion.

Conclusion: This pilot study demonstrated occasional elevations of cardiac biomarkers during cisplatin administration. Administration of cisplatin-containing chemotherapy caused significant nitroxidative stress in some patients. The relevance of cardiovascular complications in cancer patients and identification individual risk factors of developing cardiovascular toxicity merit further evaluation.

W38

The importance of determination lactates and other biomarkers in patients with acute myocardial infarction (AIM)

D Bozovic1, T Antunovic1, M Jaksic1, S Dragnic2
1Clinical Centre of Montenegro, Centre for Clinical Laboratory Diagnostic, Podgorica, Montenegro, 2Clinical Center of Montenegro, Centre for Cardiology, Podgorica, Montenegro

Introduction: After the exclusion of the patients with other possible cause of lactates growth, determination of this biomarker in patients with acute myocardial infarction (AIM) is a very important clinical contribution. Due to occlusion of blood vessels, decreased oxygenation and hypoxia forces the myocytes in anaerobic glycolysis, the final result is accumulation of lactates. The first day of the beginning of the acute myocardial infarction the lactates increase, and gradually decrease over time and so they can be used to monitor the disease in those patients.

Patients and methods: We examined 50 patients with acute myocardial infarction within 24 hours of their first chest pain, mean age 62.86±14.26 years, body mass index (BMI) 29.35±5.17 kg/m² and 30 control subjects with similar characteristics. All biochemical parameters were determined on Abbott’s Architect C 8000, TnI on Architect I 2000, IL-6 on DPC Immulite 2000, hs-CRP on nephelometer BN II Dade Behring and leukocytes were counted on Abbott’s CD 3700. All the samples were processed fresh.

Results: The values of TnI for patients with AIM were 15.83±20.38 μg/l, KV was 35.25%, and lactate was 2.91±1.02mmol/l, versus 1.12±0.35mmol/l for control group (p < 0.01); IL-6 was 6.28±3.98 pg/ml (p < 0.05); LDL was 3.86±1.27 mmol/l (p < 0.05). Leukocytes were 11.4±3.92 G/l versus 7.56±2.78 (p < 0.01); hs-CRP values were significantly higher in patients 2.77±3.83mg/L compared to control group 1.05±0.19mg/L (p < 0.001).

Conclusion: The lactate is an important indicator of tissue ischemia as well as a good predictor of outcome in critically ill patients. The lactate concentration in blood contributes to the triage in patients with a doubt of developing an acute coronary syndrome. Lactates have a prognostic significance in patients with acute myocardial infarction. Lactate levels correlate well with the degree of the myocardial infarction as well as with the severity of patients condition.
Diabetes

W39

Can HbA1c predict cancer risk in people with and without diabetes?

A Robertshaw, E English
University of Nottingham, School of Medicine, Derby, United Kingdom

Aims: Cancer is a major public health problem. In 2012, it accounted for 8.2 million deaths worldwide. For its prevention, it is important we acquire greater knowledge of what contributes to increased risk. HbA1c has been shown to predict cancer risk, though the existing evidence is sparse and somewhat conflicting. The purpose of this systematic review was to identify any correlations between HbA1c and cancer risk, in people with or without diabetes. Novel approaches to cancer screening, incorporating HbA1c, would immensely benefit the global burden of cancer.

Methods: Embase, Medline, Cinahl and Cochrane Library were systematically searched for studies, published after 1990, investigating the role of HbA1c in predicting cancer risk. Eligible articles were: randomised-controlled trials, cohort studies, case-control studies, systematic reviews and meta-analyses. Participants of either sex; with or without type 1 or 2 diabetes, were included. Children (< 18 years), pregnant women and non-human species were excluded. The methodological quality of included studies was assessed in accordance with appropriate criteria.

Results: 926 studies were identified; 18 of which meeting the inclusion criteria. Methodological quality was good overall, though assessment of confounding wasn't thoroughly addressed. Correlations between HbA1c levels and colorectal, pancreatic, respiratory, female genital, post-menopausal breast and all-cause cancer, were revealed. Positive correlations were predominant; particularly for HbA1c levels of approximately 53 mmol/mol (≥ 7.0%). Some evidence indicated that low HbA1c (31 mmol/mol, < 5.0%) increases all-cause cancer and colorectal cancer risk. In contrary, null relationships were also found for the above cancers, and for gastrointestinal, kidney/urinary and prostate cancers.

Conclusion: The identified studies report a range of findings. Generally, HbA1c appeared to affect cancer risk more so in women than in men. Overall, the maintenance of normal HbA1c levels (42 mmol/mol, < 6.0%), and good glycaemic control of diabetes, may reduce the burden of cancer.

W40

A patient focussed approach to the investigation of hyperglycaemia

L Palmer, G Sanki, BP Tennant, KL Mitchem, P Baglioni, J Geen
Cwm Taf Health Board, Biochemistry, Merthyr Tydfil, United Kingdom

Case history: A 35-year old female was diagnosed with type 2 diabetes mellitus when aged 30. She had a family history of diabetes mellitus affecting her father and paternal great grandmother, and her younger sister having gestational diabetes. The patient was referred to the Endocrine Department as she wished to start a family and was concerned about her medication, which included gliclazide plus a gliptin and metformin. On referral it was noted that over the previous 4 years she had only one fasting plasma glucose measurement within the diabetic range at 7.1 mmol/L, but her HbA1c values consistently ranged from 47-52 mmol/mol, suggesting persistent but stable hyperglycaemia. In light of these observations a 75g OGTT was performed to reassess the patient’s glycaemic status. Baseline fasting venous plasma glucose was 74 mmol/L and 2 hour post glucose load venous plasma glucose was 13.1 mmol/L. This confirmed a diagnosis of diabetes mellitus but not the aetiology. The patient had no evidence of diabetic nephropathy indicated by her normal U+E, eGFR and urine MCR. Her total cholesterol and LDL-cholesterol were raised at 5.3 mmol/L (target < 4.0 mmol/L) and 2.9 mmol/L (target < 2.0 mmol/L) respectively, possibly secondary to diabetes. Given the patient’s young age, strong family history, the presence of mild fasting hyperglycaemia, stable hyperglycaemic control, this raised the possibility of maturity-onset diabetes of the young (MODY) was raised. This often overlooked diagnosis, can have a significant impact on patient management and education. The potential benefits of investigating the aetiology of her diabetes was discussed with the patient and consent was obtained for genetic analysis. Mutational analysis identified a heterozygous c.1319_1323dup frameshift mutation in the GCK gene encoding glucokinase confirming the diagnosis of MODY type 2 in this patient. All medication was subsequently stopped and the patient’s HBA1c has continued to remain stable and unchanged after 6 months.

W41

Case of interference in HbA1c measurement on the Bio-Rad Variant II Turbo

C Hyam, T Pearson, S Sankaralingam
Princess Alexandra Hospital, Biochemistry, Harlow, United Kingdom

An 83 year old white British man was investigated for diabetes mellitus. HbA1c was measured by ion-exchange high-performance liquid chromatography (HPLC) on the Bio-Rad Variant II Turbo and a value of greater than 184 mmol/mol was obtained. The chromatogram was
examined and the only abnormality seen was a very large HbA1c peak. The fasting glucose was 5.1 mmol/L and therefore discrepant from the grossly elevated HbA1c. A repeat sample was requested for HbA1c and haemoglobinopathy screening. Repeat measurement of HbA1c on the Bio-Rad Variant II Turbo again was greater than 184 mmol/mol. However HbA1c measured by a referral laboratory using boronate affinity gave a value of 35.5 mmol/mol (20-42 mmol/mol non diabetic reference range). Haemoglobinopathy screening detected a heterozygous variant of 25.5% of the haemoglobin which was likely to be an alpha chain variant and of no clinical significance. Therefore in conclusion the HbA1c measured on the Bio-Rad Variant II Turbo was falsely elevated due to the presence of a haemoglobin variant which co-eluted with HbA1c. This example illustrates the importance of following recommendations that a haemoglobin variant interference should be strongly suspected when a HbA1c value of greater than 140 mmol/mol is obtained. Corresponding or recent plasma glucose results can be used to give an indication whether interference is likely. The case also highlights that interference may not be obvious on inspection of the chromatogram. In these cases HbA1c should be measured using an alternative method to ion-exchange chromatography that is not affected by haemoglobin variants such as boronate affinity. It is important that laboratory professionals and clinicians are aware of the factors that may suggest that a haemoglobin variant is interfering in HbA1c measurement to prevent an incorrect diagnosis or inappropriate management of diabetes mellitus.

W42

HbA1c in the diagnosis of type 2 diabetes mellitus in a primary care population: the prevalence and effect of chronic kidney disease

KE Shipman, KM Sullivan, M Jawad, C Ford, R Gama
New Cross Hospital, Dept of Clinical Chemistry, Wolverhampton, United Kingdom

Aim: Glycated haemoglobin (HbA1c) has been adopted for the diagnosis of type 2 diabetes mellitus (T2DM). Chronic kidney disease (CKD) has been reported as affecting HbA1c independent of glycaemia. We studied the prevalence and effect of CKD on HbA1c in a primary care population.

Methods: Data on age, gender, HbA1c (HPLC, Tosoh), fructosamine, fasting glucose, creatinine (Roche), and haemoglobin (Sysmex) were recorded. Variables were compared between those with different CKD stages using chi squared or t-tests. The effect of CKD on HbA1c was studied after correcting for other variables using multivariable linear regression analysis. Data are expressed as mean (standard deviation).

Results: CKD < 3 occurred in 829 (83%) and CKD 3 in 170 (17%). Only 2 had CKD ≥4 and were excluded from further analyses. Compared with CKD < 3, patients with CKD 3 were older [71.5(13.8) vs. 51.4(17.3) years, p < 0.001], had higher HbA1c [42.1(8.2) vs. 39.5(6.5) mmol/mol, p < 0.001], fasting glucose [5.5(1.8) vs. 5.2(1.1) mmol/L, p < 0.001] and fructosamine [237(43.2) vs. 225.8(25.1) umol/L, p < 0.001] but lower haemoglobin [13.8(1.5) vs. 14.2(1.6) g/L, p = 0.004]. After adjustment, HbA1c was positively associated (p < 0.05) with increasing age, fasting glucose and fructosamine and negatively with haemoglobin but not with CKD [coefficient -0.67(95% CI:-1.59-0.26)].

Conclusion: CKD 3 affects 17% of primary care patients being screened for T2DM and CKD ≥3 is very rare. Despite higher HbA1c among patients with CKD 3 compared with CKD < 3, multivariable linear regression modelling revealed this to be due to age, fasting glycaemia and haemoglobin rather than CKD. These findings support the use of HbA1c for T2DM diagnosis in primary care since HbA1c is unaffected by CKD < 4 and CKD ≥4 is rare.

W43

Increased central adiposity may not underlie marked elevation of IL-6 in Nigerian diabetes mellitus patients

EK Oghagbon1,2,3
1Benue State University, Department of Chemical Pathology, Makurdi, Nigeria, 2Federal Medical Centre, Department of Chemical Pathology, Ido-Ekiti, Nigeria, 3University of Greenwich at Medway, School of Sciences, Medway, United Kingdom

Introduction: Reported increase in proinflammatory IL-6 in diabetes mellitus patients has not been reported in Nigerian diabetics in spite of huge number of patients. This study was to assess the level of the cytokine in Nigerians and to determine associated factors.

Materials and method: The twenty-three DM2 patients and 18 controls recruited for this study had their their BP , BMI, waist circumference (WC) and waist-hip-ratio (WHR) measured. They also had fasting plasma IL-6, fasting plasma glucose, total cholesterol (TC), Triglyceride (Tg), high density lipoprotein cholesterol (HDLC), urea, creatinine, aspartate transaminases (AST), alanine transferases (ALT), total protein (TP) and albumin determined.

Results: Population mean age was 51.83 years ± 13.28, diabetics (56.61yrs. ± 9.62) and controls (41.54 years ± 14.53) P ≤ 0.05. The mean IL-6 in diabetics (194.77pg/ml ± 166.16) was significantly higher than in controls (26.29pg/ml ± 6.65) at p ≤ 0.01.

No significant difference in mean BMI. WC and WHR of diabetics (194.77pg/ml ± 166.16) was significantly higher than controls (26.29pg/ml ± 6.65). The mean IL-6 in diabetics (194.77pg/ml ± 166.16) was significantly higher than in controls (26.29pg/ml ± 6.65) at p ≤ 0.01.

No significant difference in mean BMI. WC and WHR of diabetics (194.77pg/ml ± 166.16) was significantly higher than controls (26.29pg/ml ± 6.65). The mean IL-6 in diabetics (194.77pg/ml ± 166.16) was significantly higher than in controls (26.29pg/ml ± 6.65) at p ≤ 0.01.

No significant difference in mean BMI. WC and WHR of diabetics (194.77pg/ml ± 166.16) was significantly higher than controls (26.29pg/ml ± 6.65). The mean IL-6 in diabetics (194.77pg/ml ± 166.16) was significantly higher than in controls (26.29pg/ml ± 6.65) at p ≤ 0.01.
Among diabetics, there were significant correlations between IL-6 and Tg (p ≤ 0.01**), IL-6 and LDL-C (p ≤ 0.05*), IL-6 and AST (p ≤ 0.05*) and IL-6 and ALT (p ≤ 0.01**)

**Conclusion:** Elevated plasma IL-6 in DM2 patients in Nigeria is more related hepatopathy rather than body weight indices.

W44

**A regional audit of laboratory practice for HbA1c analysis**

**DM Kennedy¹, RR Round², D Vallance¹, SE Manley¹**

¹Heart of England NHS Foundation Trust, Clinical Biochemistry, Birmingham, United Kingdom, ²University Hospital of Birmingham, Clinical Biochemistry, Birmingham, United Kingdom, ³The Dudley Group of Hospitals, Clinical Biochemistry, Dudley, United Kingdom

**Introduction:** The West Midlands Diabetes Specialist Interest Group (WMDSIG) aims to bring together staff from many different laboratories to help improve and harmonize biochemistry services. An audit questionnaire was sent to all participants in June 2013; 14 labs replied (12 within the West Midlands, 1 in London and 1 in Derby).

**Methodology:** The preferred lab method was ion exchange HPLC (11/14), then immunochemistry using POCT analysers (2/14) and affinity chromatography (1/14). The majority (9/14) used Tosoh G7/G8. POCT HbA1c analysis was performed in 8/14 sites with most (5/8) using Siemens DCA 2000 or Vantage, usually in paediatric and/or adult diabetes clinics.

**Use of HbA1c:** HbA1c was provided for diagnostic use in 11/14 labs but only 2 could specifically identify requests used for diagnosis. No labs considered HbA1c an acute marker of glycaemia. Only 6/14 labs offered fructosamine as an alternative with most referring the test elsewhere.

**Flagging results:** There was no consensus on flagging results; only 8/14 labs flagged low HbA1c results and 9/14 flagged high results. The majority of labs (9/13) flagged HbE, however there was no consensus on a cut-off for HbF above which HbA1c was not reported; range 5-22% even amongst labs using the same analyser. Only 3/13 labs reviewed samples with labile HbA1c above a cut-off (range 4-6%).

**Reporting:** A minority of labs visually checked chromatograms (6/14). The majority (10/14) reported HbA1c if there was a heterozygous abnormal Hb but only 2/14 labs reported HbA1c if it was homozygous. Only 3 labs phoned abnormal HbA1c results. The standard comments used for reporting HbA1c were compared.

**Conclusions:** Despite the majority of labs using similar equipment, there is no consensus on flagging of results or reporting issues. Further work is needed to identify best practice and standardise the approach within the region.

W45

**Non-analytical factors affecting HbA1c independently of glycaemia and erythrocyte function**

**KE Shipman¹, M Jawad¹, KM Sullivan¹, C Ford¹, R Gama¹,²**

¹New Cross Hospital, Dept of Clinical Chemistry, Wolverhampton, United Kingdom, ²Wolverhampton University, Wolverhampton, United Kingdom

**Aims:** HbA1c is determined by a wide range of factors besides glycaemia including haematological. The effect of non-analytical factors, specifically age, gender and ethnicity, were examined using univariate multivariable linear regression modelling whilst controlling for glycaemia and erythrocyte function.

**Methods:** After applying selection criteria to 1006 consecutive patient samples without diabetes received from primary care over a 4 month period 947 were selected. Inclusion criteria included adult, non-pregnant, white and South Asian ethnicity. HbA1c (G7 HPLC analyser, Tosoh Corporation, Kanagawa, Japan), fasting plasma glucose and fructosamine (hexokinase and nitrotetrazolium-blue, MODULAR® P analyser, Roche Diagnostics GmbH, Mannheim, Germany) and haemoglobin (Sysmex XN-10®, Sysmex Corporation, Kobe, Japan) were analysed with demographic data collected from request cards, laboratory system and hospital records. A forced multivariable linear regression model with A1C as the dependent variable (StataCorp LP , Texas, USA), with haemoglobin, fructosamine and glucose as dependent variables, was performed. Age, gender and ethnicity were also included in the model and the means (standard deviations) and coefficients [95% confidence interval] were calculated. Multicollinearity diagnostics confirmed reasonable independence. Probability values of < 0.05 were considered statistically significant.

**Results:** Mean age was 55 (18.2) years, haemoglobin 141.9 g/L (15), HbA1c 40.1 (6.4) mmol/mol, glucose 5.9 mmol/L (0.9), fructosamine 2272 (23.3) μmol/L, 75% were white and 42.5% male. Controlling for haemoglobin, glucose and fructosamine, age (p = 0.000) and South Asian ethnicity versus white (p = 0.000) significantly affected HbA1c with linear regression coefficients of 0.080 [0.061-0.100] and 2.002 [1.215-2.790] respectively. Gender (female versus male) was not associated with HbA1c (p = 0.116) with a coefficient of -0.631 [-1.419-0.157].

**Conclusions:** Controlling HbA1c for erythrocyte function and glycaemia, by using haemoglobin, glucose and fructosamine, age and South Asian ethnicity were significantly and positively associated with HbA1c. Gender was not significantly associated with HbA1c.
HbA1c for diagnosis; how is it being used in practice?

NJ Pullan
Royal United Hospital Bath NHS Trust, Biochemistry, Bath, United Kingdom

Aim: To determine how HbA1c testing for the diagnosis of Diabetes Mellitus (DM) is being utilised in Bath and the surrounding area, UK.

Background: In 2011, the World Health Organisation (WHO) recommended that HbA1c may be used for diagnosis of DM (HbA1c ≥ 48 mmol/mol). In 2012, the UK Expert Working Group (UKEWG) also recommended HbA1c cut-off limits to indicate patients with high risk of progression to DM (HbA1c 42-47 mmol/mol). WHO and UKEWG indicated situations in which HbA1c should not be used for diagnosis. HbA1c testing for DM now also features in the NHS Health Check Programme for England.

Method: A retrospective audit was performed; clinical details, history of previous DM diagnosis, simultaneous glucose results, evidence of anaemia or CKD stage 4/5 were examined in association with 100 HbA1c results from patients without previous history of HbA1c analysis.

Results: During a two month period, 2189 first-time HbA1c requests were made. Of the 100 results examined closely, 96 appeared to be for DM diagnosis. Almost 40% of the requests examined were received simultaneously with a request for glucose analysis; the discordance rate between the glucose and HbA1c results for diagnosis or exclusion of DM was 21%. Evidence of anaemia or ferritin deficiency was found in >10% of the first-time HbA1c results examined.

Conclusion: Although simultaneous analysis of HbA1c and glucose for the diagnosis or exclusion of DM has not been recommended by WHO or UKEWG, a large proportion of clinicians are using this approach, which will lead to diagnostic dilemmas. HbA1c is also being used in patients where red blood cell turnover may be affected e.g. anaemia, which may lead to misdiagnosis. Whilst HbA1c is being used as a diagnostic test for DM, it is often not being used as intended, therefore further education of laboratory users is required.
Methods: Whole blood (fluoride-EDTA) patient and quality control samples were used in the method comparison study. Samples were analysed using: Menarini 8160 (current analyser), Bio-Rad variant II Turbo, Tosoh G8 (all based on ion exchange chromatography), and Menarini 9210 analysers (based on boronate affinity chromatography).

Results: Menarini 8160 showed good agreement with all analysers: Bio-Rad variant II Turbo (n=99) (y=1.10x+0.48, R²=0.98); Menarini 9210 (n=127) (y=1.01x+0.92x, R²=0.97). Comparisons were also made between Menarini 9210 vs. BioRad (n=99) (y=1.10x+0.27, R²=0.99) and Tosoh G8 (n=99) (y=1.09x+0.48, R²=0.98) and BioRad vs. Tosoh G8 (n=99) (y=0.99x+3.26, R²=0.99). Inter-assay precision using BioRad IQC material (n=4) showed CVs of 2.3, 2.9, 1.6 and 3.1% at 32mmol/mol, respectively and 2.9, 1.1, 0.7 and 2.4% at 85mmol/mol, respectively for the Menarini 8160, Bio-Rad Variant II Turbo, TOSOH G8 and Menarini 9210, respectively. Analysis of WEQAS External Quality Assurance samples showed close agreement with target IFCC values for all four analysers.

Conclusions: Measurement of HbA1c by all four analysers show good agreement, the closest agreement was observed between the Menarini 8160 and Tosoh G8. Superior analytical precision was achieved using the Tosoh G8 at both concentrations of quality control material evaluated.

W49

Zinc transporter 8 autoantibodies improves diagnostic accuracy of discriminating type 1 diabetes from MODY in early-onset diabetes

AC Cooper1, T McDonald1,2
1Royal Devon & Exeter NHS Foundation Trust, Blood Sciences Laboratory, Exeter, United Kingdom; 2Exeter NIHR Clinical Research Facility, University of Exeter Medical School, Exeter, United Kingdom

Aim: Maturity-onset diabetes of the young (MODY) is a rare (~1-2%) form of genetic diabetes. MODY is often misdiagnosed as Type 1 diabetes (T1D), resulting in inappropriate treatment with insulin. T1D is characterised by the presence of glutamic acid decarboxylase (GAD) and islet antigen-2 (IA-2) autoantibodies, making them useful to differentiate T1D from MODY. Zinc transporter 8 (ZnT8) is a novel marker of diabetes, but its prevalence in early-onset diabetes is unknown. We aimed to determine the prevalence of ZnT8 autoantibodies in Using pharmacogenetics to Improve Treatment in Early-onset Diabetes (UNITED) study samples that had undergone genetic-testing for MODY. We investigated whether testing for ZnT8 autoantibodies in addition to GAD and IA-2, could improve diagnostic accuracy in identifying patients requiring genetic-testing for MODY in the UNITED testing pathway.

Methods: Using RSR Ltd ELISA kits, we measured serum ZnT8, GAD and IA-2 autoantibodies in 256 UNITED samples. Autoantibodies were considered positive if ≥ 99th centile of 595 adult control subjects.

Results: GAD and IA-2 antibodies were detected in 105/256 (41%) of samples, vs. 131/256 (51.2%) when ZnT8 antibody testing was included. ZnT8 antibodies were present in 87/131 (66%) of all antibody-positive patients. The addition of ZnT8 increased detection-rate of T1D by 10.2%, excluding an additional 26 patients from genetic-testing. None of the 7 MODY-positive patients had detectable autoantibodies.

Conclusion: The prevalence of ZnT8 was 66% of all autoantibody-positive patients. Testing for ZnT8 autoantibodies in addition to GAD and IA-2 was able to identify 26 (10.2%) additional patients with T1D, thereby excluding them from molecular genetic-testing at a potential cost saving to the NHS of ~£795/patient. This supports the addition of ZnT8 antibody testing in routine clinical use.

W50

An in depth biochemical study of glycated keratin

A Sadiki Kishabongo, P Katchunga, E Van Aken, M Speeckaert, S Lagniau, R Coopman, J Delanghe
Ghent University Hospital, Department of Clinical Chemistry, Microbiology and Immunology, Gent, Belgium

Background: Glycated proteins are involved in diabetes complications. Glycated nail proteins have recently been demonstrated as a marker for diabetes diagnosis. Although its proven usefulness in the monitoring of diabetes nephropathy and retinopathy, the nature and formation of glycated nail proteins are still poorly understood. Similarly, lens proteins are subject to life-long glycation causing cataract.

Aim: To assess the nature of the glycated nail proteins and to demonstrate if the degree of glycation in nails is representative for the glycation of clinically important target organs, such as the eye lens.

Design: 34 patients (17 non-diabetics, 17 diabetics) who underwent lens extraction were enrolled in the study. Fructosamine was assayed in both nail and lens proteins; extracted nail proteins eluted by boronate affinity chromatography were further analyzed using SDS-PAGE. Furthermore, differential analysis of glycated proteins was performed in material originating from deep and superficial nail layers.

Results: Nail proteins were 5.1% glycated, containing keratins K4, K5, K6, K14, K16, and K17 with molecular masses from 46 kDa to 59 kDa. Fructosamine in deeper layers was found higher (median 3.6 μmol/g nails) than in superficial layers (median 1.12 μmol/g nails). A marked correlation was found between nails and lens glycated proteins (r²=0.53, p < 0.001). Multiple regression analysis also revealed a significant correlation between glycated keratins and HbA1C (r²=0.73, p<0.001).
Conclusion: The glycation of keratins occurs in the deep layers of finger nails which are in close contact with blood vessels and interstitial fluid. Glycation of nail keratins can be regarded as a representative marker for diabetic glycation-associated target organ damage.

W51

The relationship of serum bioavailable- and free-25-hydroxycholecalciferol and with glycaemic status in a diabetic cohort

D Smith1,2, M Melhuish2,3, BP Tennant4, G Sanki5, E Palmer1, P Baglioni1, O Okosime1, N Agarwal1, R Adams2, K Morris1, J Geeni1
1Cwm Taf University Health Board, Clinical Biochemistry, Merthyr Tydfil, United Kingdom, 2Cardiff Metropolitan University, Cardiff School of Health Sciences, Cardiff, United Kingdom, 3Cwm Taf University Health Board, Clinical Immunology, Merthyr Tydfil, United Kingdom, 4Cwm Taf University Health Board, Endocrinology and Diabetes, Merthyr Tydfil, United Kingdom, 5University of South Wales, Pontypridd, United Kingdom

Reduced serum 25OH-vitamin D (25OHD) in patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus has been widely reported. However, little research has investigated free- or bioavailable-25OHD, with aspects of diabetes. We aimed to identify associations between free- or bioavailable-25OHD and indicators of glycaemic status, in cohorts of T1DM or T2DM patients. Diabetic patients recruited from outpatient clinics (T1DM n=21; T2DM n=52) were matched to healthy controls. Total-25OHD was measured by LC-MSMS (Chromsystems/Agilent 6410), VDBP by an automated immunoturbidimetric method (Dako/ Roche Modular), fructosamine (Roche Modular) and all other measurements by routine methods. Free- and bioavailable-25OHD was calculated as described by Powe et al. J Bone Miner Res (2011)26:1609. Normality was tested by Shapiro-Wilk, groups by unpaired t-test or Mann-Whitney and correlations by Pearson’s or Spearman’s statistics, as appropriate. P-value <0.05 was deemed significant (GraphPad, Prism). Serum total-25OHD concentrations were confirmed to be lower in diabetic groups compared to controls. VDBP concentrations were only lower (17%) in the T2DM group compared to controls (p<0.0001). However, bioavailable-25OHD, but not free-25OHD, was lower in the T1DM group only, compared to controls (4.7 v 6.3nmol/L, p<0.05).

Intriguingly, a strong significant negative correlation was observed in T1DM between fructosamine and both bioavailable- and free-25OHD (r = -0.63, p<0.01; r = -0.62, p<0.01, respectively), whereas no such association was observed in T2DM, nor either diabetic group compared to HbA1c. This suggests total-25OHD does not reflect the free/bioavailable-25OH in T2DM. However, bioavailable-25OHD appears to be lower in T1DM, with bioavailable- and free-25OHD being inversely proportional to fructosamine. The mechanism and reasons for T1DM/ fructosamine association specifically are unclear. We are exploring alterations in VDBP:25OHD binding affinity upon VDBP modification (glycation, actin binding or conversion to Gc-macrophage activating factor) as part of our ongoing research study. Increased inflammation in T2DM compared to T1DM could be a factor.

W52

An audit of HbA1c vs oral glucose tolerance test in the diagnosis of diabetes and pre-diabetes

P McGing1, C Gavir2, E Burke-Murphy1, M Davis1, M Fitzgibbon1
1Mater Misericordiae University Hospital, Department of Clinical Chemistry and Diagnostic Endocrinology, Dublin, Ireland, 2Mater Misericordiae University Hospital, Department of Endocrinology, Dublin, Ireland

Introduction: Diagnosis of Diabetes Mellitus (DM) has traditionally been glucose-based, with the ‘gold standard’ test being the oral Glucose Tolerance Test (oGTT). In the past few years HbA1c has also been recommended as a diagnostic test. To help determine the optimal testing procedure for high-risk individuals we conducted a retrospective audit of concordance between oGTT and HbA1c for the diagnosis of Diabetes and ‘Pre-diabetes’.

Materials and methods: All GTTs performed by the Biochemistry Department of MMUH between July 2010 and June 2012 were gathered from the Laboratory Information system (LIS); n=2694. A subset of oGTts with HbA1c measured within ±3 days of the oGTT were studied (n=838). Results: 41% of patients (n=342) had DM diagnosed by at least one of the following criteria (‘DM Group’) - oGTT Fasting Plasma Glucose (FPG) ≥ 7.0 mmol/L, oGTT 2hPG ≥ 11.1 mmol/L, HbA1c ≥ 48 mmol/mol. Of the DM group, 32% were diagnosed by all 3 measurements, 7% were diagnosed by FPG alone, 29% by 2hPG alone, 11% by HbA1c alone, 8% by both FPG and 2hPG, 4% by FPG and HbA1c, and 9% by 2hPG and HbA1c. HbA1c was diagnostic in 56% of the DM group, and oGTT in 89%.

In terms of HbA1c / oGTT discordance, 64% (152/342) of the DM group did not reach the HbA1c criterion for diabetes. Using HbA1c plus FPG diagnosed 71% of the DM group, with 29% diagnostic only by 2hPG. However, 92% of these patients had HbA1c (80%) or FPG (12%) in the pre-diabetes range (by ADA criteria).

Discussion: We propose the optimal protocol for high-risk screening for DM is HbA1c with FPG, followed by oGTT for patients meeting Pre-diabetes criteria. Compared to using HbA1c plus full oGTT, our audit showed 98% of DM diagnoses would have been made using this more practical protocol.
Audit of changes in HbA1c values of 147 primary care patients during 13 years of target driven care

K Stuart1, J Raju2, S Allen3, E Clarke4, A Chithiramohan5, S Ramachandran2
1University Hospitals Birmingham NHS Foundation Trust, Clinical Biochemistry, Birmingham, United Kingdom, 2Heart of England Foundation Trust, Clinical Biochemistry, Birmingham, United Kingdom, 3University of Birmingham, Medical School, Birmingham, United Kingdom, 4Keele University, NIHR Academic Clinical Fellow, Keele, United Kingdom, 5University College London, Medical School, London, United Kingdom

Aim: The aim of the audit was to determine the long-term effectiveness of target driven incentivisation following the National Service Framework (NSF) in 2003 and the Quality and Outcomes Framework since 2004 by assessing glycaemic control in diabetic patients.

Method: We published a paper of HbA1C change in a cohort of 272 primary care patients in Sutton Coldfield, comparing audits in 1999/2000 (in preparation for the NSF) and 2007/8. The findings suggested that the focus was mainly on poorly performing patients not meeting targets. A re-audit was carried out in 2012/3 (147 of the 272 patients had HbA1c measured) from the laboratory information and management system. Statistical analysis included paired t-tests (comparison of sequential HbA1c results) and multivariate regression analysis (factors predicting change in HbA1c).

Results: The distribution of HbA1c remained the same in all 3 audits. However, the pattern that we observed in 2007/8 was replicated in 2012/3. The change in HbA1c between 1999/2000 and 2012/3 was associated inversely with the baseline level (Coefficient (95% CI): -0.69 (-0.83/-0.56), P<0.001, r²=0.41). Patients were stratified into quartiles (Q1-4) based on their initial HbA1c in 1999/2000 and an inverse pattern of HbA1c change was clearly seen; Q1 (< 6.1%): +1.32%; Q2 (6.1-7.0%): +0.66%, Q3 (7.1-8.3%): +0.33% and Q4 (> 8.3%): -1.34%. The proportion of individuals meeting the HbA1c target of 7.0% compared to the initial audit was lower (1999/2000: 50%, 2008/9: 35%, 2012: 34%) while that achieving the 10% target remained similar (93-95%).

Conclusion: A pattern of convergence of HbA1c values was observed perhaps due to target driven care. The changes that we observed in 2007/8 were still evident in 2012/3. If our findings are validated the whole concept of target based incentivised care must be reconsidered to ensure that individuals meeting targets are not neglected resulting in worsening glycaemic control.

HbA1c testing frequency is associated with serum cholesterol result in a diabetic population

CJ Duff1,2, D Holland1, OJ Driskell1,2, AA Fryer1,2
1University Hospital of North Staffordshire, Department of Clinical Biochemistry, Stoke-on-Trent, United Kingdom, 2Keele University, Institute for Science and Technology in Medicine, Stoke-on-Trent, United Kingdom, 3Keele University, Keele University Benchmarking Service, Stoke-on-Trent, United Kingdom

Background: Glycated haemoglobin (HbA1c) measurement is the mainstay for monitoring diabetic control. Undesirable levels are associated with diabetic complications and adverse clinical outcomes. Recent work has shown that there may be an optimal frequency for HbA1c testing, leading to desirable changes in HbA1c level (i.e. a reduction). Diabetic patients often develop other long-term conditions (co-morbidities), including cardiovascular disease (CVD).

Aims: The aim of this study was to investigate whether HbA1c monitoring frequency was associated with serum cholesterol concentration, a known risk factor for CVD.

Methods: Anonymised test data for HbA1c over a 5-year period (Jan 2009-Dec 2013) was extracted from the Laboratory Information Management System (LIMS) at the University Hospital of North Staffordshire NHS Trust. These data were linked to serum total cholesterol test data from a 12-month period (2013) (n=30,970 patients). Patients were assigned a mean HbA1c testing interval (in months), calculated over the 5-year period. The proportion of patients with a serum cholesterol level (mean over 12 months) above a target value of 5 mmol/L for each interval category was calculated.

Results: A relationship was observed between HbA1c test interval and serum cholesterol result. The proportion of patients with a serum cholesterol result above the 5 mmol/L target was lowest (15%) when the HbA1c test interval was 5-6 months. Little difference was observed up to 10-month interval (17%), after which there was a steady increase; at a >18 month interval, 35% of patients had a cholesterol level above 5 mmol/L.

Conclusions: These data suggest that HbA1c testing frequency is associated with serum cholesterol result. Optimising HbA1c testing frequency may result in fewer diabetic patients with serum cholesterol levels above recommended targets, reducing CVD risk and potentially leading to better clinical outcomes.
Endocrinology

W55

The impact of a positive bias in serum IGF-1 concentrations on subsequent patient investigations and outcomes

L Bernstone, B Rabbani, D Vallance, M Labib
The Dudley Group NHS Foundation Trust, Clinical Biochemistry, Russells Hall Hospital, Dudley, United Kingdom

Insulin-like growth factor-1 (IGF-1) is a peptide hormone produced primarily by the liver in response to growth hormone (GH) secreted by the pituitary gland. Raised IGF-1 concentrations alone have been shown to be sufficient in establishing the diagnosis of acromegaly in the majority of clinically suspected cases. In 2012, the manufacturer of our IGF-1 method withdrew their IGF-1 immunoassay kit following the discovery of a 20% positive shift in patient means since 2010. We realised that this could have led to the over-investigation of patients with raised IGF-1 levels and potential misdiagnosis. We have therefore audited the outcomes of all patients with raised serum IGF-1 concentrations obtained in our laboratory between January 2010 and December 2012. Patients with known acromegaly or pituitary adenoma were excluded.

Over the 2-year period, 142 patients had raised serum IGF-1 levels. Of those, 44 had a subsequent GH suppression test (GHST), 28 had a pituitary MRI without prior GHST, 9 had no further investigations as they previously had normal MRI scans, and 1 had a normal repeat IGF-1 result. 60 patients (37%) had no further follow up investigations. Of the 72 patients investigated by GHST and/or MRI, 15 were found to have a pituitary adenoma (20.8%).

Our audit showed that for almost half of the patients with raised IGF-1 levels subsequent investigations proved negative. It is possible that some of this group had unnecessary investigations due to the analytical issue at the time. As well as the anxiety caused to these patients, these additional tests would have resulted in significant costs to the health service and inappropriate use of limited resources. We conclude that shifts in patient means such as the one identified for IGF-1 can potentially have far reaching consequences for patient care, and clinical laboratories and manufacturers of diagnostic assays should remain vigilant.

W56

Reference range enigmas in the transgender population

AS Mallard
Royal Cornwall Hospitals NHS Trust, Clinical Chemistry, Truro, United Kingdom

GIRES (the Gender Identity Research and Education Society) reported that the incidence in 2007 of persons with gender dysphoria aged 15 years and over in the UK was 3 in 100,000, with 1500 presenting for treatment. By 2010, presentations had increased to 12,500 with 7,500 having transitioned. The median age for presentation is 42 years with around 80% male-to-female and 20% female-to-male transitions.

Treatment usually involves hormone therapy, with regular monitoring of oestradiol and testosterone to ensure levels are within appropriate reference ranges, +/- surgical intervention. It is therefore important for laboratory staff to know which way the transition is occurring and when a name change has taken place, so that correct reference ranges can be utilised - information often not provided by service users.

However, not all individuals can undergo hormone treatment, e.g. a previous DVT precludes oestrogen therapy, and some therefore just change their names and live as the opposite gender. It has become apparent from transgender workshops, held courtesy of the charity Intercom (Exeter), that this population has no understanding of biochemical reference ranges and the importance of the medical fraternity having knowledge of their actual birth gender, which can never be altered.

Issues include:

Case 1: A female-to-male undergoing hormone therapy but not changed name - high testosterone levels could therefore be attributed to late-onset CAH or a testosterone-producing tumour.
Case 2: A male-to-female transition with some/no surgery - the laboratory receives request for PSA. Our computer system would automatically reject a PSA request on a female.

By flagging transgender patients and/or having a third “Sex” field within hospital/laboratory computer systems it would highlight to staff when sex-related test requests have been made, requiring particular interpretation, so that unnecessary, involved and costly investigations could be avoided and individual’s legal rights to maintain confidentiality would be upheld.
W57

Early postoperative changes of plasma polyunsaturated fatty acids after laparoscopic sleeve gastrectomy

M Aslan¹, I Aslan², F Ozcan³, R Eryilmaz³, CO Ensari⁴, T Bilecik⁵
¹Akdeniz University Faculty of Medicine, Medical Biochemistry, Antalya, Turkey, ²Antalya Research and Education Hospital, Endocrinology Clinic, Antalya, Turkey, ³Akdeniz University Faculty of Medicine, Surgery, Antalya, Turkey, ⁴Antalya Research and Education Hospital, Surgery Clinic, Antalya, Turkey

Background: This study aimed to determine early postoperative changes of plasma polyunsaturated fatty acids (PUFAs) following laparoscopic sleeve gastrectomy (LSG).

Methods: Ten obese patients (mean BMI: 51.10±11.59 kg/m²) underwent LSG and eleven normal weight control patients (mean BMI: 24.37±2.33 kg/m²) underwent laparoscopic abdominal surgery. Fasting blood samples were collected prior to surgery, at day 1 after surgery and after post-operation oral feeding. Plasma levels of arachidonic acid (AA, C20:4n6), dihomo-gamma-linolenic acid (DGLA, C20:3n6), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Prostaglandin E2 (PGE2) was measured in serum samples by enzyme immunoassay.

Results: A significant decrease was observed in insulin and HOMA IR levels in sleeve gastrectomy patients after postoperation oral feeding compared to preoperation. Plasma AA levels and AA/EPA ratio were significantly increased in sleeve gastrectomy patients after postoperation oral feeding compared to postoperation day 1. Serum PGE2 levels and AA/DHA ratio was significantly higher in sleeve gastrectomy patients at preoperation, postoperation day 1 and after postoperation oral feeding when compared to control group patients.

Conclusion: Increased peripheral insulin sensitivity associated with LSG may play a role in the significant increase of plasma AA levels in sleeve gastrectomy patients following postoperation oral feeding. The significant increase in PGE2 levels and AA/DHA ratio in sleeve gastrectomy group patients also confirms the presence of a proinflammatory state in obesity.

W58

Increased heterophile antibody interference in the Beckman Coulter, Inc. (BCI) access luteinizing hormone (LH) assay causes potential clinically significant errors

NA Baumann, BK Hendrix, DR Block
Mayo Clinic, Laboratory Medicine and Pathology, Rochester, United States

Introduction: Heterophile antibodies, or human anti-animal antibodies, are known to affect a variety of immunoassays. Serum luteinizing hormone (LH) concentrations are often used in the clinical assessment of infertility and to diagnose pituitary disorders and conditions associated with dysfunction of the ovaries or testes. Erroneous LH results caused by heterophile antibody interference could pose a risk to accurate diagnosis and treatment of patients.

Aim: To evaluate the prevalence and clinical impact of heterophile antibody interference in the patient population when using the BCI Access® LH assay.

Methods: All samples from physician-ordered LH (May 2013-May 2014; n=1900) were re-assayed using the Roche Elecsys® LH immunoassay. Potential heterophile antibody interferences were identified when results differed by >30% between methods and confirmed by demonstrating a decrease in LH concentration of >30% after treatment with Scantibodies HBR heterophile antibody blocking reagent. LH results were classified according to age- and gender-specific reference intervals.

Results: Heterophile antibody interference using the BCI LH assay was confirmed in 127/1900 patient samples. Eighty-six patients would have been misclassified based on reference intervals. Clinical misclassification of patient LH results due to heterophile antibody interference in the BCI assay as follows: 3 high to within reference interval, 1 high to low, 5 high to undetectable, 6 within reference interval to low, 32 within reference interval to undetectable, and 39 low to undetectable.

Conclusion: The prevalence of heterophile antibody interference was 6.7% in the patient population using the BCI Access® LH assay. In 68% of confirmed heterophile antibody interference cases, the erroneous BCI LH result would have changed the clinical classification of the patient’s LH result leading to possible inappropriate treatment or missed diagnoses. Laboratories using the BCI Access LH assay need to be aware of the high prevalence of heterophile antibody interference and potential risk to patients.

W59

TB or not TB? A case of hypopituitarism

R Bray
St. George’s Hospital, London, United Kingdom

Mrs. E, 48, initially presented to her GP with symptoms of tiredness, weight loss and haemoptysis. Her blood tests showed suppressed T4 (< 3.3 pmol/L) and low TSH (0.44 mU/L), with low gonadotrophins.
(LH < 0.2 IU/L, FSH 1.6 IU/L). For this reason, she was referred to endocrinology for investigation. She underwent a short synacthen test and insulin tolerance test (ITT) which revealed inadequate cortisol and growth hormone (GH) responses. She was therefore diagnosed with hypopituitarism and started on hydrocortisone, thyroxine and GH replacements. Investigations for the haemoptysis were also carried out but chest X-ray revealed a normal cardiothoracic ratio and clear lungs. The patient had had the BCG vaccination in the past with a positive result to the Mantoux test. These results led to TB being excluded from the differential diagnosis and later discussions with the patients revealed that the symptoms had resolved without treatment. However, 5 months after initial presentation, the patient had an MRI of the pituitary which revealed significant inflammation. This indicated possible infiltrative causes for her hypopituitarism. The patient continued to be monitored for her hypopituitarism at the outpatient clinic and 6 months later underwent a chest CT scan which showed a 2.5cm lymph node. This was investigated by fine needle aspiration biopsy and TB PCR revealed the presence of Mycobacterium tuberculosis complex. A diagnosis of panhypopituitarism secondary to TB infection was therefore made and the patient was started on TB treatment. The patient is currently still on TB treatment and continues to be monitored by the endocrinology team for her panhypopituitarism.

W60

Measurement of serum testosterone, androstenedione and dehydroepiandrosterone (DHEA) levels using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS)

RM Büttler1, F Martens2, MM Kushnir2, MT Ackermans1, MA Blankenstein1, AC Heijboer1
1VU University Medical Center, Clinical Chemistry, Amsterdam, Netherlands, 2University of Utah, Pathology, Salt Lake City, United States

The adrenal and gonadal androgens testosterone, androstenedione and dehydroepiandrosterone (DHEA) play an important role in sexual development and fertility as well as in several other processes. We developed a method for simultaneous quantitative analysis of serum testosterone, androstenedione and DHEA levels using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS). Sample preparation consisted of addition of internal standards (13C3-testosterone, 13C3-androstenedione and 2H6-DHEA) and a liquid-liquid extraction using hexane-ether (4:1). The samples were analyzed on an Acquity 2D-UPLC-system (Waters), equipped with a C4-column (Waters) and a Kinetex Fluorophenyl-column (Phenomenex), and a Xevo TQ-S tandem mass spectrometer (Waters). The intra-assay CVs were < 4%, < 6.3% and 7% for testosterone, androstenedione and DHEA, respectively. The inter-assay CVs were < 6% for testosterone and < 8% for androstenedione and DHEA. At the lower concentrations inter-assay CVs were 9%, 7% and 9%, for testosterone (0.08 nM), androstenedione (0.48 nM) and DHEA (1.18 nM), respectively. Recoveries of spiked analytes were 93-107%. Linearity was evaluated by dilution (mean r2 was >0.999). This method tested negative for interference from other steroid hormones and did not show signs of ion suppression. The method was shown to be suitable for analysis of serum as well as EDTA and heparinplasma. The present testosterone method compared well (y = 1.00x + 0.04; r=0.998) to a published ID-LC-MS/MS method for testosterone in our lab. The latter method being concordant with a published reference method (Bui et al. 2013). The present method compared well to a published ID-LC-MS/MS method for the same analytes (Kushnir et al. 2010) (y = 0.94x + 0.06; r=0.996 for testosterone; y = 0.96x + 0.04; r=0.995 for androstenedione and y = 0.97x-0.01; r=0.991 for DHEA).

W61

A case of spontaneous undetectable prolactin

K Shipman, A Haddon, M Labib
Russells Hall Hospital, Dept of Clinical Biochemistry, Dudley, United Kingdom

Low or undetectable serum prolactin concentrations have been described after hypophysectomy, secondary to pituitary apoplexy or during treatment with high dose dopamine agonists. Spontaneous prolactin deficiency is rare and is associated with puerperal alactogenesis in women. We describe a case of undetectable prolactin (< 14 mU/L) in a 42 year old woman presenting with oligomenorrhoea. The GP contacted the laboratory to discuss the unexpected finding and the result was confirmed on another analytical platform. The patient was referred to the chemical pathologist and she was found to have persistently undetectable serum prolactin despite having successfully breast fed in the past. MRI scan of the pituitary was normal and Metoclopramide Stimulation Test showed no prolactin response. Insulin Stress Test showed normal cortisol response but undetectable serum prolactin throughout the test and subtle growth hormone deficiency. Quality of life score (QoL-AHGDA) was high and the patient was referred to an endocrinologist for consideration of GH replacement therapy. Prolactin and GH share a common tertiary structure and transcription factors. The pituitary transcription factor (POU1F1 or Pit-1) regulates GH, prolactin and thyrotropin stimulating hormone β subunit expression and is essential for the maintenance, differentiation and proliferation of lactotrophs, somatotrophs and thyrotrophs in the anterior pituitary. Pit-1 has been found to be mutated in cases of combined GH, PRL and TSH deficiency and anti-Pit-1 antibodies have been identified in patients with a picture of combined pituitary hormone deficiency. The cause of the undetectable prolactin and mild GH deficiency in our patient remains undetermined.
W62

Comparison of the Abbott Architect immunoassay and LC-MS/MS for the measurement of 25-hydroxyvitamin D

S Johnston, LA Jack
Department of Clinical Biochemistry, Glasgow, United Kingdom

This study compared the performance of the Abbott Architect immunoassay for the measurement of total 25-hydroxyvitamin D (25OHD) with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method traceable to the National Institute of Standards and Technology (NIST) standard reference material (SRM 972).

A cohort of 867 serum samples (containing total 25OHD concentrations ranging from 8 nmol/L to 261 nmol/L, as measured by LC-MS/MS) were used to compare the correlation of the Abbott Architect assay with the LC-MS/MS method using Passing-Bablok regression. Assay bias was calculated using the Bland-Altman plot. The classification of vitamin D status was compared using the two methods. A cohort of 13 serum samples containing 25-hydroxyvitamin D2 (25OHD2) concentrations that were greater than 50% of the total 25OHD, ranging from 17 nmol/L to 50 nmol/L (as measured by LC-MS/MS), were used to determine the Abbott Architect's cross-reactivity with 25OHD2.

Passing-Bablok analysis demonstrated a constant positive bias for the Abbott Architect immunoassay (intercept 16.42) and the Bland-Altman plot revealed a mean positive bias of 36.8% (95% limits of agreement -41.8% to +115.5%). The methods classified patients' vitamin D status differently with the prevalence of vitamin D deficiency (< 25 nmol/L) being 4% for the Abbott Architect immunoassay and 30% for the LC-MS/MS method. The mean 25OHD2 cross-reactivity for the immunoassay was 75%.

The Abbott Architect assay had unacceptable concordance with LC-MS/MS, demonstrating an overall positive bias but with highly variable individual results. It is important that clinical laboratories are aware of the limitations of their 25OHD assay.

W63

Vitamin D measurements in daily clinical routine with a new generation of vitamin D assays

D Enko1, D Rinner2, E Worf1, N Dirsch3, R Stolba1, E Rezanka1, G Halwachs-Baumann1
1Central Hospital Steyr, Department of Laboratory Medicine, Steyr, Austria, 2University of Applied Sciences for Health Professions Upper Austria, Bachelor Program Biomedical Science Steyr, Steyr, Austria, 3RECIPE Chemicals + Instruments GmbH, Munich, Germany

Background: Recently several diagnostic manufacturers have launched new 25-hydroxy-vitamin D (25[OH]D) assays, that are aligned to the National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM). Considering the difficulties of 25(OH)D measurements in the past, this study was conducted to compare the 25(OH)D determination results between one liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, one enzyme linked immunosorbent assay (ELISA), and one recalibrated and previous version of a chemiluminescence immunoassay (CLIA).

Methods: A total of 198 blood samples from routine 25(OH)D serum-level determination were measured by the ClinMass® LC-MS/MS Complete Kit (RECIPE Chemicals + Instruments GmbH), the ORGENTEC 25(OH)D3/D2 ELISA (ORGENTEC Diagnostika GmbH), the recalibrated IDS-iSYS 25(OH)D and the previous used IDS-iSYS 25(OH)D CLIA (Immunodiagnostic Systems Ltd). Pearson's correlation coefficients, Bland-Altman plots and Deming regression analyses were calculated.


Conclusions: The highly positive correlations and the low-to-moderate biases demonstrated between the new 25(OH)D assays are a clear indicator for a widespread introduction of a well standardized 25(OH)D assay generation in clinical laboratories within the next few years.

W64

Hyponatraemia audit: laboratory and clinical performance

A Francis1, EV Reynolds2, SJ Waterman2
1Gloucestershire Royal Hospital, Acute Medicine, Gloucester, United Kingdom, 2Gloucestershire Royal Hospital, Clinical Biochemistry, Gloucester, United Kingdom

Background: Hyponatraemia is the most commonly encountered biochemical abnormality and can be life-threatening. Hyponatraemia is often managed poorly, with inadequate investigations performed.
Gonadotrophin requests in older women

L. Wingsi, A.E. Armstrong
University of Southampton NFT, Clinical Biochemistry, Southampton, United Kingdom

Measurement of gonadotrophins to identify the peri-menopause, is not recommended in women over 45 when convincing symptoms are present. Biochemistry alone cannot identify the menopause as this is a clinical diagnosis. The presence of normal gonadotrophins levels does not exclude the perimenopause and measurements cannot be used to decide when contraception should be withdrawn. In addition gonadotrophins do not always suppress on HRT, nor are there guidelines suggesting appropriate levels. These unnecessary FSH and LH requests place avoidable demands on healthcare resources.

All computer held requests for FSH were examined during a 3 month period in women over 45 years (533 requests). An FSH of >20IU/L was considered to be consistent with the perimenopause providing the LH level did not indicate a mid-cycle peak.

335 requests had been made to establish the peri/menopausal status, with 40% including clinical details that indicated the menopause transition had commenced. We therefore estimate that by preventing these inappropriate requests £7000 could be saved (based on unnecessary LH and FSH requests at a cost of £6.70 each) however this figure could be a gross underestimate as many requests merely stated “perimenopause.”

During the 3 months 113 FSH requests had insufficient information regarding the reason for the test so potentially additional savings could be made. Given that some profiles also include oestradiol and/or progesterone there is scope for further reductions.

Conclusions: Training issues with junior laboratory staff not recognising the severity of the condition or having to deal with a very demanding workload caused delays but the time taken from venipuncture to receipt is a major barrier in meeting the new KPIs. Increased awareness and advertisement of recommended protocols via multidisciplinary presentations and the creation of an aide memoire card for Junior Doctors should improve clinical management. Progress will be monitored by re-auditing in a year.

Protocol for interpretation of thyroglobulin results in differentiated thyroid carcinoma

S. Brady, G. Jones, A. Dawnay
University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

Guidelines for managing differentiated thyroid carcinoma recommend measurement of thyroglobulin (Tg) to detect disease recurrence or residual thyroid tissue. Tg antibodies (TgAb) may interfere in Tg measurement, causing under- or over-estimation of Tg in non-competitive and competitive immunoassays, respectively. Assay discordance may be used as a hallmark of Tg assay interference.

We developed and validated a protocol for local Tg monitoring, designed to detect samples with a high risk of interference requiring further investigation. TgAb (Roche Elecsys) and Tg (non-competitive Siemens Immulite and competitive RIA Queen Elizabeth Hospital Birmingham) were determined on 117 patient samples. The prevalence of assay interference (i.e. discordance) was 12.8%. The optimal TgAb cut-off to predict assay discordance was 40 IU/mL (sensitivity 93%, specificity 95%, ROC curve AUC 0.973), which is within the TgAb reference range of <115 IU/mL. We established a policy of routinely referring samples with undetectable Immulite Tg and TgAb >40 IU/mL for second-line Tg analysis by competitive RIA, with assay concordance or discordance being reported as appropriate.

An audit of the first year of the Tg service revealed that 702 samples were analysed for Tg (Immulite) and TgAb. Of these, 36 samples with TgAb >60 IU/mL were referred for Birmingham RIA. Tg results were discordant in 61% (Immulite Tg all < 0.9 μg/L, RIA Tg 5.6-38.1 μg/L, TgAb
Laboratory Medicine at the Clinical Interface, United Kingdom, 7th–10th October, 2014

49-3832 IU/mL and concordant in 39% (Immumite Tg all < 0.9 μg/L, RIA Tg all < 5.0 μg/L, TgAb 42215 IU/mL). Our results show that TgAb levels within the reference range cause significant interference with Tg measurements. Raising the TgAb cut-off to increase the PPV is not an option without significant loss of sensitivity because of variable interference at any given level of TgAb. A small number of samples (5%) will continue to require dual Tg analysis to determine if interference is present.

W67

Traceability of testosterone assays in the UK-comparison with a JCTLM listed reference method

DH Ducroq, HL Fraser, C Strevens, SE Thompson, MA Thomas
Cardiff and Vale University Health Board, WEQAS Quality Labs, Cardiff, United Kingdom

With the implementation of ISO15189 accreditation, labs are becoming more aware of the need for traceability of their routine methods. Traceability of results to the SI unit utilising reference target values is the preferred method of comparison of returned EQA results where available. This ensures the transfer of accuracy from definitive methods to routine methods. Four individual donations (3 males, 1 female) of human serum, all endogenous steroid, encompassing the analytical range for testosterone (125nmol/L) and one donation consisting of 2 female sera pooled together, were analysed by a validated reference method utilising a JCTLM listed Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS) reference method. Traceability of analysis was assured by the inclusion of certified reference standard material within the analytical run.

The prepared pools were circulated over a period of 19 months to all participants of the WEQAS endocrine scheme. One distribution consisted of 5 samples that all had reference target values, enabling both linearity and traceability to be assessed. The deviations from the ‘true’ result (the reference method) for the main analyser groups were plotted in the form of bias plots (Bland-Altman plots). The Abbott method showed a negative proportional bias in the order of -20% across the range. The Roche method showed a crossover at approximately 8mmol/L with a positive bias above this level and negative bias below. The Advia method showed the opposite with a positive bias at low concentrations and negative bias at higher concentrations. The Tandem MS method showed reasonable agreement across the whole range.

Peer review of performance against method mean and overall mean data cannot identify true errors in accuracy. This can only be achieved by comparison with traceable reference methods such as ID-GCMS.

W68

A rare case of metastatic pituitary carcinoma secreting ACTH-precursors: the ‘state of the art’ in ACTH measurement

A Kyriacou1, C Higham1, C Sturgeon1, A Davies1, PJ Trainer1, A White1, PJ Monaghan1
1The Christie NHS Foundation Trust, Department of Endocrinology, Manchester, United Kingdom, 2Royal Infirmary of Edinburgh, UK NEQAS [Edinburgh], Department of Laboratory Medicine, Edinburgh, United Kingdom, 3University of Manchester, Faculty of Life Sciences & Medical and Human Sciences, Manchester, United Kingdom, 4The Christie NHS Foundation Trust, Department of Biochemistry, Manchester, United Kingdom

Objectives: We present the case of a 53 year old male with stable Nelson’s syndrome for 12 years until being incidentally diagnosed with spinal metastases and subsequently diagnosed with an ACTH-secreting pituitary carcinoma. The patient underwent debulking surgery and responded well to spinal radiotherapy. ACTH-precursors (pro-opiomelanocortin [POMC] and pro-ACTH) known to be increased in pituitary macroadenomas and the ectopic-ACTH syndrome, were grossly elevated at 6660 pmol/L. This initial ACTH-precursors result prompted the laboratory to review cross-reactivity in the ACTH immunoassay. A formal cross-reactivity assessment of POMC in commercial ACTH assays was performed in collaboration with UK National External Quality Assessment Service (UK NEQAS). To complement this study, a survey of practice was undertaken by way of an interpretative exercise to evaluate the awareness of laboratory professionals to cross-reactivity issues in contemporary ACTH immunoassays.

Results: 86 laboratories participated in this study. Two manufacturers dominated the market from a total of 4 different assay platforms. Cross-reactivity ranged from 1.6 to 4.7%. 20% of laboratories interpreted results as consistent with ectopic ACTH syndrome, yet only 1 laboratory suggested measurement of ACTH-precursors.

Conclusions: ACTH-precursors secreted by ectopic tumours and aggressive pituitary tumours may produce variable results in different ACTH immunoassays. Laboratory professionals must be aware of the degree of cross-reactivity of ACTH-precursors in their assays and be suspicious that tumours of this nature may be secreting very high concentrations of precursors. Measurement of ACTH-precursors in this context may be appropriate to aid diagnosis and to monitor response to treatment.
W69

Utilizing salivary cortisol and cortisone analysis by LC-MSMS as an alternative to serum cortisol during a short synacthen test

MP Cornes1, Y Kahlid1, HL. Ashby1, C Ford1, HN Buch1, R Gama1,2

1New Cross Hospital, Clinical Chemistry, Wolverhampton, United Kingdom, 2New Cross Hospital, Wolverhampton Diabetes Centre, Wolverhampton, United Kingdom, 3Research Institute, Healthcare Sciences, Wolverhampton University, Wolverhampton, United Kingdom

Background: Cortisol circulates as biologically active free cortisol (< 5%) but mostly as biologically inactive cortisol bound to cortisol binding globulin (CBG) and albumin. Serum cortisol, a measurement of free and bound cortisol, may be misleading in conditions of altered CBG levels, such as acute illness or oestrogen therapy as it does not accurately reflect serum free cortisol. Salivary cortisol is unbound and therefore unaffected by CBG accurately reflecting serum free cortisol. We investigated utilizing salivary cortisol and/or its salivary metabolite cortisone as alternatives to serum cortisol during a standard 0.25 mg synacthen test.

Methods: Paired serum and saliva samples were collected from 36 outpatients (16 females) with a mean age of 46 yr (range 15-80) before and then 30 and 60 minutes following intravenous administration of 0.25 mg synacthen. Serum cortisol was analysed using an electrochemiluminescence immunoassay on a Roche Modular E170 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Salivary cortisol and cortisone were analysed simultaneously on an ABSciex 3200 mass spectrometer with a Shimadzu HPLC system mass spectrometry.

Results: Correlation of salivary cortisol with serum cortisol was bimodal, intersecting at a serum cortisol of 600 nmol/L (y = 0.0214x - 2.0105 R² = 0.6037 < 600 nmol/L; y = 0.0852x - 42.666 R² = 0.6836 > 600 nmol/L). Correlation of salivary cortisone with serum cortisol was linear (y = 1.072 - 13.939 R² = 0.7511). Based on these correlations and a serum cortisol cut-off of 500 nmol/L, adequate salivary cortisol and cortisone responses to synacthen were defined as 15 nmol/L and 45 nmol/L respectively.

Conclusions: The bimodal correlation of serum cortisol with salivary cortisol is indicative of CBG saturation with cortisol at 600 nmol/L. Compared to salivary cortisol, the close linear correlation of salivary cortisone with serum cortisol suggests that salivary cortisone is the preferred analyte in the assessment for adrenal function. We plan to offer synacthen tests with salivary cortisone measurement routinely for suspected adrenal hypofunction.

W70

Re-evaluation of urinary free metadrenalines reference range

AS Davison, A Banks, G Higgins, R Pruden, JJ Dutton
Royal Liverpool and Broadgreen University Hospitals Trust, Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

Background: Diagnosis of phaeochromocytoma can be challenging, current recommendations suggest the measurement of total fractionated urine metadrenalines or plasma free metadrenalines for initial biochemical assessment. Few laboratories offer urinary free metadrenalines (UFM) despite them potentially offering greater diagnostic specificity than total fractionated urine metadrenalines. In house urinary free metadrenalines have been available for several years. The aim of this study was to re-evaluate our current reference ranges in a large local population.

Methods: 24h urinary free normetadrenaline (NMA) and metadrenaline (MA) results, measured by HPLC-ECD were gathered from the laboratory computer system (Telepath) between 2009-2014. All samples received from proven cases of phaeochromocytoma, where multiple urine collections on the same patient and requests from external laboratories, were excluded.

Results: 5664 urine samples were received over the five year period studied. After making the relevant exclusions there were 1559 requests remaining. Median age of requests on men and women were 54 (18-92) and 58 (19-99) years. Urine NMA and MA concentrations were significantly higher in men compared to women (p = 0.0001 and p = 0.0001, respectively). Requests were received from cardiology and pharmacology (60% M, 53 F), endocrinology (27% M, 32% F) and surgery (13% M, 15% F). NMA and MA were not significantly different in men (p = ns, in all cases) or women (p = ns, in all cases) when results were compared from the three requesting locations.

Conclusions: A reliable sex specific NMA and MA reference range has been re-evaluated from a large population of patients that have had phaeochromocytoma excluded. The new reference ranges are lower than our previous laboratory reference range.

W71

An audit of extracted testosterone requests sent to a reference laboratory

J Scott
Mid Cheshire Hospitals NHS Foundation Trust, Biochemistry Department, Crewe, United Kingdom

Introduction: It is well recognised that some female samples give falsely high results when measured in direct (non-extracted) testosterone immunoassays, probably due to the presence of water soluble steroid conjugates. Consequently all elevated testosterone levels with elevated
free androgen index (FAI) are sent to a reference laboratory for analysis by an extracted testosterone assay. This has time and cost implications for the trust.

**Aim:** To review testosterone results obtained by both assays and identify a level at which further testing is not required, giving possible cost savings.

**Methods:** Samples tested for testosterone between 01.11.2013 and 31.12.2013 by both the direct assay and the extracted assay were obtained from the Pathology IT system and the results compared.

**Results:** A total of 26 requests for testosterone had both the direct and extracted assay performed during the study period. Criteria and results obtained were as follows:

1. Result variations for testosterone >2.1 nmol/L or FAI > 5.6 in females < 50 years should not exceed 20% of total requests. Outcome 24/25 (96%) showed a difference. (2) Result variations for testosterone >1.7 nmol/L or FAI > 4.5 in females > 50 years should not exceed 20% of total requests. Only 1 patient fell in this category. Outcome—difference seen. (100%) Testosterone levels reduced in 22 (85%), no change in 1 (4%) and increased in 3 (12%). Similarly for the FAI.

**Conclusion:** The difference between the extracted testosterone and the direct testosterone results was not consistent and any change could not be predicted, as the amount varied from person to person. Therefore there is a need to continue with current practice of sending samples with elevated testosterone/FAI, obtained using a direct assay, for further investigation.

**W72**

First-trimester reference intervals for thyroid hormones-comparison of two immunoassays

N Gligorovic Barhanovic1, V Nikolic1, A Djogo2

1Clinical Center of Montenegro, Center for Clinical-Laboratory Diagnostic, Podgorica, Montenegro, 2Clinical Center of Montenegro, Clinic for Internal Diseases, Podgorica, Montenegro

**Objective:** The importance of diagnosis of thyroid dysfunction during pregnancy has been widely recognized. The objective of our study was to establish first trimester specific reference intervals for thyroid function tests in our population using immunoassays from two manufacturers.

**Materials and methods:** Serum samples were collected from 360 pregnant women (9-13 weeks of gestation) who underwent the combined screening for chromosomal abnormalities in Clinical center of Montenegro from February till May of 2016. Levels of serum thyrotropin (TSH), free thyroxin (fT4), free triiodothyronine (fT3), thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) were measured by two chemiluminescent immunoassays (Abbott, Architect i2000 and Siemens, Immulite 2000). Reference intervals based on 2.5th and 97.5th percentiles for TSH, fT4 and fT3 were calculated after exclusion of women with positive TPOAb and/or TgAb (n = 68). All statistical analysis was carried out using SPSS statistical package (version 17.0).

**Results:** Derived reference intervals for thyroid function tests during first trimester of pregnancy on Architect i2000 were: TSH 0.04-3.33 mU/L, fT4 12.6-19.5 pmol/L and fT3 3.7-6.1 pmol/L. Reference values on Immulite 2000 were: TSH 0.04-2.94 mU/L, fT4 10.7-17.2 pmol/L and fT3 4.6-9.0 pmol/L. 1.8% of tested population had TSH values within reverence interval with one assay, and classified as higher than upper range with other. Correlation coefficient (r) for TSH assays was high (r = 0.97), and lower for fT4 (r = 0.74) and fT3 (r = 0.60) assays, with significance p < 0.001. Regression results Architect i2000 versus Immulite 2000 were: TSH-slope 1.03, intercept 0.04; fT4-slope 0.83, intercept 4.87; fT3-slope 0.30, intercept 2.86.

**Conclusions:** Accurate evaluation of thyroid function tests in first trimester of pregnancy should be based on the gestational specific reference range. Interpretation of results should take into account differences between thyroid assays from different manufacturers.

**W73**

Plasma metanephrines by LC-MS/MS: method development, validation and application in a tertiary referral centre

SL Pitkin, F Lam, MC Serrano, A Dawnay

UCL Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

Plasma metanephrines are the method of choice for the diagnosis and monitoring of catecholamine secreting tumours (pheochromocytomas and paragangliomas). Due to a growing number of requests and to reduce turnaround time, we developed and validated an in-house LC-MS/MS method for the quantification of normetanephrine, metanephrine and 3-methoxytyramine.

Blood was collected in to K2-EDTA tubes on ice and plasma separated and frozen within 2h. Calibrants were weighed and made up in steroid stripped serum. Internal standards (normetanephrine-d3, metanephrine-d3 and 3-methoxytyramine-d3) were added and samples solid phase extracted using Oasis WCX uElution plates on a Tecan automated liquid handling system. The eluent was injected on to a Waters ACQUITY UPLC-TQD mass spectrometer. Gradient elution on an ACQUITY BEH amide column was followed by quantification by electrospray ionisation mass spectrometry in multiple reaction monitoring mode, with a run time of 5min.
Standard curves were linear ($r^2 > 0.99$) across the calibration ranges. The intra- and inter-assay coefficients of variation were < 10% and the lower limits of quantification for all three analytes were 100 pmol/L. Post-column infusion revealed ion suppression for normetanephrine and metanephrine in agreement with previous reports. Comparative sample analysis ($n = 215$) with the LC-MS/MS method at Newcastle Upon Tyne Hospitals NHS Foundation Trust, showed a bias of +141 pmol/L for normetanephrine ($r^2 = 0.97$) and +26 pmol/L for metanephrine ($r^2 = 0.98$). Over 12 months, the assay was consistently within the acceptable performance limits of the RCPAQAP plasma metanephrines EQA scheme for all three analytes. We adopted the diagnostic cut-offs used by Newcastle, and validated these against the final diagnoses of our patient population where possible.

In conclusion, a novel LC-MS/MS method for the simultaneous quantification of normetanephrine, metanephrine and 3-methoxytyramine was developed and validated. This was implemented for the diagnosis and monitoring of catecholamine-secreting tumours and retrospective review of the results revealed that the diagnostic cut-offs implemented were appropriate.

W74

Reference ranges for serum total and monomeric prolactin for the current generation Abbott Architect assay

SJ Whitehead, MP Cornes, C Ford, R Gama

1Royal Wolverhampton NHS Trust, Clinical Chemistry, Wolverhampton, United Kingdom, 2Wolverhampton University, Research Institute, Healthcare Sciences, Wolverhampton, United Kingdom

Background: Although biologically inactive, macroprolactin remains immunoreactive and is detected to varying degrees by different prolactin assays. Laboratory screening to exclude macroprolactinaemia is essential in order to avoid potential misdiagnosis and mismanagement of patients. A change from using the commonly employed percentage recovery based approach in favour of absolute post-polyethylene glycol (PEG) prolactin concentrations has been advocated. As part of the change in our laboratory’s approach, we derived gender specific serum total and post-PEG precipitation monomeric reference ranges the recently re-standardised Abbott Architect prolactin assay.

Methods: Prolactin was measured in serum samples obtained from males ($n = 69$) and females ($n = 52$) using the re-standardised Abbott Architect assay pre- and post-PEG precipitation. Gender specific reference ranges were derived for both total and monomeric (post-PEG) serum prolactin. A series of routine patient samples ($n = 175$) with a serum total prolactin ≥ 700 mIU/L were screened for macroprolactinaemia in order to compare classification of the new monomeric prolactin approach with our previous percentage recovery based method.

Results: Reference ranges for serum total prolactin were 58-419 (male) mIU/L and 63-561 (female) mIU/L. Male and female monomeric prolactin reference ranges were 32-309 mIU/L and 39-422 mIU/L respectively. Of 175 patient samples screened for macroprolactinaemia, 149 had monomeric prolactin levels (median monomeric prolactin = 1035 mIU/L; median recovery = 83%) above the gender specific reference range. Monomeric prolactin levels (median monomeric prolactin = 162 mIU/L; median recovery = 20%) in the remaining 26 were within the derived reference ranges. Two patients (one macroprolactin positive and another macroprolactin negative) would not have been identified as such using the previous percentage recovery-based approach.

Conclusions: The use of post-PEG monomeric reference ranges not only identifies hyperprolactinaemia due solely to macroprolactin but has the added advantage of identifying patients who have simultaneous true monomeric hyperprolactinaemia and elevated levels of macroprolactin.

W75

Thyroxine binding globulin deficiency spuriously affects free T4 measurement in some current immunoassays

C Moran, O Blake, S Bradbury, VK Chatterjee, DJ Halsall

1Institute of Metabolic Sciences, Metabolic Research Laboratories, Cambridge, United Kingdom, 2University Hospital Limerick, Clinical Biochemistry, Limerick, Ireland, 3The Pathology Partnership, Biochemistry Department, Cambridge, United Kingdom

Thyroid function testing of two adult males (P1, P2) using their local hospital assay (Siemens Immulite) showed elevated serum free thyroxine (fT4) (P1 25, P2 21.1 pmol/L (RR 9.9-20.1)) but normal Thyroid Stimulating Hormone concentrations (P1 2.1, P2 1.5 mIU/L (RR 0.15-3.2)). When retested using different assays, fT4 measurements with the PE Delfia method remained high (P1 25.2, P2 25 (RR 9-20 pmol/L), but were normal using the Siemens Centaur assay (P1 16.7, P2 16 pmol/L (RR 10-19.8)). fT4 measurement by equilibrium dialysis was within the reference interval (P2 25.8 (RR 10.3-35 pmol/L). Further analyses showed low total T4 (TT4) values using two different methods (Siemens Immulite P1 30, P2 29 (RR 58-161 nmol/L); PE Delfia P1 31 (RR 69-141 nmol/L) and reduced Thyroxine Binding Globulin (TBG) concentration (< 5.0 mg/L (RR 14-31)) in both patients. Genetic testing confirmed TBG deficiency, with each individual being hemizygous for known TBG gene mutations (P1, p.Arg221fs*5 “TBG Poland”; P2, p.Trp300Ter “TBG Buffalo”).

Measurement of fT4 by immunoassay, involving competition between tracer and free hormone, assumes existence of the same equilibrium between T4 and serum binding proteins in both test and assay standard samples. However, it is recognised that the T4-TBG equilibrium is perturbed, particularly when samples with very low TBG concentrations are analysed at non-physiological (37°C) temperature (Ross & Benraad Clin Chem 1992 38: 880-886). It is likely that such disequilibrium, occurring to varying extent with different assay conditions, leads to falsely increased fT4 values in cases of TBG deficiency. Whether this can be circumvented by altering assay conditions or alternative measurement methods (fT4 by equilibrium or symmetrical dialysis), remains to be determined.
W76

Local experience of an intraoperative PTH service

F Lam1, T Kurzawinski2, G Rumsby1
1University College London Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, London, United Kingdom, 2University College London Hospitals NHS Foundation Trust, Centre for Endocrine Surgery, London, United Kingdom

Intraoperative parathyroid hormone (ioPTH) measurement is the perioperative quantitation of PTH. The circulatory half-life of PTH is \( \sim 4 \) mins, with sequential decline in the peptide concentration utilised to confirm tissue resection. Rapid parathyroidectomy confirmation reduces operation time with benefits including decreased surgery-related complications, lower recurrence and re-operation rates, and shorter hospital stays. At UCLH, clinical biochemistry is an integral part of the ioPTH provision, operating monthly. Here, features of the service are reviewed.

Scheduled peripheral EDTA plasma samples were taken from the patient prior to surgical incision, upon gland localisation, and at 5 min intervals following resection, for up to 15 min. Additional measurements were performed at the surgeon’s discretion. ioPTH was quantified on a STAT-IntraOperative system (Future Diagnostics), which utilised a two-site chemiluminescent immunometric assay for intact PTH. Sample-to-result time was \( \sim 8 \) mins.

For 142 patients reviewed over a period of 3 years, a median of 5 samples/operation (as per schedule) were taken, range = 2-12. The patient with 2 measurements underwent total thyroidectomy in addition to removal of a single parathyroid gland. Due to the nature of this procedure, only 2 measurements were required to confirm resection. The patient with 12 measurements initially underwent single-gland parathyroidectomy, the hyperactive gland identified through imaging. Following resection, scheduled ioPTH measurements did not decline as expected, prompting further neck exploration. Two other glands with hyperplasia were identified and removed.

Median duration of operation, guided by the pre-incision and final ioPTH sample time interval, was 65 mins (range 21-233). The median interval between gland localisation and the final ioPTH measurement was 17 mins (range 6-136), the mode was 15 mins. This data suggests that, in most cases, ioPTH quantitation confirmed successful parathyroidectomy at the first exploration. Neck re-exploration was often prompted by ioPTH measurements that did not decline as expected.

W77

An investigation into the clinical utility of serum IGF-I and IGFBP-3 measurement in adult patients receiving parenteral nutrition

J Hamilton, G Wark, C Livingstone
Royal Surrey County Hospital NHS Trust, Clinical Biochemistry, Guildford, United Kingdom

Background: In hospitalised patients receiving parenteral nutrition (PN), it is desirable to optimise the regimen so as to meet patients’ nutritional requirements and improve clinical outcome. Currently this is achieved by monitoring anthropometric and biochemical indices. Serum concentrations of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) have potential utility in this situation because they are known to correlate with recent dietary intake. However, there are limited data available on IGF-I and IGFBP-3 in patients receiving PN.

Aim: This study aimed to provide data pertinent to the clinical utility of IGF-I and IGFBP-3 measurement in hospitalised patients receiving PN by examining the response to PN and correlation with known nutritional indices and potential confounding factors.

Methods: Serum concentrations of IGF-I and IGFBP-3 were measured by immunoassay at baseline and serially during PN in 20 patients referred for nutrition support. Malnutrition Universal Screening Tool (MUST) score was calculated in all patients.

Results: IGF-I increased significantly from a baseline (pre-PN) concentration of 9.79 \( \pm 1.19 \) (mean \( \pm \) SEM) to 14.8 \( \pm 2.03 \) nmol/L on day 8 of PN (\( p=0.047 \)). IGFBP-3 increased significantly from a baseline concentration of 1.83 \( \pm 0.22 \) (mean \( \pm \) SEM) to 3.42 \( \pm 0.37 \) mg/L on day 11 of PN (\( p=0.045 \)). Bivariate analysis of baseline parameters showed that IGF-I and IGFBP-3 concentrations were strongly correlated (\( p<0.001 \)). Neither IGF-I nor IGFBP-3 concentrations correlated with markers of the acute-phase response or with BMI, recent weight loss, duration of fasting, MUST score or length of hospital stay.

Conclusions: This study supports utility for measurement of IGF-I and IGFBP-3 in patients receiving PN. Their concentrations climb promptly during nutrition support and do not appear to be confounded by the degree of inflammation.

W78

An LC-MS/MS method for the panelling of 12 steroids in serum

DR Taylor, L Ghataore, L Couchman, CF Moniz, NF Taylor
King’s College Hospital, Department of Clinical Biochemistry, London, United Kingdom

Analysis of serum steroids by LC-MS/MS is increasingly replacing immunoassay, especially for those most subject to cross-reaction. However, much more is possible. By using a cocktail of deuterated internal standards, multiple steroids can be simultaneously quantified to provide
Thyroid function test (TFT) retesting intervals in patients treated with thyroxine-effect of TSH, free T4 and location

**W79**

**If Scargill**, D Holland, A Heald, AA Fryer
1 Salford Royal NHS Foundation Trust, Department of Clinical Biochemistry, Manchester, United Kingdom, 2 Keele University, National Pathology Benchmarking Service, Keele, United Kingdom, 3 Leighton Hospital, Department of Medicine, Crewe, United Kingdom, 4 University Hospital of North Staffordshire, Department of Clinical Biochemistry, Stoke-on-Trent, United Kingdom

**Introduction:** Guidelines suggest patients stabilised on thyroxine therapy should have TFTs checked annually and target a TSH within the reference range, with the minimum period to achieve stable concentrations after change of dose of thyroxine being 2 months. This retrospective study assessed the effect of TSH, free T4 and location of request on TFT retesting interval.

**Method:** All TFTs where the set code indicated that the patient was on thyroxine treatment performed by the Department of Clinical Biochemistry at Salford Royal NHS Foundation Trust, UK from January 2009-September 2012 were included. TSH/Free T4 were measured on a Roche Cobas E170 platform.

**Results:** 52,221 TFT results were identified from 16,663 patients on thyroxine therapy, with 12,025 patients having an initial TFT and a repeat TFT during the period reviewed. Median TFT retesting interval was 133 days, with peaks in repeat TFT requesting at 2, 6 and 12 months. 22% of repeat tests were <2 months and 13% were >12 months.

In 11,380 patients with initial TSH within the reference range (0.27-4.20mU/L), median retesting interval was 22.1 weeks (IQR=41.4). In 1,167 patients with a raised TSH, median retesting interval was 22.7 weeks (IQR=60.6). In patients with a low TSH (n=4,116) median retesting interval was 14.1 weeks (IQR=29.0). 8,476 (75%) of patients with a normal TSH had a normal fT4 (12-22pmol/L).

**Discussion:** Median TFT retesting intervals were 3-5 months, with significant variation in retesting intervals evidenced by wide inter-quartile ranges. Variation in repeat testing did not appear to be predicated upon the initial TSH or free T4 concentration, but more related to the location of the request.

**W80**

Evaluation of the Roche Elecsys calcitonin assay

C Boot, L Hughes, D Neely
Newcastle upon Tyne Hospitals NHS Trust, Blood Sciences, Newcastle upon Tyne, United Kingdom

Calcitonin is a tumour marker for medullary thyroid carcinoma and is important in diagnosis and monitoring of the disease. Doubling time of plasma calcitonin is often used to determine patient follow-up so a precise, reliable assay is required. The new Roche Elecsys calcitonin assay has a high claimed precision across the measured range (CV < 2.3% between 4.2 and 1613 ng/L). An evaluation of the assay was performed including comparison to the DIAsource CT-EASIA immunoassay currently in use in our laboratory. Assay acceptance criteria included equivalent or improved interassay precision and functional sensitivity compared to the DIAsource assay (CV of 8.8% at 60 ng/L and sensitivity of 11 ng/L) as well as a high degree of correlation with the existing method.
Interassay CVs were <2.4% at concentrations of 1.8, 7.6, 9.3 and 93.0 ng/L (the diagnostically important upper limits of the reference range are 6.4 ng/L and 10.2 ng/L for females and males respectively). 130 patient samples submitted routinely for calcitonin measurement were analysed using the DIAsource and Roche assays. Linear regression and Bland-Altman analysis indicated a high degree of correlation between the assays ($R^2=0.99$) with a proportional bias for Roche of -43%. This was expected as the DIAsource assay is known to over-recover. Analysis of nine EQA specimens (UKNEQAS) indicated a high degree of correlation ($R^2=1.00$) with the all-laboratory mean. To allow uninterrupted monitoring of plasma calcitonin during the change of assay in our laboratory, a re-baselining exercise was carried out so that for each patient there was at least one specimen where calcitonin had been measured using both assays. Comparison of results for the two assays over multiple specimens for individuals showed similar temporal trends. The Roche Elecsys calcitonin assay is precise, displays a high degree of correlation with our existing assay and appears suitable for clinical use.

**W81**

The visfatin levels in thyroid dysfunction

U Yildiz, N Bukan, F.B Toruner

1Aksaray State Hospital, Medical Biochemistry, Aksaray, Turkey, 2Gazi University, Medical Biochemistry, Ankara, Turkey, 3Gazi University, Endocrinology and Metabolism, Ankara, Turkey

Visfatin, an adipose tissue-derived protein is reported that may play a role in cholesterol homeostasis however, the literature about visfatin’s physiology remains controversial. Recent studies have shown multiple roles of hormones on visfatin expression and downregulation of visfatin expression by T3. We aimed to investigate the relationship between thyroid functions and visfatin in this study. Twenty-seven patients with hypothyroidism, 27 patients with hyperthyroidism and 31 euthyroid subjects as control group were selected from patients referred to the hospital of Gazi University Medical Faculty, Ankara.

Serum TSH, fT3, fT4, fasting glucose, lipid profile and visfatin levels were determined. Fasting glucose, triglycerides, total cholesterol, HDL-C levels were measured by enzymatic colorimetric method with auto analyzer.(Architect c-16000, Abbott Laboratories) TSH, fT3, fT4 levels were established by directly-chemiluminescent method with double sandwich immunoassay.(ADVIA Centaur-XP, Siemens-Healthcare Diagnostics) Visfatin levels were determined by enzyme linked immunosorbent assay (ELISA) method (Phoenix-Pharmaceuticals Visfatin C-Terminal (Human) Enzyme Immunoassay(Katalog No:EK-003-80) Sensitivity:2.42ng/mL, Linear-Range:2.42-38.1ng/mL, Intra-assay C.V.(%):<10, Inter-assay C.V.(%):<15. All analyses were performed using SPSS program(Version 16.0 for Windows).

Serum visfatin levels were markedly higher in the hypothyroidism group(8.96±4.27ng/mL) as compared with the hyperthyroidism(5.8±3.78ng/mL) and control(3.57±2.24ng/mL) groups (p<0.0001). Groups of hyper- and hypothyroidism demonstrated a significant difference(p<0.005), hypothyroidism and control groups showed no significant difference(p>0.0167), hypothyroidism and control groups exhibited a significant difference(p<0.001). According to this result, hypothyroidism group was found to show statistically significant difference from other groups in visfatin levels. Visfatin, positively correlated with TSH, total cholesterol, LDL-C and negatively correlated with fT3 and fT4. Visfatin is thought as a partly mediator to the effect of hyper/hypothyroidism on several metabolic parameters. Thyroid dysfunction may affect the visfatin clearance and may trigger visfatin secretion from visceral adipose tissue. The decrease in visfatin level due to thyroid hormones can be an additional result of the influence of thyroid hormones on the whole body metabolism.

**W82**

Direct relationship between aldosterone and parathyroid hormone levels

JM Bauça, D Morell-Garcia, S Cabello, A Barceló

1Hospital Universitari Son Espases, Servei d’Anàlisis Clínees, Palma de Mallorca, Spain, 2Hospital Universitari Son Espases, Servei de Nefrologia, Palma de Mallorca, Spain

**Introduction:** Cardiovascular disease is the leading cause of death worldwide. Endocrine function is globally accepted to have direct impact on the pathogenesis of the metabolic syndrome and the development of atherosclerosis. There is also growing evidence that aldosterone and parathyroid hormone (PTH) axes interact directly, and the dysregulation of either has a great effect on cardiovascular health. In this study, we evaluate the relationship between the two endocrine axes, according to different variables, such as gender, levels of both hormones and salt intake.

**Methods:** A total of 53 patients with simultaneous determination of plasma aldosterone (IDS iSYS, Vitro) and serum PTH (Abbott Diagnostics) were included. At the moment of analysis, 57% of them were under medication for the renin-angiotensin-aldosterone system. The reference interval for aldosterone was 4-300pg/mL, and for PTH was 11-67pg/mL. Salt intake was assessed as 24h-urine sodium excretion (Abbott Diagnostics). Comparison was performed between the upper tertile and the rest.
Kolmogorov-Smirnov test was used to assess normality, and Pearson’s correlation coefficient to quantify the relationship of variables. After Fischer’s transformation, strengths of correlation were compared.

**Results:**
- The correlation between aldosterone and PTH was greater for women than men ($p=0.041$).
- The relationship aldosterone-PTH does not vary depending on salt intake.
- The relationship between aldosterone-PTH is greater for pathological values of either hormone ($p=0.047$).

**Conclusions:** Our study partly supports the growing evidence of clinically relevant interactions between aldosterone and PTH, which might have direct therapeutic implications in patients with cardiovascular disease, since the management of either pathology could help improving the other by reducing the levels of both hormones.

In further randomized studies, assessment of a greater population and consideration of confounding variables is needed, such as age, body mass index, hypertensive status, smoking status, 25-hydroxyvitamin D levels and calcium-phosphorus metabolism.

**W83**

**Faecal calprotectin audit-establishing patient pathways following the issue of NICE guidelines**

**J Pethick**, V Lee, C Trzcinski, B Rathbone, W Madira

1University Hospitals Leicester, Department of Chemical Pathology, Leicester, United Kingdom, 2Markfield Medical Centre, Gastroenterology GP Lead for Three CCGs, Markfield, United Kingdom, 3University Hospitals Leicester, Department of Gastroenterology, Leicester, United Kingdom

NICE guidelines (DG11) recommend measurement of faecal calprotectin (FCALP) to aid in the differential diagnosis of irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) in patients with gastrointestinal symptoms where cancer was not suspected. Our local guidelines for the use of FCALP in patient pathways were drawn up by Gastroenterology in conjunction with Primary Care and the Laboratory. We carried out a retrospective audit to assess the use of the faecal calprotectin assay in adults in Primary Care five months after the issue of the guidelines and an education programme. The reason/s for requesting FCALP were obtained from the request forms, and we contacted GP Surgeries and interrogated the hospital information systems to determine which patients were referred to Gastroenterology. FCALP was measured using the Buhlmann ELISA with values < 50ug/g considered normal and those >200ug/g indicating active organic disease with inflammation in the gastrointestinal tract.

During the five month study period, 122 FCALP requests were received from Primary Care, of which 15 (12%) were >200ug/g and 34 (28%) were between 50-200ug/g. Of the 15 cases (FCALP >200ug/g) four were already known to Gastroenterology. Apart from one case where symptoms were thought to be related to antibiotic use the remainder had documented symptoms of IBD and all were referred to Gastroenterology. Of the 34 patients (FCALP 50-200ug/g) 29 had documented symptoms to suggest IBD and all were referred, the remainder were not referred and in the majority of these symptoms improved. Of the 71 patients (FCALP <50ug/g) six were already known to Gastroenterology, 12 were referred and the remainder were not referred.

This audit demonstrates the implementation of an effective patient pathway using FCALP to aid in the investigation of patients suspected of IBD / IBS.

**W84**

**Vitamin D status in chronic liver disease**

C Marinova, L Mateva, D Svinarov

Medical University of Sofia, Sofia, Bulgaria

**Background:** Vitamin D (VD) status is crucial for improving health, and for prevention of disease. The main objective of this study was to determine the prevalence of VD deficiency and insufficiency in patients with chronic liver disease (CLD), and to assess its relationship to the severity of disease.

**Methods:** Study encompassed 36 healthy control subjects (HCs) and 288 patients with proven CLD who consented to participate: 117 with nonalcoholic liver disease (NALD), 32 with alcoholic liver disease (ALD), 32 with chronic HCV infection (CHCV), 36 with chronic HBV (CHBV) infection, and 71 with liver cirrhosis (LC). Determination of 25-hydroxyvitamin D (250HD, sum of 250HD, and 250HD) was performed by a validated, DEQAS certified ID-LC-MS/MS method with accuracy and precision within 7.5% and linearity range 3.0-300.0 nmol/L.

**Results:** (mean ±SD): Total 250HD for all patients was 34.4±22.0 nmol/L (range 3.6-113.9), and for HCs=64.0±18.5 (range 20.6-105.6); 39% of patients (3% of HCs) had 250HD below 25 nmol/L (deficiency); profound insufficiency (25-50 nmol/L) was found in 41% of patients (17% in
HCs); another 17% were in the range 50-80 nmol/L, assessed as mild insufficiency (58% of HCs), and only 3% of patients (22% for HCs) were in insufficiency. 25OHD levels were registered in LC patients (17.6±13.6 nmol/L), with predominance of deficiency and severe insufficiency, without effect of season or cause for LC; VD status was lower in de-compensated vs compensated LC, p < 0.005. ALD patients had lower 25OHD levels compared to NALD, p < 0.005; VD status in CNCV and CHBV was comparable to NALD.

**Conclusion:** Most of our CLD patients were with vitamin D deficiency and insufficiency and there was an inverse relationship between 25OHD levels and severity of disease.

**W85**

**Diagnostic performance of faecal calprotectin in primary care**

*N Hunt*, R Allcock, A Sharma, M Myers

1Lancashire Teaching Hospitals, Clinical Biochemistry, Preston, United Kingdom, 2Lancashire Teaching Hospitals, Gastroenterology, Preston, United Kingdom

Faecal calprotectin is recommended by NICE for distinguishing between irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) in patients with lower gastrointestinal symptoms in primary care. We are extending our calprotectin service to primary care however, a number of GPs have requested calprotectin on an ad-hoc basis for a year, giving us insight into test performance in primary care. Primary care calprotectin data was audited over a 1 year period (Dec 2012-2013) and compared to 1 month of secondary care data (June 2013). Clinical details, including endoscopy and histology results were extracted from electronic patient records. In total 198 requests for calprotectin came from primary care however, 40 were unsuitable. Worryingly, 17% of requests had inappropriate clinical details such as bleeding; such patients’ referral to Gastroenterology was potentially delayed by requesting calprotectin.

Of the primary care requests, 29% of results were consistent with intestinal inflammation (≥50μg/g). If GPs use our proposed algorithm which suggests only referring patients with a calprotectin ≥50μg/g, and those where strong clinical suspicion remains, there is potential for up to 71% reduction in patients referred to Gastroenterology with IBS/IBD symptoms.

Diagnostic performance of faecal calprotectin compared with endoscopy and histology in secondary care is excellent with a sensitivity of 100% and a specificity of 91%. In primary care the corresponding data gives a sensitivity of 93% and a specificity of 79%.

These data show that offering calprotectin testing to primary care alone is not sufficient, it is critical to offer the service in a controlled way as part of a locally agreed care pathway. We are producing a GP information leaflet to advise on sample collection, result interpretation and the proposed patient pathway. We will re-audit following introduction to investigate whether a targeted approach leads to improved diagnostic performance of calprotectin in primary care.

**W86**

**Pre-operative assessment of satiety gut hormones does not correlate to weight loss after Roux-en-Y gastric bypass surgery**

*GF Cross*, I Fändriks, RP Vincent, CW le Roux, T Olbers, M Werling

1Department of Clinical Biochemistry, King’s College Hospital NHS Foundation Trust, London, United Kingdom, 2The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 3Diabetes Complications Research Centre, UCD Conway Institute, School of Medicine and Medical Sciences, University College Dublin, Ireland

**Background:** Morbid obesity can be effectively treated surgically by Roux-en-Y gastric bypass (RYGB). Nonetheless, post-operative weight loss can vary markedly and currently no biomarker can predict weight loss outcome. It is well established that post-prandial responses of the satiety gut hormones; glucagon like peptide-1 (GLP-1) and peptide YY (PYY) are attenuated in the obese but, exaggerated after RYGB. We aimed to evaluate whether the pre-operative response of these gut hormones to a standard meal could predict post-surgery weight loss. We hypothesised that poor GLP-1 and PYY responses pre-operatively, would predict poor weight loss after RYGB surgery.

**Methods:** The prospective study recruited 43 participants (F=25). Blood samples were collected before and every 30 min for 180 min after a standard 400 kcal mixed meal. Fasting and post-prandial plasma GLP-1 and PYY were measured by in-house radioimmunoassay. The assay detection limit was 2pmol/L, with a CV of 9.1% and 5pmol/L, with a CV of 10.2% for PYY and GLP-1 respectively. Delta change (difference between fasting and maximal response) in gut hormones were calculated. Weight loss was assessed as weight stability after RYGB [mean 16.2 months (CI 15.5 to 16.9)].

**Results:** Following RYGB, the Body Mass Index (BMI) decreased from 44.0 kg/m² (CI 42.2-45.7) to 30.3 kg/m² (CI 28.4-32.2), p < 0.001. Pre-operative GLP-1 and PYY response expressed as delta value as well as area under the curve during 180 minutes, did not correlate to total weight loss
(GLP-1; rho=-0.060 and rho=-0.089, PYY; rho=-0.03 and rho=-0.022 respectively) or to excess weight loss% (GLP-1; rho=0.051 and rho=-0.064, PYY; rho=-0.1 and rho=-0.088 respectively).

**Conclusion:** In the morbidly obese, pre-operative responses of satiety gut hormones, GLP-1 and PYY to a 400 kcal meal does not correlate to weight loss after RYGB surgery.

**W87**

**Could the Bühlmann Quantum Blue® POCT be used in the primary care setting, a comparison with a laboratory based method**

TI Staughton  
*Royal Berkshire NHS Foundation Trust, Biochemistry, Reading, United Kingdom*

**Introduction:** Calprotectin is increasingly being used in the differential diagnosis of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Availability of point of care testing (POCT) means this could be carried out in primary care, resulting in fewer patients being referred to secondary care and reduced workload for laboratories. This study compares the Bühlmann Quantum Blue® POCT with Thermo Scientific EliA™ Calprotectin.

**Method:** 27 samples were analysed simultaneously by both methods using identical extraction devices. Clinical notes were obtained to determine where possible the diagnosis of the patient. All patients diagnosed with IBD had a colonoscopy or sigmoidoscopy as confirmation.

**Results:** There were 13 patients diagnosed with IBD. The median and mean values for Quantum Blue® POCT in these patients were 1081ug/g and 1049ug/g. The median and mean values for the EliA™ were 698ug/g and 995ug/g. The Quantum Blue® POCT produced higher results than the EliA™ in the mid range (250-1500ug/g), but lower results than the EliA™ method above this range. Between values of 100-250ug/g the two assays showed agreement. Comparison of results produced a correlation coefficient r=0.3256. Both methods gave results that agreed with the clinical outcome of IBD.

**Conclusions:** A difference in calibration between the two assays would explain the difference in results. Both assays provided the same clinical answer. The preparation of the samples was identical apart from the differing buffer solutions. The preparation whilst simple is open to operational error which would require training in the POCT setting. If the test is used to determine a requirement for secondary care referral the POCT would provide that information. Indeterminate calprotectin results may be repeated following a 4-6 week period. With the difference between assays for calprotectin being considerable the repeat should be performed by the same assay as the original.

**W88**

**Lactose malabsorption testing in daily clinical practice**

D Enko, E Rezanka, R Stolba, G Halwachs-Baumann  
*Central Hospital Steyr, Department of Laboratory Medicine, Steyr, Austria*

**Background:** The purpose of this study was to establish a retrospective evaluation and comparison of the genetic test (C/T_13910 polymorphism) and the combined hydrogen/methane (H₂/CH₄) breath test results of lactose malabsorption testing in daily clinical routine.

**Methods:** A total of 263 patients were included in this retrospective study. The C/T_13910 polymorphism was performed using a melting curve analysis on the LightCycler Instrument (Roche Diagnostics). After the ingestion of 50 g lactose, gaschromatography (QuinTron Model DP Plus MicroLyzer™ [QuinTron]) was employed to measure the end-expiratory breath H₂ and CH₄ concentration at 15, 30, 45, 60, 75, 90 and 120 minutes. The breath test result was considered positive if the H₂ and/or the CH₄ peak was >20 ppm over the baseline value.

**Results:** All in all 51 patients (19.4%) had a C/C_13910 genotype, indicating primary lactose malabsorption. Only 19 patients (7.2%) had also a positive H₂/CH₄ breath test. In total 136 patients (51.69%) had a C/T_13910 and 76 patients (28.91%) a T/T_13910 genotype, indicating lactase persistence. Four patients (1.5%) with the C/T_13910 genotype and one patient (0.4%) with the T/T_13910 genotype had a positive H₂/CH₄ breath test result, indicating secondary lactose malabsorption associated with gastrointestinal diseases. The sensitivity of the genetic test compared to the gold standard lactose breath test was 79%, the specificity 87%, the positive predictive value 60%, and the negative predictive value was 98%. Cohen’s Kappa for agreement between the two methods was 0.44.

**Conclusions:** In conclusion, only moderate agreement between the genetic test and the breath test results was shown in daily clinical lactose malabsorption testing. Secondary lactose malabsorption as well as user-related pre-analytical deviations such as handling the gas-chromatography measurements or instructing the patients to exhale end-expiratory breath can contribute to discrepant results. Both methods should be performed in daily clinical routine.
W89

Stability of stool collected in traditional faeces containers for immunochemical faecal test

HD Abraha, NA Agbi, C Stone
Viapath, Kings College Hospital, Reference Biochemistry Laboratories, London, United Kingdom

There is evidence that the globulin molecule in faeces is steadily degraded by colonic bacteria. Previous studies have suggested using a freshly passed stool for faecal occult blood detection. Recent reviews and guidelines of regional and national screening programs have strongly supported the use of immunochemical test (FIT) for detecting occult blood in faeces. It is also recommended that stool for FIT analysis should be collected directly into the tube or onto the card of FIT collection device to minimize haemoglobin degradation and that will remain stable up to several days at ambient temperature. It was also proposed that stool samples collected in traditional faeces containers are likely to have false negative results due to haemoglobin degradation. The aim of this study is to evaluate the stability of stool collected in traditional collective device for FIT analysis. FIT is evaluated using the qualitative assay Hema-screen™ SPECIFIC.

Four FIT positive EQA and patient’s samples were evaluated for stability of stool at 4 °C. Samples are stored at 4°C and analysed on week 1, 2, 3, and 4. FIT remained positive in all samples for four weeks. In addition 11 patients who had FIT positive report on first examination were used to evaluate stool stability at ambient temperature. FIT were performed in all samples every day at the same time for five consecutive days and after 10 days. 2/11 samples were found to be negative on day 2 and 3/11 on day 10.

In conclusion, traditional stool collection tubes can be used for qualitative FIT analysis but stool should be analysed within 48h of collection. A stool sample that has been kept more than 48h at room temperature should be rejected to avoid false negative reports. It was also noted that stool samples remained stable for four weeks when kept at 4°C.

W90

The effect of blood contamination on the faecal calprotectin ELISA assay

P Mohammed, S Kaur-Sunnar, J Berg
City Hospital, Clinical Biochemistry, Birmingham, United Kingdom

Faecal Calprotectin (FC) is a protein used in the assessment of intestinal inflammation. It is a major protein in the neutrophilic granulocytes and macrophages, which accounts for 60% of the total protein in the cytosol fraction of these cells. FC is increasingly being used as a front line test to distinguish between organic and non organic intestinal disease. Since the publication of NICE guidelines there has been a significant increase in work load.

Faecal samples contaminated with blood are commonly encountered however there is a lack of information about how the results are affected. The aim of this study was to look at how blood contamination affects the FC concentration and if contaminated samples should be used.

We homogenised and made 1g aliquots of faecal samples with varying concentration of calprotectin (< 30 ug/g, 597 ug/g, 869 ug/g). We spiked the aliquots with different amounts of blood ranging from 50ul to 500ul and measured the FC using the Buhlmann ELISA assay. We also took blood samples from patients who provided contaminated faecal samples and measure the FC in the blood. Finally we took blood samples from patients with severe inflammatory conditions and measured the FC.

Spiked faecal samples were not affected the CV obtained ranged from 4.9 to 12.48% which is below the between assay variation for FC. Our results showed that in patients with active inflammatory conditions calprotectin concentrations may be significantly increased. Blood calprotectin ranged from < 30 to 145 ug/g in IBD patient blood samples. We found detectable levels of calprotectin (51 ug/g) in patients with acute inflammation who did not have IBD.

In summary blood contamination may significantly affect results especially if the faecal samples are taken during an acute / severe inflammatory response. FC in contaminated samples may be reported with appropriate comments.

W91

Vitamin D levels after gastric bypass surgery: do they reflect vitamin D status?

R Pitt, A Abeysekera, M Labib
Russells Hall Hospital, Clinical Biochemistry, Dudley, United Kingdom

There have been several reports of vitamin D deficiency in patients following gastric bypass (GBP) surgery. Vitamin D supplementation is recommended following GBP but there is no agreement on which regimen should be used. It has been suggested that vitamin D supplementation should be individualised to avoid hyperparathyroidism. We audited serum vitamin D and PTH levels in a group of patients who had GBP and who were on vitamin D supplements.
The records of 34 patients (15 men and 19 women) who were followed up in the post-bariatric surgery clinic at Russells Hall Hospital, Dudley, UK, were audited. 27 patients were on daily combined calcium/vitamin D supplements (1000-1200 mg/400-800U) and 7 patients were on vitamin D only (Fultium 800U/day) for 1-6 years after surgery.

The mean serum vitamin D was 58 ± 27 nmol/L. 21% of patients were vitamin D deficient (<25 nmol/L), 44% were insufficient (25-75 nmol/L) and 35% were sufficient (>75 nmol/L). The mean PTH level in vitamin D deficient/insufficient patients (7.5 ± 3.3 pmol/L) was not significantly different from that in vitamin D sufficient patients (6.7 ± 2.4 pmol/L) \(p=0.46\).

Our audit showed that, despite vitamin D supplementation following GBP, two-thirds of our patients were either deficient or insufficient in serum vitamin D levels. It has been suggested that hyperparathyroidism may reflect inadequate vitamin D supplementation; however, there was no significant difference in plasma PTH levels between our deficient/insufficient patients and those with sufficient vitamin D levels. We conclude that serum vitamin D levels may not accurately reflect vitamin D status in these patients. More research is required to clarify the relationship between adipose storage of vitamin D and serum levels in obesity, and how that might change after bariatric surgery. This could lead to improved management of vitamin D status in this growing clinical population.

W92

**Development of an LC-MS/MS assay for 7-α-hydroxy-4-cholesten-3-one for use as a biomarker for bile acid malabsorption**

**LC Cobbold, DR Taylor, RA Sherwood**

*Viapath, Kings College Hospital, Biochemistry, London, United Kingdom*

Bile acid malabsorption (BAM) is an under-recognised and under-diagnosed condition resulting in chronic watery diarrhoea. Many cases are misclassified as irritable bowel syndrome and patients may therefore not receive the correct treatment. Bile acid synthesis is up-regulated in BAM and therefore the measurement of bile acid precursors in patients suffering from chronic diarrhoea may offer a non-invasive and sensitive method of distinguishing BAM from other diarrhoeal causes. This study describes the development of a method for the quantification of the bile acid precursor 7-α-hydroxy-4-cholesten-3-one (C4), the product of the rate limiting step of bile acid synthesis. C4 was quantified in serum by LC-MS/MS using the atmospheric-pressure chemical ionization mode. The quantitation limit for C4 was found to be 2.5 nmol/L (signal to noise ratio 87). Inter-assay variability was calculated at three concentrations and gave CVs of <3.5% for each. Inter-assay variability was calculated and gave CVs of <8.5%. Potential isobaric interferences in the method were excluded and a C4 reference range was derived in serum by LC-MS/MS using the atmospheric-pressure chemical ionization mode. The quantitation limit for C4 was found to be 2.5 nmol/L (signal to noise ratio 87). Inter-assay variability was calculated at three concentrations and gave CVs of <3.5% for each. Inter-assay variability was calculated and gave CVs of <3.5%.

Bile acid malabsorption (BAM) is an under-recognised and under-diagnosed condition resulting in chronic watery diarrhoea. Many cases are misclassified as irritable bowel syndrome and patients may therefore not receive the correct treatment. Bile acid synthesis is up-regulated in BAM and therefore the measurement of bile acid precursors in patients suffering from chronic diarrhoea may offer a non-invasive and sensitive method of distinguishing BAM from other diarrhoeal causes. This study describes the development of a method for the quantification of the bile acid precursor 7-α-hydroxy-4-cholesten-3-one (C4), the product of the rate limiting step of bile acid synthesis. C4 was quantified in serum by LC-MS/MS using the atmospheric-pressure chemical ionization mode. The quantitation limit for C4 was found to be 2.5 nmol/L (signal to noise ratio 87). Inter-assay variability was calculated at three concentrations and gave CVs of <3.5% for each. Inter-assay variability was calculated and gave CVs of <3.5%. Potential isobaric interferences in the method were excluded and a C4 reference range was derived in serum by LC-MS/MS using the atmospheric-pressure chemical ionization mode. The quantitation limit for C4 was found to be 2.5 nmol/L (signal to noise ratio 87). Inter-assay variability was calculated at three concentrations and gave CVs of <3.5% for each. Inter-assay variability was calculated and gave CVs of <3.5%.

W93

**The effect of different storage conditions on faecal calprotectin analysis**

**I French1, M Brookes2, S Whitehead2**

1Birmingham Quality [UK NEQAS], Birmingham, United Kingdom, 2Royal Wolverhampton NHS Trust, Wolverhampton, United Kingdom, 3Russells Hall Hospital, Dudley, United Kingdom

Calprotectin is a 36kDa calcium and zinc binding protein accounting for ~40% of neutrophils’ cytosol. Measurement of calprotectin in faeces has been shown to be strongly correlated with 111-indium-labelled leucocytes-the gold standard measurement of intestinal inflammation. Such measurements may be used to help distinguish inflammatory bowel disease (IBD) from functional bowel disease (IBS). A nationwide study was undertaken to determine whether the conditions under which the stool sample was stored prior to analysis affected the results obtained. Full-day stool collections were provided by three different patients with IBD. Each stool was mixed well and split into six portions. Each of the six portions was stored under different conditions (room temperature for one week, room temperature for two weeks, 4°C for one week, 4°C for two weeks and two portions at -40°C). Following storage, the portions not already in the freezer were immediately frozen at -40°C to arrest the degradation process.

Sub-aliquots from each of the six portions from Patient 1 were dispatched through the UK NEQAS external quality assessment (EQA) programme for Faecal Markers of Inflammation at the same time. Sub-aliquots from Patients 2 and 3 were dispatched one month and two months later respectively. Participants were asked to analyse the specimens on receipt or perform the extraction step immediately and then freeze the extract.

Faecal calprotectin levels in stool fell on storage for prolonged periods at both room temperature and 4°C, but to varying degrees depending upon the initial concentration. There was no set pattern to the results obtained on the frozen specimens. Some increased slightly while others fell. The conditions under which stool samples are transported and stored prior to calprotectin analysis can affect the results obtained in the laboratory. This could result in delays in diagnosis or treatment with serious consequences for the patient.
W94

Effect of one year B and D vitamins supplementation on telomere length in elderly

J Pusceddu1, SH Kirsch1, C Werner2, R Obiedt1, U Hübner1, M Herrmann1, M Bodis1, U Laufs1, J Geisel2, W Herrmann2
1University of Saarland, Department of Clinical Chemistry and Laboratory Medicine, Homburg/Saar, Germany, 2University of Saarland, Department of Cardiology, Homburg/Saar, Germany

Background: Telomeres are essential for the maintenance of genomic integrity. Telomere length declines with age and telomere shortening/dysfunction has been proposed as a biomarker for age-related diseases. B and D vitamins are essential cofactors for numerous cellular processes including the synthesis of purines and nucleotides, DNA methylation, cell differentiation, proliferation and apoptosis. B and D vitamin deficiencies are risk factors for the development of age-related diseases. The aim of this study was to evaluate the effects of B and D vitamin supplementation on telomere biology in elderly people.

Methods: In a double-blind study 60 subjects (>54 years) were randomly assigned to receive a daily combination of vitamin D3 (1200 IU), folic acid (0.5 mg), vitamin B12 (0.5 mg), vitamin B6 (50 mg) and calcium carbonate (456 mg) (Group A) or vitamin D3 and calcium carbonate alone (Group B) for 1 year. Blood concentrations of 25-hydroxy-vitamin D, vitamin B12, folate forms and several metabolites were measured. Furthermore, LINE-1 methylation and telomere length in peripheral blood were analyzed at baseline and after 1 year of supplementation.

Results: Baseline gender- and age-adjusted telomere length correlated with methyl-tetrahydrofolate (r=0.35), 5,10-methenyl-tetrahydrofolate (r=0.36) and total folate (r=0.33). At the end of the study gender- and age-adjusted telomere length showed the following correlations: Group A: methylmalonic acid (r=-0.46), and choline (r=-0.39); Group B: 5,10-methenyl-tetrahydrofolate (r=-0.57), dimethyl-glycine (r=-0.39), and LINE-1 methylation (r=-0.43).

Conclusions: The present results provide evidence for an association between vitamin B status and telomere length in elderly subjects. One year of B and/or D vitamin supplementation substantially changes the pattern of correlations observed at baseline. This suggests an active involvement of these vitamins in telomere biology and genomic stability. The inverse relationship between telomere length and DNA methylation could be an appealing explanation that links telomere length with B vitamins.

W95

A single centre prospective evaluation of thiopurine metabolite monitoring in an Inflammatory Bowel Disease clinic

BK Mccann1, S Mackinnor2, K Smith1, AJ Morris2, DR Gaya2
1Glasgow Royal Infirmary, Department of Clinical Biochemistry, Glasgow, United Kingdom, 2Glasgow Royal Infirmary, Gastroenterology Unit, Glasgow, United Kingdom

The thiopurines, azathioprine and 6-mercaptopurine (6MP) are cheap and effective drugs used in the management of Inflammatory Bowel Disease (IBD). Increasing evidence suggests monitoring the metabolites of these drugs can personalise therapy to increase efficacy and limit toxicity. We set out to analyse the impact of metabolite testing in a tertiary referral IBD clinic.

The first 60 IBD patients (median age 42 years, 21 males) with normal TPMT activity, in whom initial thiopurine metabolite monitoring was requested, were identified and prospectively followed. Information on patient demographics, indication for testing, metabolite results and the effect of testing on clinical management were collated using the NHS Greater Glasgow and Clyde clinical portal. All patients had received either 2 mg/kg azathioprine or 1 mg/kg 6MP for at least three months prior to testing.

Using a weight-based dosing regime, only 12 patients (30%) had therapeutic concentrations (235-450 pmol/ 8 x 108 RBCs) of the active metabolite, 6TGN. In all, the clinical management of 32 patients (80%) changed following metabolite measurement..

Out of 24 patients with adverse side effects such as leucopenia or deranged LFTs, 18 (75%) had a change in therapy following metabolite measurement. Their dose of thiopurine was either reduced 12 (50%), stopped 2 (8%), or changed to a regime of allopurinol and low-dose thiopurine 4 (17%).

The introduction of thiopurine metabolite testing has been of major clinical utility in our IBD cohort. It allows personalisation of therapy and avoids escalation onto anti-TNF therapy or surgery in a significant proportion of patients.

W96

The prevalence of vitamin B1 (thiamine) deficiency in hospital patients referred for the assessment of thiamine status

K Deuchande1,2, DJ Card3, A Sołczyńska-Malefora3
1Viapath, Nutristasis Unit, Haemostasis & Thrombosis, London, United Kingdom, 2University of Surrey, Guildford, United Kingdom

Classical thiamine deficiency manifesting as beriberi, presents with severe cardiovascular, neurological and/or gastrointestinal disturbances. Other diseases relating to thiamine deficiency include Wernicke’s encephalopathy, Korsakoff’s (memory impairments) and Strachan’s (optic...
Relationship of selenium, zinc and copper with inflammatory and nutritional markers and impact on clinical outcome in malnourished patients

AS Davison, A Young, FJ Stokes, P Turner, V Mishra
Royal Liverpool and Broadgreen University Hospitals Trust, Department of Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

Aims: Malnourished patients usually have low serum selenium (Se), zinc (Zn), copper (Cu) concentrations either due to poor nutritional intake, gastrointestinal or cutaneous losses and redistribution into tissues.

Methods: This observational study was carried out using MUST (malnutrition universal screening tool), which showed poor nutritional status in all patients. Serum Cu, Zn and Se, pre-albumin, albumin and CRP concentrations were collected from the laboratory computer database. Statistical analysis included the assessment of significant differences (t-test and Mann Whitney) and correlations (Spearman’s rank) between the groups and the respective analytes studied.
Results: Study patients were split into two groups: survivors \((n=65)\) and non-survivors \((n=44)\). Serum Se, Zn and Cu concentrations were lower in non-survivors compared to survivors. Borderline significance observed with serum Se \((p=0.05)\). There was no significant difference in CRP, albumin and Pre-albumin between the two groups. Serum Se and Zn showed a significant negative correlation with CRP in survivors and non-survivors. A significant negative correlation with Cu and CRP was only seen in survivors. Se showed a positive correlation with pre-albumin and albumin in both groups. Cu showed a positive correlation with albumin in both groups. Zn showed a positive correlation with albumin in both groups, but only in the non-survivors did Zn show a positive correlation with pre-albumin. There was a significant negative correlation between CRP and albumin and pre-albumin in both survivors and non-survivors.

Conclusion: Our study suggests that serum Se, Zn and Cu should be interpreted in malnourished patients with the inflammatory markers as metabolic stress influences their concentration in blood. Serum Se, Zn, Cu cannot be considered as prognostic markers of clinical outcome in malnourished patients.

W99
Verification of an automated serum hyaluronic acid assay in NAFLD

L Russell, E Dow, WA Bartlett
NHS Tayside, Dept of Blood Sciences, Dundee, United Kingdom

Introduction: Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver disease from benign steatosis to non-alcoholic steatohepatitis, featuring inflammation and fibrosis. Liver fibrosis is an important feature to define as it may silently progress to cirrhosis and hepatocellular carcinoma. Definitive diagnosis and staging of fibrosis requires liver biopsy, which is expensive, unpleasant, has recognised technical difficulties and incurs a 1% risk of complication. Thus, a serum liver fibrosis marker is sought to improve patient safety and care. The efficacy of a promising serum biomarker, hyaluronic acid (HA), was investigated.

Methods: An automated serum assay for hyaluronic acid was verified and tested on a NAFLD cohort \((n=71)\). Results were compared to gold standard liver biopsy on paired samples.

Results: Assay verification demonstrated that within-run and between run imprecision, linearity, recovery and effects of common interferents were acceptable and comparable to manufacturer’s findings. Comparison with an alternative commercial HA assay identified a proportional positive bias.

Liver biopsy was avoidable in 78.9% of this cohort from exclusion of those with non-significant fibrosis \((HA<50\text{ ng/mL})\) and cirrhosis \((HA>200\text{ ng/mL})\). Those with intermediate concentrations of HA would require further investigation \((21.1\% \text{ of cohort})\). Serum HA < 50 ng/mL confirmed non-significant fibrosis with 82% sensitivity, 80% specificity, 91% positive predictive value \((PPV)\) and 64% negative predictive value \((NPV)\). Serum HA > 200 ng/mL confirmed cirrhosis with 89% sensitivity, 97% specificity, 80% PPV and 98% NPV.

Discussion: This assay performed well and showed HA to be promising serum marker for reducing the number of liver biopsies performed. Larger and longitudinal studies are warranted in order to fully assess the merit of HA in diagnosis and monitoring of liver disease. An automated assay such as this would be preferable to manual assays generating fewer errors, being available on existing commercial platforms and minimizing additional labour.

W100
Soluble transferrin receptors versus traditional iron studies as markers of iron status in the post-operative inflammatory response

AU Kelly, K Tober, A Duncan, P Galloway, D Talwar
1Glasgow Royal Infirmary, Department of Biochemistry, Glasgow, United Kingdom, 2Glasgow Royal Infirmary, Department of Anaesthetics, Glasgow, United Kingdom

Introduction: Non-invasive assessment of iron stores is desirable in patients with chronic inflammatory conditions to diagnose subclinical iron deficiency. Iron is also implicated in mediating oxidative stress, and a suitable marker of stores would aid research in this area. However, traditional biochemical markers of iron status (ferritin, iron, transferrin and transferrin saturation) are affected by the acute phase response.

There is evidence that soluble transferrin receptors \((s\text{TfR} - \text{the truncated form of membrane-bound transferrin receptors})\) are not affected by inflammation, and may be a more robust marker of intracellular iron content. This hypothesis does not appear to have been tested in severe inflammation, eg major surgery.

Aim: To assess whether sTfR values change in response to post-operative inflammation, and compare utility of sTfR to traditional markers of iron status.

Methods: Serum samples were collected pre-operatively and on day 6 post-operatively from 17 patients undergoing elective oesophagectomy at Glasgow Royal Infirmary, and analysed for sTfR, iron studies, CRP, albumin and haemoglobin. A reference range for soluble transferrin receptors was determined on samples from healthy volunteers.
**Results:** There was a CRP rise (median 1.2mg/L pre-op, 147.5mg/L day 6) and albumin fall (median 39g/L pre-op, 19g/L day 6) in response to inflammation.

Significant changes in iron parameters were noted, with rise in ferritin (median 179ng/ml pre-op, 568ng/ml day 6) and fall in calculated transferrin saturation (median 26.2% pre-op, 10.5% day 6). Most day 6 values were outwith reference intervals.

An sTfR fall was observed (median 18.73nmol/L pre-op, 15.19nmol/L day 6), which was not statistically significant (Friedman test p value 0.09). No post-operative results exceeded the upper reference interval.

**Conclusion:** We demonstrated no clinically significant changes in sTfR values during post-operative inflammation, compared to traditional markers of iron status. sTfR may be a more accurate marker of iron stores in the presence of inflammation.

**W101**

The value of direct antibiotic sensitivity testing on acute dysentry stool cultures, a novel laboratory approach

**FE Oronsaye¹, MT Michel², OMD Imasuen¹**

¹University of Benin, Medical Laboratory Science, Benin City, Nigeria, ²University of Benin, Benin City, Nigeria, ³University of Benin, Geology, Benin City, Nigeria

**Background:** Acute bacillary dysentery is a common health problem in the tropical countries. The routine method of microscopy culture and sensitivity (MC/S) for specimens sent to the medical microbiology laboratory, takes a minimum of 72hours for provisional diagnosis to be made and in developing countries where there is lack of modern technology for rapid diagnosis. Patients with acute bacillary dysentery suffer for longer period and this could be fatal. The purpose of this study therefore, was to determine the possibility of reducing the time spent on MC/S on stools from patients suffering from acute bacillary dysentery.

**Methods:** A direct sensitivity test was carried out on carefully selected portion of blood stained stool specimens from 100 patients with acute bacillary dysentery sixty of the patients were in patients in paediatric wards while forty of them were adults from out patients department, whose specimens were collected in the laboratory.

**Results:** Fifty of the specimens yielded a heavy pure growth of *Shigella flexneri* type 2, while the remaining fifty yielded a heavy pure growth of *Shigella flexneri* type 3. All the strains were sensitive to gentamicin, neomycin and kanamycin, but resistant to ampicillin, erythromycin, chloramphenicol and tetracycline. Al the patients were treated with 500mg/l of neomycin, mixed with kaolinTD/S; Those on admission were discharge after three days, to continue their treatment at homeDirect sensitivity test can speed up treatment for patients with acute bacillary dysentery.

**Conclusion:** This study presents a novel method of diagnosis for dysentery stools that mainly consist of mucus and blood stained. There was no significant difference between the in-patients and out-patients (P > 0.05) using the Chi-square comparison method.

**W102**

A retrospective audit of abnormalities in TPN patients

**R Bray**

St. George's Hospital, London, United Kingdom

Studies have shown that hospital patients on total parenteral nutrition (TPN) are vulnerable to abnormalities in their blood test results. A retrospective audit was carried out to identify common abnormalities in patients on a standard TPN regime based at one hospital. The standard TPN regime contains (per litre of TPN solution): 900-1200kcal energy, 100-175g glucose, 35-50g protein, 25-50g lipid, 25-3mmol potassium, 30-40mmol sodium, 2.5-5mmol magnesium, 7.5-20mmol phosphate, and 1L fluid. The audit used results from 39 TPN patients. The patients were split into two groups: those on short-term TPN (defined as 7-14 days of TPN) and those on long-term TPN (15-35 days of TPN). Results were taken for a period of 7 days during their time on TPN and included electrolytes, liver function tests, bone profile, CRP and glucose. Abnormalities were noted and the following observations were made. Low albumin results were observed in 95% of short-term and 85% of long-term patients. Raised CRP results were observed in 89% of short-term and 65% of long-term patients. Low albumin and raised CRP in these patients indicates the presence of inflammation and/or infection with an acute phase response. Raised ALP and GGT results were seen in 53% of short-term and 60% of long-term patients. This pattern indicates the presence of cholestatic liver disease. Raised ALT was seen in 60% of long-term patients indicating the presence of hepatocellular damage. However, 50% of these were transiently raised. Raised glucose results were seen in both short-term and long-term TPN patients (51%); however 75% of these were transiently raised. This audit has confirmed a common pattern seen in TPN patients; that of inflammation with liver cholestasis. This was consistent with both short-term and long-term patients and identifies a potential area of improvement in the monitoring of TPN patients.
W103

Can an automated faecal immunochemical test (FIT) determine whether faecal haemoglobin (f-Hb) concentrations can aid in stratifying symptomatic patients referred for colonoscopy

IM Godber1, LM Todd1, CG Fraser2, L Smith4, L McDonald4, H BenYounes4
1Monklands Hospital, Department of Biochemistry, Lanarkshire, United Kingdom, 2University of Dundee, Centre for Research into Cancer Prevention and Screening, Dundee, United Kingdom, 3University of Strathclyde, Department of Mathematics and Statistics, Glasgow, United Kingdom, 4Wishaw General Hospital, Department of Surgery, Lanarkshire, United Kingdom

This study aimed to determine whether faecal haemoglobin concentrations (f-Hb) could aid in stratifying symptomatic patients referred for lower gastrointestinal (LGI) tract endoscopy and whether it could be used to fast-track the need for a colonoscopy.

f-Hb were measured on single samples from 507 patients who also underwent a LGI endoscopy within NHS Lanarkshire in 2013-14. Age ranged from 15 to 89 years (median: 60). f-Hb was measured using an automated Faecal ImmunochemicalTest (FIT) on the HM-JACKarc analyser (Kyowa Medex, Japan).

Results showed f-Hb increased significantly with age (p=0.018) and was higher amongst men (p<0.0001); on average men were 5 years older than women. F-Hb was higher in those with benign and malignant organic bowel disease (p<0.0001) but had no association with diverticular disease. No association was found between f-Hb and number and/or size of polyps.

11 (2.2%) participants were found to have adenocarcinoma, all of whom had f- Hb >150 μg Hb/g faeces thus illustrating potential use in the referral and treatment pathway for colorectal cancer. At a cut-off of 10 μg Hb/g, the NPV for cancer and high-risk adenoma combined was 94% with a sensitivity of 54% and a specificity of 78%; receiver operating characteristic curves are presented.

Paediatrics and IEM

W104

Alkaline phosphatase reference intervals

A Dodd1,2, P Thomas1
1Bristol Royal Infirmary, Bristol, United Kingdom, 2East Kent Foundation Hospital Trust, Biochemistry, Ashford, United Kingdom

Introduction: Paediatric reference intervals that change with age are notoriously difficult to define. Reference intervals for alkaline phospha tase for paediatric patients at the University Hospitals Bristol Trust were considered to be due for review. Recent published data are available for healthy children using Abbott Architect methodology in Canada (CALIPER study), and for a Danish population between ages five to nineteen years using Roche modular, but the applicability of these to a British population using Roche modular methodology was unclear.

Methods: Three and three quarter years of data were collected from the laboratory database (N=1,036,063 total adults and children). All results extracted from the laboratory database were analysed on Roche modular analysers, with the exception of 25-OH vitamin D by LC-MS/MS. Data were filtered to include only results requested from primary care, and exclude requests with abnormal creatinine (Roche enzymatic), bilirubin, gamma glutamyl transferase, alanine transaminase, calcium, 25-OH vitamin D, parathyroid hormone, glucose or HbA1c. Data were then filtered by age and parametric reference intervals calculated by least-squares fitting. For teenage years, a bimodal distribution was seen and these were fitted with a bimodal Gaussian to calculate reference intervals.

Results: Results indicated that the current paediatric intervals in use were not suitable. Similarly, these local data did not match reference intervals at the closest local trust or those recommended by Pathology Harmony. Paediatric results were seen to closely mirror the results from Norwegian study and the CALIPER study performed in Canada.

Conclusions: Our data locally validate the reference intervals proposed by the CALIPER study, and regional harmonised reference intervals have been proposed based upon this. It may be appropriate to reconsider Pathology Harmony recommendations on paediatric alkaline phosphatase reference intervals in light of recent publications.

W105

Ivacaftor: a new treatment for cystic fibrosis which results in reduced sweat chloride and improved clinical outcomes

L Nedevska1, N Bell1, K Bateman1, L-H Simon1, A Bowron1
1Bristol Royal Infirmary, Clinical Biochemistry, Bristol, United Kingdom, 1Bristol Royal Infirmary, Bristol Adult Cystic Fibrosis Centre, Bristol, United Kingdom, 1Bristol Royal Hospital for Children, Department of Paediatric Respiratory Medicine, Bristol, United Kingdom

Background: Cystic fibrosis (CF) is a life-limiting disorder caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Treatment aims to minimising the effects of impaired chloride transport and mucus accumulation on the respiratory and gastrointestinal systems.
Ivacaftor is a CFTR channel modulator which corrects the defect caused by the CFTR gating mutation G551D found in 4% of CF patients. Treatment of those carrying at least one G551D mutation started in England in January 2013.

**Aim:** To assess biochemical and clinical response to ivacaftor in patients attending a regional adult and paediatric CF service.

**Method:** 9 adults (mean age 27 years, range 22-36) and 2 children (aged 6 and 10 years) began treatment with ivacaftor. Sweat chloride was recorded before and 6 weeks and 6 months after starting. Number of days spent in hospital, number of days of intravenous antibiotics and in adult patients, forced expiratory volume (FEV$_1$) and weight were compared in the year before versus the year after starting treatment.

**Results:** Sweat chloride was 99.2 (87.0-111.4) mmol/L (mean, [95% CI]) at baseline and fell to 44.3 (37.2-51.3) mmol/L after 6 weeks’ treatment (p < 0.0001, t-test). The fall in sweat chloride was sustained at 6 months 42.0 (31.6-52.4) mmol/L (p < 0.0001).

During the year before treatment, 123 days were spent in hospital and 345 days on intravenous antibiotics. These fell to 82 and 206 respectively the following year. In adults, FEV$_1$ increased by 7.4% (-3.4-22.3) (mean, range) and weight increased by 1.5kg (-1.7 -3.8kg).

**Conclusion:** Treatment of CF with ivacaftor results a sustained fall in sweat chloride and improvements in lung function, weight, hospital admission and antibiotics use. This is the first treatment of CF at the molecular level; early results are encouraging and demonstrate the potential for treatments of the effects of other CFTR mutations.

**W106**

**Zellweger syndrome and Cri Du Chat in a baby boy**

**KM Stepien**, TH-Y Wu, A Morris

Salford Royal Foundation Hospital Foundation Trust, Clinical Biochemistry Department, Salford, United Kingdom, Central Manchester University Hospitals NHS Foundation Trust, Willink Biochemical Genetics, Manchester, United Kingdom

**Case:** A boy born to Jewish non-consanguineous parents presented shortly after birth with congenital hypotonia, apnoea, bradycardia. He was noted to have classical dysmorphic features associated with Zellweger Syndrome, including high arched palate, hypertelorism, shallow supraorbital ridges, epicanthic folds, micrognathia, low set ears, large fontanelles, redundant skin folds in the neck and a single palmar crease. Echocardiogram showed Ventricular Septal Defect, Patent Ductus Arteriosus, Patent Foramen Ovale. His cranial ultrasound showed multiple subtle dysplastic features. There was mild hepatic splenomegaly on the abdomen ultrasound.

The definite diagnosis was confirmed by VLCFA analysis (raised C26/C22 and C26/C22 ratios) on two separate samples. Additionally, the chromosome analysis via micro-array indicated Cri Du Chat syndrome (25Mb deletion of the terminal short arm of chromosome 5, bands p14.1 to p15.33). He required CPAP for the first 7 days of life at the Neonatal Intensive Care Unit. His CRP was raised at 131 mg/L and the management included a hyoscine patch in an attempt to control secretions and antibiotics: Benzylpenicilin, Gentamycin, Vitamin K, Cefotaxime. Baby had a slowly increasing oxygen requirements during his stay (0.9 L/min). He also developed episodes of apnoea, which progressively become more frequent in the last few days of his life and led to respiratory distress. Boy died at the age of 4 weeks.

He was the fourth child of his parents and the second with this condition. His sister died of Zellweger syndrome (PEX2 gene, c.355C>T, p.Arg119X) complications at the age of 2 months. Despite the family history of the condition, parents refused prenatal testing.

**Conclusions:** This case presents an extremely rare coincidental diagnosis of both metabolic conditions in a newborn. Zellweger Syndrome is a life limiting condition. Cri Du Chat has some implications for any future pregnancies. Both conditions warrant prenatal diagnosis and genetic counselling.

**W107**

**Delayed neonatal argininosuccinic aciduria presentation with a good outcome following therapeutic cooling; CSF, plasma and urine amino acid findings**

O Jackson, V Powers, T Thorpe, E Chronopolou, A Bowron

Bristol Royal Infirmary, Department of Clinical Biochemistry, Bristol, United Kingdom, Bristol Royal Hospital for Children, South West Regional Metabolic Department, Bristol, United Kingdom

Argininosuccinic aciduria (ASA) is a rare inborn error of metabolism caused by deficiency of the urea cycle enzyme argininosuccinate lyase, resulting in elevated argininosuccinate. Delayed diagnosis results in severe hyperammonaemia, causing neurological damage and if untreated may cause encephalopathy and death in the neonatal period. We present a case of ASA with a delayed presentation and good outcome possibly due to therapeutic cooling.

Patient X was unexpectedly born in a poor condition suffering from hypoxic ischaemic encephalopathy. He was immediately therapeutically cooled and put on 10% dextrose infusion. After 72 hours, cooling was stopped and oral feeding (breast milk) slowly commenced. Over the next 24-48 hours the patient became quiet, floppy, unresponsive and developed seizures. He suffered a cardiac arrest on day 6. He was intubated, feeds were stopped and dextrose infusion resumed. A metabolic screen demonstrated he was severely hyperammonaemic (1700 μmol/L). He was given sodium benzoate and Carbiglu, and haemofiltration was commenced in order to lower his plasma ammonia.
CSF, plasma and urine amino acids showed high concentrations of argininosuccinate and citrulline as well as increased ammonia transporter amino acids glutamine and alanine. Urine orotic acid was also increased. These findings were consistent with ASA. He improved neurologically on treatment and was discharged home on a low protein diet, sodium benzoate and arginine. He is making good developmental progress and will continue to be monitored.

A good outcome in neonates with urea cycle disorders is very rare. This patient underwent therapeutic cooling (which reduces enzyme activity in-vivo) within 1 hour of birth and was not given protein until day 4. These two factors may have avoided the early accumulation of ammonia and contributed to the unexpectedly good outcome.

W108

Specific allergens, triggers for urticaria in children

CA Grigore¹, NN Grigore²
¹Universitary Children’s Hospital, Laboratory Medicine, Sibiu, Romania, ²University Hospital Sibiu, Sibiu, Romania

Background: Many children have urticaria and the first clinical suspicion is allergy. In order to help clinicians in their diagnosis, laboratory has to give the biological evidence of allergy and one way we prove this is to detect the level of specific IgE antibodies against different allergens and find a connection with the disease.

Methods: We use an enzyme immunoassay on a nitrocellulose membrane (immunoblot), test that gives a semi-quantitative detection of IgE antibodies specific for 20 different respiratory and food allergens in human serum. We read the results on a photometric analyzer that gives results in 6 EAST classes.

Results: We tested 81 children with urticaria. In 53% of the patients (43/81) there were no high levels of specific IgE antibodies. The other 47% of the patients (42/81) had high levels of different specific IgE antibodies. Most of them, 42% (18/42) had high levels of antibodies against respiratory allergens. From the food allergens tested, 33% (14/42) had high levels of antibodies for Casein, Pasteurised milk, alpha lactoglobulina, 21% (9/42) for beta lactoglobulina, 12% (5/42) for white egg, 9% (4/42) for wheat.

Conclusions: Using this panel with 20 IgE specific allergens, we found which are the most frequent allergens that give high levels of specific IgE antibodies in children with urticaria and combining this with the clinical history we can advise what triggers be avoided.

W109

Analysis of the impact of new European guidelines for coeliac diagnosis in paediatrics

A Dodd¹,², P Thomas²
¹Bristol Royal Infirmary, Bristol, United Kingdom, ²East Kent Foundation Hospital Trust, Biochemistry, Ashford, United Kingdom

Introduction: Recent guidelines from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition have suggested using a combination of serology and genotyping to avoid need for biopsy to diagnose coeliac disease in children with tissue transglutaminase (TTG) results greater than ten times the upper limit of normal. However, the evidence behind their final guidelines is unclear.

Methods: All positive paediatric (age < 18 years of life) TTG results (>10 U/L, Orgentec immunoassay) were extracted from the laboratory database over one year and any further relevant serology, genotyping or biopsy results on these patients were reviewed. Previously diagnosed coeliac patients were excluded.

Results: One hundred and sixteen new patients had a positive TTG result. Of these, 92 were biopsied, six of which were negative and the remainder were all diagnosed with coeliac disease. The TTG result was below 50 U/L for all negative biopsies. Under the new guidelines, using a cutoff of 100 U/L, 58 patients would have not required biopsy. A further 21 biopsies would have been avoided if a cutoff of 50 U/L had been used.

Conclusions: The suggestion by the European Society to avoid biopsy in children with strongly positive TTG results appears to be safe and efficient, with benefits for both the NHS and patients. However, no evidence of potential further value was observed for additional genotyping or for endomysial antibody testing. The proposed serology cutoffs are based on limited evidence, and are likely to require assessment before introduction due to variation between assays.

W110

Alkaptonuria: an LC-MS/MS method for diagnosis, treatment and monitoring

AT Hughes¹, AM Milan¹, JA Gallagher², JJ Dutton¹, L Ranganath¹
¹Royal Liverpool and Broadgreen University Hospital Trust, Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom, ²University of Liverpool, Musculoskeletal Biology, Liverpool, United Kingdom

Introduction: Alkaptonuria (AKU) is a rare, autosomal recessive, inborn error of metabolism caused by a deficiency of homogentisate 1,2-dioxygenase. An accumulation of homogentisic acid (HGA) in plasma and increased urine excretion of HGA subsequently occurs. HGA
oxidises to a melanin-like ochronotic pigment which deposits in cartilage and connective tissues. This results in early onset degenerative arthritis, pain and early joint replacement. The National AKU Centre at Liverpool currently provides nitisinone (NTBC) to AKU patients to evaluate its effectiveness in the treatment of AKU.

Methods: A method has been developed and validated to measure serum tyrosine, HGA and NTBC, and urine tyrosine and HGA. Using an Agilent 6490 Triple Quadrupole mass spectrometer, chromatographic separation was achieved on an Atlantis C18 column (100mm x 3.0mm, 3μm, Waters). The primary outcome was to monitor the response to NTBC therapy by measuring the change in urine HGA excretion. In addition the safety aspects of treatment were monitored, due to the concurrent rise in serum tyrosine.

Results: The LC-MS/MS assay was fully validated:- inter- and intra-batch accuracy was +/- 15% for all analytes; precision was < 10% for all analytes both intra- and inter-batch with no carryover in either serum or urine matrices. HGA showed linearity up to x10 dilution. Patient results demonstrated a 60% drop in circulating HGA by day 4, and >94% reduction in HGA excretion at 12 months. Serum tyrosine was significantly elevated post NTBC treatment.

Summary: We have established and validated methods for serum tyrosine, HGA and NTBC and urine tyrosine and HGA. The assays are linear over several orders of magnitude to encompass pre and post NTBC treatment levels of HGA. The methods have been demonstrated as fit for purpose for NTBC clinical trials and in conjunction with SOBI, provide trial data to enable licensing of NTBC for AKU treatment.

W111

An audit of specialist enzyme diagnoses made by the south west regional specialist enzyme laboratory at Bristol Royal Infirmary

V Powers1, A Bowron1, G Pierre2, H Stroud1, P Thomas1
1Bristol Royal Infirmary, Bristol, United Kingdom, 2Bristol Royal Hospital for Children, Bristol, United Kingdom

Background: The Bristol Royal Infirmary specialist enzyme laboratory provides a South West UK service for diagnosis of lysosomal storage diseases (LSDs). LSDs are rare inherited metabolic disorders with defective lysosomal degradation of macromolecules, resulting in accumulation of storage products, tissue and organ dysfunction and often severe, progressive disease. They are diagnosed biochemically by demonstrating enzyme deficiency in vitro. It is our laboratory’s protocol to confirm positive diagnostic cases by repeat enzyme analysis or alternative testing e.g. genetics. The audit aim was to review diagnostic cases made over the last 10 years (from January 2003 to August 2013) to determine whether this protocol was followed.

Audit standards:
– A repeat sample requested on the laboratory report in positive diagnostic cases (target 100%);
– If repeat enzyme analysis was not done, a second-line test should have been carried out (target 100%, excluding affected siblings).

Results: 110 cases were reviewed. A request for a confirmatory sample was made in 63 cases (57%). Of these, a repeat sample was received in 39 and the initial results confirmed in all. 13 of the 71 cases in which the diagnosis had not been confirmed by repeat enzyme analysis were siblings of index cases and therefore excluded. Of the remaining 58 cases, evidence of second-line testing was found in 44 (76%). 14 cases were lost to follow up.

Discussion: This audit has demonstrated the laboratory’s protocol for confirmation of diagnoses was not always followed, and that when requested these samples were not always received. All repeat tests performed confirmed the diagnosis. The laboratory has introduced clear guidelines for reporting diagnostic cases and a follow-up system to chase repeat samples and ensure the laboratory is made aware of confirmatory testing if done elsewhere. This provides confidence in the diagnostic service. Re-audit is planned in 2 years.

W112

Can haemoglobin A1c levels be used to diagnose diabetes mellitus in children and adolescents?

FM Ivison, CM Chaloner
Central Manchester University Hospitals NHS Foundation Trust, Clinical Biochemistry, Manchester, United Kingdom

Introduction: In a symptomatic adult patient who does not have any contraindication to measurement, blood haemoglobin A1c (HbA1c) level above 48 mmol/mol is accepted as a diagnostic test result for Diabetes Mellitus (DM). However there is no consensus on use of HbA1c for diagnosis of DM among children.

Aim: To evaluate whether HbA1c and a standard Glucose Tolerance Test give concordant results for diagnosis of DM among children.

Methods: The Laboratory Information Management System was interrogated to extract contemporaneous GTT and HbA1c data between April 2012 and July 2013. HbA1c was measured using the Siemens DCA Vantage. Data were analysed for agreement using diagnostic cut-offs as described by the WHO (2006 and 2011 update).

Results: There were 28 individuals who had GTT and HbA1c done at the same time (19 girls and 9 boys), median age was 13.2 years (range 5.5 to 17.6 years). The reasons for testing were Cystic Fibrosis annual review in 17 (59%); obesity in 5 (17%); and other endocrine indications
in 6 (24%). In our cohort 2 subjects (7%) had fasting plasma glucose below 6.1mmol/L but HbA1c >48mmol/L and 6 subjects (21%) had glucose >11.1mmol/L but HbA1c < or = 48mmol/mol. 

**Conclusions:** Further studies are required to understand whether HbA1c is under diagnosing DM, or GTT is over diagnosing the condition. Evaluation of diagnostic accuracy is undermined by the absence of a diagnostic definition for DM that is independent of the diagnostic test used. Our results support a cautious approach before HbA1c is adopted as a diagnostic standard for DM in children.

**W113**

Development of a rapid liquid chromatography tandem mass spectrometry dried blood spot screening test to exclude classical galactosaemia in neonates

*R Curd, D Cregeen, M Jackson, S Gillingwater, R Carling*

*Viapath, Guys’ and St Thomas’ NHS Trust, London, United Kingdom*

**Introduction:** Classical galactosaemia is a rare inherited metabolic disease caused by a deficiency of galactose 1-phosphate uridyltransferase (GALT). This results in the accumulation of toxic metabolites including galactose 1-phosphate. Galactosaemia presents with non-specific symptoms (including liver failure) following the introduction of milk feeds and if untreated can be fatal during the neonatal period. Existing screening tests for galactosaemia are prone to false positives and false negatives.

**Aim:** The aim was to develop a rapid dried blood spot (DBS) screening test to exclude galactosaemia in neonates.

**Method:** An ultra performance liquid chromatography tandem mass spectrometry method measuring aldose monophosphate (AMP) as a surrogate marker of galactose 1-phosphate in DBSs has been developed and validated. This method utilises a HILIC column with gradient elution and has a run time of 6.4 minutes.

**Results:** The method was linear up to at least 8 mmol/L (y = 0.347x + 0.0379, R^2= 0.9977, n=3). The method has acceptable precision (intra-assay precision less than 11.04%, n=10 and inter-assay precision less than 6.74%, n=20 tested at three concentrations of galactose 1-phosphate 0.31 mmol/L blood spot standard, 1 mmol/L solvent standard and 2 mmol/L blood spot standard). The limit of detection is 0.06 mmol/L and the limit of quantification is 0.1225 mmol/L. It was not possible to assess the accuracy of this test because there is no EQA scheme, no gold standard test and no other laboratory in the UK measuring galactose 1-phosphate in DBSs. However, clinical utility was demonstrated by analysis of 100 control samples and 40 samples from confirmed galactosaemic patients.

**Conclusion:** A rapid DBS screening test has been developed that can be used to exclude classical galactosaemia in neonates.

**W114**

Demand management of urine amino acid analysis?

*RS Carling¹, I Tooth¹, A Bowron²*

¹Viapath, Biochemical Sciences, London, United Kingdom, ²St George’s Hospital, Clinical Biochemistry, London, United Kingdom, ³Bristol Royal Infirmary, Clinical Biochemistry, Bristol, United Kingdom

**Aim:** To determine current practice and opinion on the use of urine amino acid (UAA) analysis in clinical biochemistry laboratories and to establish guidelines for service users.

**Methods:** A survey was prepared, peer-reviewed and distributed via the Metabolic Biochemistry Network (MetBioNet). Responses were received from 18 laboratories, who together receive approximately 15,000 UAA requests annually.

**Results:** Respondents agreed that UAAs should be analysed to investigate patients with renal stones and renal tubular disorders, both primary and secondary to inherited metabolic disease (IMD). However, there was no agreement on the clinical utility of UAA in the investigation of IMD. Some consider it an appropriate first line investigation for IMD, others view it as an important second line test in a small number of patients and some have reduced their workload significantly (up to 90%) by introducing demand management protocols. These differences of opinion did not correlate with laboratory size, workload or MetBioNet stakeholder status.

**Discussion:** UAA analysis costs the NHS approximately £1.2M annually. If a most of these requests are inappropriate, as a profession we have an obligation to act. Whilst the lack of published standards makes it difficult to establish evidence-based protocols, this must not be used as an excuse and our goal must be to agree on a common approach. The move to specialised commissioning arrangements for metabolic laboratory services reinforces this.

The authors would not recommend UAA analysis as a first line investigation for an IMD. We would recommend demand management, as already successfully implemented in some metabolic reference laboratories, combined with educating users as to the appropriate use of the test and the importance of accurate clinical information. Reflex testing UAA requests would be done by the reference laboratory on the basis of clinical details, results of other investigations (e.g. urine organic acids) and specific queries.
W115

**Validation of a short ion exchange chromatography method for the quantitation of urinary cystine, ornithine, arginine and lysine**

K Rawstron¹, L Dron², M Dowley¹, R Carling¹

¹Viapath, St. Thomas’ Hospital, Inherited Metabolic Disease Laboratory, London, United Kingdom, ²Royal Free London NHS Foundation Trust, Department of Clinical Biochemistry, London, United Kingdom

**Aim:** To develop and validate a short analytical method on the Biochrom 30+ Amino Acid Analyser (AAA) for the quantitation of urinary cystine, ornithine, arginine and lysine. To utilise this method for the analysis of patient samples sent for investigation of urinary stones and for the routine monitoring of patients with cystinuria.

**Method:** The Biochrom 30+ AAA is considered the gold standard in amino acid analysis. It utilises cation exchange chromatography coupled with post column ninhydrin derivatisation to separate and quantitate physiologically important amino acids in a 125 minute run. We have adapted this method to produce a shorter run time, suitable for the quantitation of cystine, ornithine, arginine, lysine and potentially the drug metabolite penicillamine-cysteine mixed disulphide.

**Results:** A new method was successfully developed with a total run time of 99 minutes. Within-batch (n=20) and between-batch (n=10) precision studies showed CV < 4% at two clinically relevant concentrations for cystine, ornithine, lysine and arginine. A method comparison with the current method using samples from patients with cystinuria showed an average difference of less than 7% for all analytes (n=12). Linearity was observed up to 1250 umol/L.

**Discussion:** The results demonstrate that this is a robust method that can be used for the routine diagnosis and monitoring of patients with cystinuria. This method provides decreased analysis time without loss of resolution, increasing the capacity of the analyser and reducing costs compared to the current method. There is scope to develop the method to reduce run times further and to include routine penicillamine-cysteine quantitation, potentially improving clinical utility as a monitoring tool.

W116

**Citrullinemia: a case study**

E Evans¹, L Rowbottom², C Collingwood², P Newland², A Morris³

¹Lancashire Teaching Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, Preston, United Kingdom, ²Alder Hey Children’s NHS Foundation Trust, Department of Clinical Biochemistry, Liverpool, United Kingdom, ³Central Manchester University Hospitals Foundation Trust, Paediatric Metabolic Disease, Manchester, United Kingdom

A 3 day old term male presented to A&E with poor feeding, lethargy and respiratory distress following an uncomplicated birth. He was born to non-consanguineous parents with no family history of metabolic disorders. Blood gas analysis revealed mixed acidosis (pH 7.20) with plasma lactate of 3.9mmol/L [0.7 -2.1mmol/l]. Further investigations showed raised plasma ammonia (1361 μmol/L [0-70 μmol/L]) and plasma amino acid analysis identified significantly raised glutamine (4975 μmol/L, [46-1400 μmol/L]), glycine (712 μmol/L, [178-248 μmol/L]), citrulline (2500 μmol/L, [0-55 μmol/L]) and low arginine (44 μmol/L [53-71μmol/L]). Urine organic acids analysis also revealed the presence of orotic acid. Hyperammonemia with elevated citrulline, low/normal levels of the other urea cycle intermediates and increased excretion of orotic acid are suggestive of citrullinemia.

Citrullinemia is an autosomal recessive urea cycle disorder (UCD), caused by deficiency of argininosuccinate synthetase. Patients with the acute neonatal form appear normal at birth, however shortly thereafter, they develop hyperammonemia, become progressively lethargic and feed poorly. Without prompt intervention, hyperammonemia and the accumulation of other toxic metabolites (e.g. glutamine) result in encephalopathy, seizures and may lead to coma and death.

Treatment for this neonate included the administration of sodium benzoate, sodium phenylbutyrate and L-arginine to promote the removal of ammonia. Despite treatment, his ammonia levels continued to rise, peaking to 2023 μmol/L. Continuous venovenous hemodialfiltration was commenced which reduced his ammonia to 55μmol/L within 24hrs. Since then his ammonia level has remained within the normal range (30μmol/L) and his feeding has improved. The long term effects of exposure to increased ammonia concentrations are still to be ascertained.

Classically, in UCDs, hyperammonemia causes respiratory alkalosis through stimulation of the respiratory centre. Neonatal hyperammonaemia with metabolic acidosis is more typical of the branched-chain organic acidemias. Metabolic acidosis can, however, occur in UCDs as hyperammonaemia leads to vasomotor instability. This case demonstrates the importance of measuring ammonia in acutely sick neonates.
W117
Establishing an internal quality control material for lysosomal enzyme analysis

F Oates, H Stroud, L Nedevska, S Woollaston, V Powers
Bristol Royal Infirmary, Bristol, United Kingdom

Background: Lysosomal enzymes are analysed in the investigation of lysosomal storage diseases (LSDs) which typically result in severe, progressive disease. The lysosomal enzyme screen for the diagnosis of LSDs in our laboratory involves manual analysis of 14 enzymes in leukocytes. There is no commercial quality control (QC) material for lysosomal enzyme analysis in leukocytes. Diagnosing a LSD is life-changing, therefore control of assay performance is critical.

Aim: To prepare in-house QC material for use in monitoring lysosomal enzyme assay performance in our laboratory.

Method: Discarded K-EDTA blood samples (n = 40 per batch of QC material) were obtained following completion of routine full blood count analysis. They were less than 24 hours post-venepuncture and were anonymised. Leukocytes were extracted from these samples in batches of eight using a modification of the red cell lysis method used for routine patient sample preparation. The extracted leukocytes were pooled with careful mixing to produce a homogeneous QC material from which 30 aliquots were prepared. The QC was stored at -70 °C until required. One aliquot was analysed with each routine batch of lysosomal enzymes.

Results: The QC has been in use for 6 years, analysed with each batch of lysosomal enzymes per week. Between batch CVs for the 14 individual assays varies from 6.7% to 13.6% (based on October 2013-Jan 2014 data). Degradation of the QC material was observed after 4 months.

Conclusion: A QC material for use in lysosomal enzyme assays has been developed and put into routine use. Due to sample degradation, fresh QC is prepared every four months. Use of this QC has overcome the challenge of control of esoteric laboratory investigations for which no commercial QC is available and has given us confidence in our assays performance and ultimately the diagnostic service we provide.

W118
Argininosuccinic aciduria: a case study

AW Beardsmore1, L Rowbottom2, C Collingwood2, P Newland2, A Morris3
1Alder Hey Children’s Hospital, Department of Clinical Biochemistry, Liverpool, United Kingdom, 2Alder Hey Children’s Hospital, Biochemistry, Liverpool, United Kingdom, 3Royal Manchester Children’s Hospital, Manchester, United Kingdom

Ammonia produced by the deamination of amino acids is converted to urea, via the urea cycle and excreted in urine. Defects in the enzymes involved in the urea cycle result in an increased concentration of plasma ammonia. Hyperammonaemia is a clinical emergency which if left unmanaged carries a high risk of morbidity and mortality due to the neurotoxic properties of ammonia. Here we present a case of a female neonate born at a district general hospital who developed poor feeding and respiratory alkalosis 24 hours after birth. She was found to have an ammonia concentration of 802umol/L [0-70umol/L]. At two days of age she was transferred to a tertiary paediatric hospital. On arrival she was commenced on IV L-arginine, sodium benzoate, sodium phenylbutyrate, 10% dextrose and received continuous hemofiltration. Urgent intervention resulted in a rapid reduction in the concentration of plasma ammonia reaching levels within the reference range 53 hours post admission. Urgent plasma and urine amino acid analysis showed the presence of argininosuccinate which when quantified in the urine was shown to be grossly increased (>1853 umol/mmol of creatinine). In addition, citrulline was shown to be increased in both plasma (319umol/L [0-55umol/L]) and urine (175umol/mmol of creatinine [0-11umol/mmol of creatinine]). High concentrations of plasma ammonia with increased argininosuccinate and citrulline in the plasma or urine supports the diagnosis of argininosuccinic aciduria (ASA), a urea cycle disorder caused by a mutation in the enzyme argininosuccinate lyase. Argininosuccinate lyase catalyses the conversion of argininosuccinate to fumarate and arginine, arginine being the final metabolite in the urea cycle prior to conversion to urea. An enzyme block in this stage of the urea cycle results in the accumulation of ASA and citrulline in the blood and prevents the conversion of ammonia to urea resulting in hyperammonaemia.

W119
An audit on intermediary metabolites in the Thames region

N Costa
Great Ormond Street Hospital, Chemical Pathology, London, United Kingdom

Aim: Intermediary metabolite analysis is an essential tool in the investigation of inherited metabolic diseases. An audit was conducted in the Thames Region to investigate how laboratories conduct analyses of intermediary metabolites. The metabolites of interest included glucose, non-esterified fatty acids, beta-hydroxybutyrate, acetoacetate, lactate, pyruvate and ammonia. The aim was to identify common practices
of their analysis, including sample types, sample handling and transportation, methods, reference ranges and clinical interpretation. The responses were used to devise Best Practice Guidelines for the analysis of these metabolites.

**Methods:** An audit questionnaire was distributed by e-mail to the laboratories of the Thames region and responses collated. Participating laboratories were asked about their provision for analysis of the intermediary metabolites listed above, and their common practices regarding the pre- and post-analytical processes, including point-of-care testing. The findings of the audit were presented to the region in a half day meeting. The results of the audit were discussed and best practice guidelines for analysis of these metabolites were agreed and distributed.

**Results:** A total of 26 laboratories responded to the audit and their questionnaire answers were collated. A number of similarities were noted between all laboratories with regard to their recommendations for specific sample types for each of the analytes. However, the audit also highlighted many differences in the pre- and post-analytical handling of the samples, in particular how or when the samples should be transported or received within the laboratory, the use of sample indices, phoning limits and reporting interpretive comments.

**Conclusions:** By highlighting the differences in practice across the region and discussing the variety of laboratory processes, we were able to establish best practice guidelines. This will enable a standardised approach for the analysis of these metabolites within the region and improve the quality of the service to our patients.

**W120**

**Serum concentrations and urinary excretion of homogentisic acid and tyrosine in normal subjects**

AS Davison, AM Milan, AT Hughes, JJ Dutton, LR Ranganath
Royal Liverpool and Broadgreen University Hospitals Trust, Department of Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

**Introduction:** Alkaptonuria (AKU) is a rare, inherited metabolic disease of tyrosine metabolism. Degradation of tyrosine is blocked at the level of homogentisic acid (HGA) due to a congenital lack of the enzyme homogentisate 1,2-dioxygenase. Medical management of AKU has been palliative relying largely on analgesia and arthroplasty. With the establishment of a National Alkaptonuria Centre in Liverpool, nitisinone (NTBC), is being prescribed to AKU patients. Monitoring of off-licence therapy is essential for patient safety. NTBC has resulted in a >95% suppression of HGA excretion with a concurrent rise in serum tyrosine (>500 μmol/L). On-going clinical trials as part of the DevelopAKUre program are assessing the dose of NTBC which suppresses HGA to within the normal healthy population reference range. This study was aimed at determining the reference range of HGA and tyrosine in serum and urine, in a non-AKU population.

**Methods:** Ethical approval was obtained (NRES No 07/H1002/11) and 22 volunteers provided fasting serum samples and 24hr acidified urine collection. Samples were analysed using liquid chromatography tandem mass spectrometry in the laboratory that provides the service to the National AKU Centre. Data were examined for normality using Kolmogorov-Smirnov and Shapiro Wilks statistics.

**Results:** Serum HGA was below the lower limit of quantification (LLOQ) of 3.1 μmol/L and urine HGA could only be determined in 7 out of 22 subjects (LLOQ 1.0 μmol/L) with the highest excretion observed being 2.91 μmol/24hr (~0.49mg/day). Serum tyrosine was 30-87 μmol/L (normally distributed) and urine tyrosine 14-147 μmol/24hr.

**Conclusions:** Tyrosine reference ranges and serum HGA are comparable with previously published. Urine HGA reference range < 2.92 μmol/24hr is significantly lower than previously published, however this is the first reference range determined by sensitive LC-MS/MS in comparison with previous methodology using indirect colorimetric assay. The determined reference range for urine HGA will provide an invaluable tool in the DevelopAKUre clinical trials.

**W121**

**Association of 25-hydroxyvitamin D with traditional and novel markers of allergic asthma in children at disease diagnosis**

J Siodmiak1, K Bergmann1, G Fulgheri1, M Krintus1, A Zawadzka-Krajewska2, S Kierat1, B Jaszowska1, R Staszak-Kowalska1, M Kuligowska-Prusinska1, U Demkow3, G Sypniewska1
1Nicolaus Copernicus University, Department of Laboratory Medicine, Bydgoszcz, Poland, 2Medical University of Warsaw, Department of Pediatric Pulmonology and Allergology, Warsaw, Poland, 3Medical University of Warsaw, Department of Laboratory Diagnostics and Clinical Immunology, Warsaw, Poland, 4Children's Hospital, Department of Pediatrics, Pneumonology and Allergology, Bydgoszcz, Poland

**Background:** Vitamin D insufficiency/deficiency is highly prevalent in children with asthma. Recent evidence suggests that vitamin D deficiency may predispose to allergic asthma phenotype. We assessed the association of 25(OH)D with traditional and novel determinants of asthma in children at asthma diagnosis.
Patients, material, methods: Study included 122 children, 59 (6.4±2.3 yrs) at diagnosis of allergic asthma, 13 with non-allergic asthma and 50 without asthma diagnosis (5.97±2.4 yrs). In all fasting blood was collected, blood cell count was performed and BMI was calculated. Blood eosinophils, serum total IgE, periostin (surrogate marker of airways remodeling), lipid profile, C-reactive protein (hsCRP), 25(OH)D total were measured (XE-2100, Sysmex, ARCHITECT, Abbott Diagnostics, Cobas e411, Roche Diagnostics, ImmunoCap-100, HVD).

Results: Mean 25(OH)D concentration was lower in children with allergic asthma compared to the reference group (28.3±9.1 vs 33.7±12.7 ng/mL, p=0.008). The prevalence of 25(OH)D deficiency (<20ng/mL) was higher in allergic asthma patients compared to the reference group (18.6% vs 8.2%); eosinophil count and IgE concentration were significantly higher in children with allergic asthma (p=0.001). Children with allergic asthma and eosinophilia (≥0.305K/μL) had significantly higher IgE (p=0.02) but similar 25(OH)D level. A clear tendency to higher periostin concentration with lower 25(OH)D was observed in allergic asthma (r= -0.43; p=0.075). In our small study group serum 25(OH)D concentration was a significant predictor of allergic asthma (OR 1.05; CI 1.01-1.09; p=0.01).

Conclusion: Vitamin D deficiency, more prevalent in allergic asthma children, may be related to increased airways remodeling and considered as predictor of the disease.

W122

Isolated increase in urinary thymine in an infant receiving the anti-retroviral drug zidovudine (AZT)

CJ Gallagher, RS Carling
Viapath, St. Thomas’ Hospital, Inherited Metabolic Disease Laboratory, London, United Kingdom

Urinary organic acid analysis performed for a 17-day-old baby showed increased thymine with no increase in uracil concentration. Increased urinary thymine occurs in the pyrimidine disorders dihydropyrimidine dehydrogenase (DPD) deficiency and dihydropyriminidase (DHP) deficiency, but in these disorders there is also an increase in urinary uracil concentration. There is no known disorder in which increased urinary thymine occurs in isolation.

Isolated increased urinary thymine has previously been reported by Srivastava et al. (Ann Clin Biochem 48(S1) 2011) and in both cases, the infant was undergoing prophylactic treatment with the anti-retroviral drug zidovudine (3’-azido-3’-deoxythymidine, AZT). AZT is a nucleoside analogue reverse-transcriptase inhibitor (NRTI) and is an analogue of thymidine that hydrolyses to form thymine. In both cases, samples taken post-therapy showed that the thymine concentrations had normalised.

Srivastava et al. proposed two explanations for the increased urinary thymine following AZT administration;

a. contamination of AZT with thymine and
b. degradation of AZT to release thymine during the extraction step of organic acid analysis.

We analysed the urine from our patient by an alternative method to investigate further. Analysis by HPLC without initial extraction showed no increase in thymine concentration, consistent with degradation of AZT during organic acid analysis rather than presence of thymine as a contaminant of AZT.

In conclusion, in patients receiving AZT, falsely increased urinary thymine concentrations may occur when the analytical conditions cause hydrolysis of AZT in the sample to produce thymine. In addition, it is possible that this could occur with other thymidine analogue drugs. HPLC can be used as a secondary method to confirm the true thymine concentration in these samples. It is also important to ensure that the uracil concentration is not increased to exclude a diagnosis of a pyrimidine disorder.

Renal Disease

W123

The use of cystatin C eGFR for staging chronic kidney disease

CJ Cockcroft, SA Madrid-Willingham, R Hoole, S Holding, ES Kilpatrick, J Shepherd
Hull and East Yorkshire Hospitals NHS Trust, Blood Sciences, Hull, United Kingdom

Chronic kidney disease (CKD) is routinely staged using the estimated glomerular filtration rate (eGFR) derived from the serum creatinine concentration. However, creatinine can also be influenced by non-GFR related factors which bias the eGFR. Recent KDIGO and NICE CKD guidelines recommend the use of cystatin C derived eGFR (cys-eGFR) for more accurate staging of patients at CKD stage 3a (eGFR 45-59 mL/min/1.73 m²) presenting without other features of kidney impairment or diabetes. This study aimed to compare cr-eGFR and cys-eGFR results for suspected CKD stage 3a patients attending GP surgeries in the Hull and East Yorkshire community.
Serum samples from 131 individuals (59% female; median age 72 yrs, IQR 65-77 yrs) with reported cr-eGFR requests were pseudoanonymised and re-tested for cystatin C in order to obtain the equivalent cys-eGFR. Measurement of serum cystatin C concentration was carried out using an immunoturbidimetric assay (Gentian) on the AU58000 automated analyser (Beckman Coulter). The cys-eGFR was calculated for each measurement using the evaluated formula specified by the manufacturer (Gentian).

Comparison of cr-eGFR and cys-eGFR CKD staging showed that 43.5% of individuals could be reclassified into stage 2 (60-89 mL/min/1.73 m²) from stage 3a using cys-eGFR. The staging was unchanged for 39.7% of individuals, confirming Stage 3a. Whereas, reclassification to stage 3b (eGFR 30-44 mL/min/1.73 m²) was observed for 16.8% of individuals. The proportion of individuals reclassified from cr-eGFR CKD stage 3a using cys-eGFR was consistent with recent meta-analysis by Shlipak M.G., et al (N Engl J Med 2013;369:932-43); showing upward (eGFR>60 mL/min/1.73 m²) and downward (eGFR< 45 mL/min/1.73 m²) reclassification for 42% and 26% of individuals respectively (34% remaining in stage 3a). In summary, this study demonstrates that CKD staging using cys-eGFR in our local population study is consistent with findings from larger populations.

W125

Biological variation of high-sensitivity troponin in stable haemodialysis patients

W Mbagaya1,2, B Lopez3
1Harrogate and District NHS Foundation Trust, Harrogate, United Kingdom, 2Leeds Teaching Hospitals NHS Trust, Clinical Biochemistry, Leeds, United Kingdom, 3Norfolk and Norwich University Hospitals, Norwich, United Kingdom

Background: High-sensitivity troponin assays have significantly lowered the detection limit and allowed detection of small changes in troponin values. Assessment of biological variation of high-sensitivity troponin (hs-Tn) is important in the determination of the optimal change required in the diagnosis of MI. More widespread availability of high-sensitivity troponin (hs-Tn) assays and a move to incorporating delta troponin changes for the diagnosis of MI means than greater clarity of the significance of the changes in troponin elevations in end stage renal disease patients is required. The aim of this study was to establish the biological variation of hs-Tn among stable haemodialysis patients.

Methods: 18 stable haemodialysis patients were recruited, of which 16 completed the study. Predialysis blood samples were collected from study participants weekly during the second dialysis session of the week for a total of 10 weeks. Analytical CV (CV_a, intra-individual CV (CV_I), inter-individual CV (CV_I), reference change value (RCV) and index of individuality (II) were computed.

Results: All samples had hs-TnT concentration above the 99th percentile for a healthy population while only 29.4% of samples had an hs-TnI concentration above the 99th percentile. For hs-TnT the long-term CV, was 2.1%, CV, 10.5%, CV, 64.2%, RCV 28.1% and log-normal RCV (rise/fall) 34.4%/25.6%. The corresponding values for hs-TnI were 7.1%, 20.2%, 100.5% and 79.8%/44.4%. The II was 0.17 for hs-TnT and 0.21 for hs-TnI.
**Conclusion:** Long-term biological variation of hs-TnI and hs-TnT in stable haemodialysis patients is similar to that in healthy individuals and stable coronary arterial disease. The index of individuality for hs-TnI and hs-TnT in stable haemodialysis patients were low thus population based clinical decision points are of limited value in the diagnosis of MI. Serial measurements are required to detect significant changes in hs-Tn concentrations and support diagnosis of MI in this group.

**W126**

**Comparison of established creatinine-based equations with iohexol clearance for the estimation of GFR in children**

Z Maunsell  
Oxford University Hospitals NHS Trust, Department of Clinical Biochemistry, Oxford, United Kingdom

An accurate assessment of glomerular filtration rate (GFR) is required in a variety of clinical situations. Formal measurement of GFR using clearance methods traditionally uses radioactive tracers such as 51Cr-EDTA, although iohexol clearance allows for safer, more convenient assessment. Plasma creatinine and creatinine-based equations have served as convenient estimates of GFR but they are subject to limitations. A compromise therefore exists between highly accurate but time consuming, technically difficult reference methods and more accessible, readily available markers of renal function.

GFR was assessed by gold-standard iohexol clearance in 210 paediatric patients and compared with GFR estimated by two widely used creatinine-based estimations, the Bedside Schwartz and Counahan-Barratt equations. An equation using plasma urea, cystatin C and creatinine (CKiD Schwartz, 2009) was also evaluated. Creatinine in all cases was measured by an enzymatic method and GFR was adjusted for body surface area.

Iohexol GFR (iGFR) ranged from 26-160 mLs/min/1.73m² (median 99, IQR 26). Of these, 24 patients had a measured iGFR of < 60 mLs/min/1.73m². Regression analysis of these results compared to their corresponding calculated GFR yielded relationships $y = 1.46x-0.90$, $y = 1.53x-0.95$ and $y = 1.67x + 13.40$ for the Schwartz, Counahan-Barratt and CKiD Schwartz equations respectively. Using equations to estimate GFR in these patients resulted in a median (min, max, IQR) overestimation of 65% (-10, 112, 24), 52% (-6, 123, 25) and 87% (38, 160, 36) for the Bedside Schwartz, Counahan-Barratt and CKiD Schwartz equations respectively.

It is therefore concluded that creatinine- and cystatin C-based equations for the estimation of GFR in children are prone to being unreliable and in many cases correlate poorly with iohexol clearance. In cases where a true measure of GFR is needed, formal measurement using a clearance method such as iohexol must remain the method of choice.

**W127**

**Proactive identification and management of early-stage chronic kidney disease patients in primary care-a laboratory solution**

K Hadfield, N Flynn, A Dawnay  
University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

Patients with chronic kidney disease (CKD) often present late with significant deterioration in renal function conferring high risk of morbidity and mortality. Strategies for earlier identification and appropriate monitoring of CKD patients are required to facilitate timely initiation of interventions to prevent/slow disease progression and ensure referral of patients to secondary care when required.

Supported by Islington CCG, we have launched a pilot service in 5 practices for proactive identification and biochemical management of CKD patients in primary care. Data from the laboratory information system (LIMS) is used to produce cumulative graphs of all eGFR and urinalysis results for all primary care patients with eGFR < 60 ml/min/1.73m². Graphs are reviewed by clinical scientists who append patient-specific interpretative comments in real-time providing recommendations for CKD diagnosis, monitoring, and referral based on UK Renal Association guidelines.

In the first 3 months, 385 graphs from 298 patients were reviewed; a CKD diagnosis was made in 69% patients (55% stable disease, 14% unstable/progressive disease). After 3 months the number of patients coded on the practices’ CKD registers increased by 11% with a total of 65 extra patients recorded: 35 stage 3A, 13 stage 3B, 7 stage 3 (eGFR fluctuating 30-59 ml/min/1.73m²), 2 stage 4 and 8 with unstable/progressive disease. In patients with eGFR < 60ml/min/1.73m² for the first time, UK guidelines suggest a repeat eGFR within 14 days. This improved from a pre-launch of 9.1% (11.4% within 30 days) to 32.7% (55.8%) post-launch, demonstrating improved adherence to national guidelines.

We have developed a laboratory-based system for identification and management of CKD patients in primary care. The service has had a positive impact on appropriate test requesting and case finding and is adding value to the results released by the laboratory. Further work is required to assess its impact on patient outcomes.
W128

Acute kidney injury (AKI) in primary care—one year’s observation

AL Barton\(^1\), AS Mallard\(^1\), APatterson\(^1\), SC Fleming\(^1\), RG Parry\(^2\)

\(^1\)Royal Cornwall Hospitals Trust, Clinical Chemistry, Truro, United Kingdom, \(^2\)Royal Cornwall Hospitals Trust, Renal Unit, Truro, United Kingdom

Introduction: Early recognition of Acute Kidney Injury (AKI) and prompt initiation of treatment is important in the reversal of injury and preventing complications. We reviewed the identification and follow-up of AKI within Primary Care.

Method: Samples identified as AKI from May 2012-April 2013 were staged according to the ‘Acute Kidney Injury Network’ (AKIN) criteria with a non-reportable test code added. Patient results were downloaded from the Pathology computer and cross-matched against the Patient Administration System for admissions to mid-May 2014.

Results: In twelve months 991 AKI episodes were identified on 970 patients. Breakdown of AKI staging was 61% AKI1, 29% AKI2 and 10% AKI3. The median age for all stages was 79 years (Inter Quartile Range, IQR AKI1 69-86, AKI2 70-85 and AKI3 71-85). The next creatinine request date and location was identified with 33% AKI1, 57% AKI2 and 69% AKI3 patients having a repeat sample within 7 days. The primary locations were 67% GP for AKI1s, 61% GP & 11% Medical Assessment Unit (MAU) for AKI2s and 31% GP & 37% MAU for AKI3s. Admissions were identified on 59% AKI1s, 68% AKI2s and 66% AKI3s. Median time from AKI identification to hospital admission was 73 days for AKI1 (IQR 15-217) and 23 days for AKI2 (IQR 3-130). AKI1s and AKI2s had a median length of stay (LoS) of 2 days (IQR 0-8.5). In contrast, AKI3 patients were admitted on the same (22%) or within 17 days (52%) (IQR 1-4.5 days) with a LoS of 7 days (IQR 4-11). Mortality during hospital stay was similar in all stages (30-33%).

Conclusions: Although a large proportion of AKI2 and AKI3 patients had repeat sampling within 7 days, there was still a significant number with delayed follow-up. Education within Primary Care is required on how to identify and manage AKI.

W129

Effect of acute kidney injury electronic alerts on patient outcomes

G White

Hampshire Hospitals NHS Trust, Biochemistry Laboratory, Winchester, United Kingdom

Aim: The aim of the study was to investigate the effect AKI e-alerts have on subsequent patient outcomes.

Method: Real time AKI e-alerts were generated using bespoke software that integrated into the laboratory computer system (WinPath v5.32). Alerts were based on the ACBLM algorithm published in July 2013 and issued only to the Medical Admissions Unit at Winchester from August 2013 onwards. No other ward/department received any e-alerts. A control ward (the Medical Admission Unit at Basingstoke, was used to assess the effect of educational and media campaigns.

Results: From 2012 to January 2014, the AKI and the ninety day mortality rates fell from 27.7% to 12.8% and 23.0% to 16.4% respectively. However, in the control ward there was a similar fall in these rates (14.5% to 9.3% and 20.2% to 10.0% respectively). The same Consultant who was also the Trust lead for AKI oversaw both wards. The Cardiology Ward at Winchester witnessed a similar trend, 26.2% to 18.9% for AKI rate and 24.4% to 16.1% for ninety day mortality.

Conclusion: Over the study period a fall in AKI and ninety day mortality rate was seen in three acute wards, only one of which received any form of AKI e-alert. This would suggest that, whilst AKI e-alerts undoubtedly have a role in highlighting patients to clinicians, the publicity that has been generated on the subject together with the clinical educational aspects associated with alerting has led to improved short-term outcomes for patients.

W130

The role of renal biomarkers as predictors of acute kidney injury in critically ill patients

EL Evans\(^1\), J Armer\(^1\), J Baldwin\(^2\), R Allcock\(^3\), MA Myers\(^1\)

\(^1\)Lancashire Teaching Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, Preston, United Kingdom, \(^2\)Lancashire Teaching Hospitals NHS Foundation Trust, Critical Care Department, Preston, United Kingdom

Background and objectives: Acute kidney injury (AKI) is a frequent complication seen in the adult intensive care unit (ICU) and is associated with increased morbidity and mortality. The identification of reliable biomarkers that allow earlier diagnosis of AKI may increase the success of therapeutic interventions. The aim of this study was to compare the predictive abilities of a panel of renal biomarkers including: plasma and urinary neutrophil gelatinase-associated lipocalin (pNGAL and uNGAL), plasma cystatin C (pCysC) and urinary N-Acetyl-β-Glucosaminidase (uNAG) to detect AKI and prognostic outcomes such as renal replacement therapy (RRT) and mortality in an adult ICU population.
Methods: A total of 69 consecutive ICU patients were included in the study. AKI was defined according to KDIGO criteria and ROC curve analysis was used to evaluate biomarker predictive abilities and prognostic outcomes.

Results: AKI occurred in 29% of patients seven days post-admission. Of all renal biomarkers measured, pCysC and pNGAL showed the best predictive ability for AKI with AUCs of 0.83 (95% CI 0.779-0.949) and 0.82 (95% CI 0.706-0.903) respectively. pNGAL showed potential as an early indicator of AKI with increased levels observed in patients that later developed AKI (>24h post critical care admission). Urinary NGAL showed the best predictive ability to predict renal replacement therapy (RRT) and 60-day mortality with AUCs of 0.87 (95% CI 0.759-0.936) and 0.81 (95% CI 0.697-0.879). In addition, uNAG was the best indicator for AKI severity.

Conclusions: The measurement of a panel of renal biomarkers is more informative than the currently used marker, serum creatinine. The predictive ability for AKI with AUCs of 0.83 (95% CI 0.779-0.949) and 0.82 (95% CI 0.706-0.903) respectively. pNGAL showed potential as an early indicator of AKI with increased levels observed in patients that later developed AKI (>24h post critical care admission). Urinary NGAL showed the best predictive ability to predict renal replacement therapy (RRT) and 60-day mortality with AUCs of 0.87 (95% CI 0.759-0.936) and 0.81 (95% CI 0.697-0.879). In addition, uNAG was the best indicator for AKI severity.

W131

The role of urea in patient outcomes following suspected AKI

CI Cockcroft, S Holding, ES Kilpatrick, J Shepherd
Hull and East Yorkshire Hospitals NHS Trust, Blood Sciences, Hull, United Kingdom

The detection and monitoring of acute kidney injury (AKI) in patients is a crucial component of inpatient care. AKI is detected by measurement of incremental change in serum creatinine concentration, as defined by the KDIGO AKI guidelines. Our laboratory, in collaboration with renal clinicians, recently developed an AKI detection algorithm to provide automated clinical alerts for inpatients attending Hull and East Yorkshire Hospitals NHS Trust. The purpose of this audit was to assess the patient outcomes in terms of all-cause mortality within 30 days of an AKI alert.

The all-cause mortality over a period of 6 months (August-2013 to January-2014) within 30 days of an AKI alert was 20.8% (557/2683 patients; 52% female; median age 83 yrs, IQR 73-88 yrs). The serum creatinine concentration was analysed by categorisation of patients into 2 groups: low/normal and high (>107 μmol/L / >127 μmol/L females/males). Chi squared statistics were used to compare observed and expected mortality. As expected, 30 day mortality was significantly associated with the high creatinine group (Obs/Exp mortality ratio=1.21, P< 0.01). We expanded this analysis to include serum urea concentration, also a marker of renal function. This showed that high creatinine and low/normal urea (≤7.6 mmol/L) resulted in significantly lower than expected 30 day mortality (Obs/Exp mortality ratio=0.51, P< 0.01). Survival analysis, using multi-variant Cox regression demonstrated that urea (hazard ratio=1.45±0.17, P< 0.01), unlike creatinine (hazard ratio=0.93±0.12, P=0.224) was the most significant risk factor following AKI.

These results suggest that an elevation in urea relative to creatinine is significantly associated with 30 day mortality following an AKI alert. This is consistent with presentation of pre-renal AKI, in that urea is typically disproportionally elevated above creatinine. In summary, these findings suggest that the serum urea concentration could be considered as an additional prognostic marker for patients identified as having possible AKI.

W132

The delta check/e-alert for identifying acute kidney injury (AKI): how robust is your system?

AS Mallard1, AL Barton1, A Patterson1, SC Fleming1, RE Parry2
1Royal Cornwall Hospitals Trust, Clinical Chemistry, Truro, United Kingdom, 2Royal Cornwall Hospitals Trust, Renal Unit, Truro, United Kingdom

Since December 2011 our laboratory computer system (Clinisys Winpath) has flagged samples exhibiting a change in creatinine from the previous result of at least 50%, or ±/−26 mmol/L. These are reviewed by the duty biochemist and AKI-staged using the Acute Kidney Injury Network (AKIN) criteria.

In the first 15 months it appeared there may have been a seasonal variation with a higher number of cases in winter than summer, i.e. 267 in December 2011, decreasing to 139 in July 2012 and peaking again at 295 in February 2013. However, in summer 2013 the numbers did not fall as expected, with July having 255 cases; numbers then fell in the autumn reaching a nadir of only 83 cases in November. In late February 2014 we discovered that the delta-check rules had been unknowingly deleted in early September 2013 which explained the decreased numbers found over that approximate six-month autumn/winter period.

Prior to this “alert-off” period the percentage of cases was 52.7% AKI1, 30.2% AKI2 and 17.0% AKI3. However, during the “alert-off” period the percentages changed to 33.1% AKI1, 36.5% AKI2 and 30.4% AKI3. This may have been influenced by the number of creatinine results >250 mmol/L being sent for clinical approval. These equated to 11.2%, 29.0% and 77.8% for the various stages before, versus 31.4%, 62.2% and 88.2% during, the “alert-off” period.

Although the most urgent cases were still being detected, we know from our previously published data that the mortality rate is higher (62.4%) for patients progressing through AKI stages compared to AKI3 patients (31.4%) so it is important to accurately detect all stages.
For the two months since the rules have been reinstated identification of AKI has returned to that expected for the time of year. A fail-safe quality assurance mechanism for the delta-check is being instigated.

W133

Mind the gap-the effect of incomplete patient coding on chronic kidney disease prevalence in North Central London

K Hadfield, N Flynn, A Dawnay
University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

Comparison of Quality and Outcomes Framework (QOF) and Health Survey England (HSE) data indicates that there is a chronic kidney disease (CKD) prevalence gap in Islington. HSE expected CKD prevalence for Islington is 4.1%, while QOF 2012/13 diagnosed prevalence was 1.79%. We hypothesised that a proportion of this prevalence gap is due to incomplete coding of CKD patients on practice registers.

Between 16/10/13 and 19/12/13 eGFR results <60 ml/min/1.73m² from patients at 5 South West (SW) Islington GP practices were interpreted according to UK Renal Association guidelines alongside any previous results recorded on the laboratory information system (LIMS) to determine whether a CKD diagnosis could be made. The stability of disease was also determined and a stage given if appropriate. The results were compared with the practices' CKD registers.

In total 110 patients were given a biochemical diagnosis of CKD. 24 (22%) of these patients were not coded on the practice registers: 15 stage 3A, 3 stage 3B, 1 stage 3 (eGFR fluctuating between 3A and 3B) and 5 patients with unstable disease. 44 (40%) patients were incorrectly coded, with the majority of practices not utilising stage 3A and 3B codes to differentiate between stage 3 patients.

Uncoded patients were significantly less likely to have had ACR/PCR measured within the stage-appropriate timeframe specified by UK Renal Association CKD guidelines than those who were on the register (11% vs 68% respectively). For 50% of these uncoded patients there was no record of urinalysis ever having been requested on the LIMS.

We have demonstrated that a proportion of the CKD prevalence gap in SW Islington is due to incomplete coding of patients on practice registers. Uncoded patients are less likely to have appropriate monitoring of their disease, highlighting the importance of electronic coding for good quality patient care.

W134

ß-trace protein as marker for GFR in renal transplant recipients

S Atef, S Abdelrahman
Ain Shams University, Cairo, Egypt

Background: After renal transplantation monitoring and detection of slight-to-moderate changes in GFR is a prerequisite for an optimal patient management. Due to the limitations of serum creatinine and lack of validation of creatinine based GFR estimation equations in transplantation setting, ß-Trace protein (BTP) has been proposed as an alternative marker for GFR.

Aim: The aim of this study was to evaluate the relationship between serum levels of beta-trace protein (BTP) and glomerular filtration rate (GFR) in renal transplant recipients

Methods: We measured true GFR by 99mTc-diethylenetriaminepentaacetic acid (99mTc-DTPA) and BTP and for comparison cystatin C and creatinine in 60 RTRs. We also conducted a study of the GFR estimates of the Cockcroft and Gault (C&G), and the abbreviated modification of diet in renal disease (aMDRD).

Results: Serum levels of BTP progressively increased with the reduction of GFR. A good correlation was found between GFR and serum levels of BTP (r=0.938), Creat (r=0.823), Cys (r=0.907). BTP has the highest sensitivity of 96% and specificity of 91% at a cutoff of 2.01 mg/L with area under the curve of 0.965. The BTP correctly classified 89% of patients compared to to only 80% with cystatin-c,75% with aMDRD) equation, 69% with the Cockcroft-Gault equation

Conclusions: On the basis of the above results, we believe that BTP may be a useful and reliable analyte to estimate GFR in RTRs.

W135

‘Adding insult to injury’: four years on from NCEPOD

C Hill1, LM Bailey1, G Philp1, TJ Hine1
1Royal Liverpool & Broadgreen University Hospitals NHS Trust, Department of Clinical Chemistry and Metabolic Medicine, Liverpool, United Kingdom, 1iSoft (a CSC company), Banbury, United Kingdom

Introduction: Acute kidney injury (AKI) is a common complication in hospitalised patients. NCEPOD published a review in 2009 that suggested that the condition frequently goes undetected thus worsening outcome for the patient. The report highlighted the increased incidence
of this condition over the weekend period. The NICE AKI briefing paper (2014) reports that mortality associated with AKI may be at least 25-30%. Following the NCEPOD report the nephrology and clinical chemistry departments at the Royal Liverpool and Broadgreen University Hospitals NHS Trust have collaborated on an electronic AKI alert system using the laboratory information management system (LIMS) to help with the detection and management of the condition. The introduction of the alert system has enabled data collection and audit on frequency of AKI in the hospitalised population.

Method: The LIMS was interrogated to provide numbers of electronic AKI alerts on the inpatient population generated daily over a period of three months in 2013. The alerts were separated into stages of AKI detected each day and provides a guide to severity of the condition.

Results: There appears to be an increased number of AKI alerts generated at the weekend. During the week (Monday to Friday), an average of 5.5% (median 5.6%) of results generate an AKI alert compared to 8.4% on Saturday and 7.2% on Sunday. 22.1% and 22.4% of AKI alerts on Saturday and Sunday respectively are stage 3, compared to a mean of 14.9% (median 15.3%) of alerts during the normal working week (Monday to Friday). Data analysis suggests that >80% of AKI alerts generated using this system are true AKI.

Conclusion: Implementation of a robust electronic AKI alert algorithm into LIMS facilitates detection of AKI in hospitalised patients and may be used as a data collection tool to assess frequency of this condition.

W136
Comparison of lipid profile in the use of two immunosuppressive drugs

A Marinkovic, M Stanojkovic
Clinical Center, Center for Medical Biochemistry, Nis, Serbia

Background: Immunosuppression has improved graft and recipient survival in transplantation but is accompanied by several adverse effects like dyslipidemia and cardiovascular disease. This study analyzed the relationship of dyslipidemia and hypertrygliceridemia and cardiovascular disease with two different immunosuppressive regimens in renal transplantation.

Methods: Complete lipid profile was assessed before transplantation and after 6 months of treatment with either cyclosporine or tacrolimus.

Results: In patients treated with cyclosporine was significant increase in mean cholesterol and mean LDL values from the pre-treatment period to 6 months post-treatment (CHOL: 5.2 +/- 1.2 vs. 6.7 +/- 1.05 mmol/L, P = 0.004; LDL: 3.04 +/- 1.25 vs. 5.2 +/- 1.05 mmol/L, P = 0.002. At 12 months, LDL-cholesterol levels were significantly elevated compared with pretransplant levels (LDL: 3.07 +/- 1.27 vs. 3.81 +/- 1.22 mmol/L, P = 0.034. In cyclosporine treated patients, plasma triglyceride levels were reduced at the 6- and 12-months follow-up (TG: 3.2 +/- 0.65 vs. 2.01 +/- 0.82 mmol/L, P = 0.023). Cholesterol levels at 12 months posttransplantation were significantly lower than the pretransplant measurements (CHOL: 4.68 +/- 1.1 vs. 4.22 +/- 0.97 mmol/L, P = 0.024.

Conclusions: Conversion from cyclosporine microemulsion to tacrolimus resulted in decreased cholesterol in stable renal transplant recipients with treated but persistent mild dyslipidemia.

W137
Phenols elimination in chronic kidney disease patients

I Ortega1, B Torrubia1, M Garbira1, C Castillo1, FJ Illana1, MJ Torrejón1, JA Herrero2, M Arroyo1
1Hospital Clínico San Carlos, Clinical Laboratory, Madrid, Spain, 2Hospital Clínico San Carlos, Nephrology, Madrid, Spain

Objective: The aim of the present study is to evaluate the phenols removal in chronic kidney disease (CKD) patients by dialysis treatments.

Methods: Sixteen patients were treated with different hemodialysis systems during four weeks (week1: BG (polymethylmethacrylate) membrane-hemodiafiltration on line (HDF-OL); week 2: TS (Polisulfone) membrane-HDF-OL; week 3: BG membrane-high-flow hemodialysis (HF-HD); week 4: TS membrane-HF-HD).

Urea, b2-microglobuline, phenol and free p-cresol were measured in control, predialysis and postdialysis samples.

Measurement of phenol and free p-cresol was performed by reversed phase HPLC with gradient elution (methanol:formic acid 1M) and PDA detection (phenol (272nm); p-cresol (275nm)).

Elimination of metabolites was calculated using the following equation:

$$\text{Elimination} = \left(\frac{X_{\text{predialysis}} - X_{\text{postdialysis}}}{X_{\text{predialysis}}} \times 100\right)$$

The Pearson correlation coefficient was used to compare the elimination of the four metabolites.

Results: The elimination of p-cresol was lower (62%) than phenol (64%), b2-microglobuline (66%) and urea (77%) and it was statistically significant (p< 0.001).

In all patients, the urea post-dialysis levels were in the reference range (15-50 mg/DL). Nevertheless, the b2-microglobuline and p-cresol post-dialysis serum level (b2-microglobuline=2.9-21.5mg/L and p-cresol= 2.245.9 mg/L) were higher than the reference range (b2-microglobuline=1.25 mg/L and p-cresol=0.12.0 mg/L).
In respect to phenol post-dialysis serum levels (0.23-9 mg/L), only 30% of patients (percentile30= 0.598mg/L) were within reference range (0.1-0.6 mg/L).

Only the Pearson correlation coefficient between the elimination of phenol and p-cresol (r=0.672) was statistically significant. **Conclusions:** Urea elimination is better than the elimination of the other molecules that we have studied.

Although the elimination of b2-microglobuline and phenol is good, it is not enough to get suitable post-dialysis levels. p-Cresol is the most difficult molecule to eliminate via hemodialysis treatments, probably due to p-cresol is bound to albumin. Despite the elimination of phenol and p-cresol show different levels of clearance, the mechanism of removal by hemodialysis must be similar because the elimination of both phenols are correlated (r=0.672).

**W138**

The effect of comorbidities on urinary constituents in patients with renal stone disease

P Mohammed, M Harrison, Y Shakil, J Berg
City Hospital, Clinical Biochemistry, Birmingham, United Kingdom

Incidence of Renal Stone Disease (RSD) has risen in recent years alongside an increased incidence of obesity, diabetes and hypertension, leading to suggestions of an association between RSD and comorbidity particularly manifestations of the metabolic syndrome.

An audit was performed to determine the relationship between pre-existing comorbidity and concentration of urinary biochemical markers for RSD and to identify if the presence of comorbidities is associated with increased predisposition to supersaturation and stone formation. Results from urinalysis of 475 RSD patients over the period 2009-2013 were recorded. Morbidity status was determined from the patient records. Mean concentration was recorded and Statistical t-tests were performed to determine significance of findings.

The mean oxalate excretion was significantly increased in the obesity and diabetes patients (0.44mmol/day and 0.42mmol/day respectively) compared to RSD patients with no comorbidity (0.35mmol/day) (normal reference range 0.08-0.49 mmol/day). Uric acid concentration was also increased between diabetic, obese compared to RSD patients without comorbidity (mean concentrations, 3.57, 4.94 and 3.06 mmol/day respectively) (normal reference range 1.23-0mmol/day). Incidence of hyperuricosuria was 16% higher in the obese RSD patients compared to those with no comorbidity 63% vs 47% respectively. Mean calcium excretion was raised in hypertensive and diabetic RSD patients against those without morbidity (5.21, 5.84 and 5.61 mmol/day respectively) (normal reference range 2.5-7.5 mmol/day).

Obesity and diabetes were the strongest influences upon urine constituents. A clear association exists between these two morbidities and uric acid concentration. The mechanisms involved could be a consequence of insulin resistance, resulting in defective ammoniagenesis and increased pH. In view of the strong association between uric acid and manifestations of the metabolic syndrome, it is suggested that RSD be considered a risk factor when metabolic syndrome is determined.

**W139**

Comparability of oxalate excretion and oxalate:creatinine ratio in the investigation of hyperoxaluria

O Clifford-Mobley, G Rumsby
1University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

**Introduction:** Urine oxalate is an important investigation in the evaluation of primary hyperoxaluria (PH) and other causes of renal stone disease. As a tertiary referral centre for the laboratory investigation of PH with a high workload of urine oxalate analyses we reviewed urine oxalate results to explore whether oxalate:creatinine ratio can substitute for oxalate excretion for the diagnosis of hyperoxaluria and whether there are potential cases of PH without adequate follow-up.

**Methods:** Urine oxalate results were extracted from our laboratory information management system from August 2011 to April 2013. Data was analysed using Microsoft Excel.

**Results:** Oxalate:creatinine ratio and oxalate excretion were moderately correlated (R=0.63) in 24h urine collections from 4666 patients aged 18 years and above. Sex-related differences were found, which were especially pronounced for oxalate:creatinine ratio and required the implementation of separate male and female upper reference limits (33 and 45 μmol/mmol respectively). 7% of samples with both oxalate:creatinine ratio and oxalate excretion above the reference limit came from patients with confirmed PH. 0.9% of samples with only one parameter elevated came from patients with confirmed PH. There was no statistically significant difference in the level of oxaluria between the three types of PH. There were 24 adult patients with repeatedly grossly elevated urine oxalate who had not been evaluated for PH.

**Conclusions:** Inter-individual variation in creatinine output is the likely cause for much of the discordance between oxalate:creatinine ratio and oxalate excretion and this was improved by use of sex-related upper reference limits for oxalate:creatinine ratio. A combination of both parameters of oxaluria provided the greatest power to distinguish PH. Our data suggest that there may be a substantial number of undiagnosed PH patients, who would be at risk of renal failure. This highlights the need for further investigation of individuals with hyperoxaluria and the consideration of additional testing.
Proteins/Enzymes

W140

An enigmatic paraprotein

SN Colyer, N Wassef
Royal Free London NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

Capillary zone electrophoresis (CZE) is a sensitive technique used to screen for monoclonal gammopathy when plasma cell dyscrasias are suspected. It is favoured over traditional gel-based electrophoresis due to its high-throughput analysis. We report a case of AL amyloidosis with an IgM Lambda paraprotein not detectable by CZE.

A 78-year-old female presented to A&E at the Royal Free Hospital in 2007 with back pain and pyrexia of unknown origin. Serum protein electrophoresis (SPE) was performed, which showed an IgM Lambda paraprotein of 4 g/L. Since bone marrow examination results were normal, she was monitored in primary care. Two years later she developed proteinuria and underwent a renal biopsy, which revealed AL amyloidosis. She was referred to the National Amyloidosis Centre where she was regularly followed up in clinic. Her paraprotein remained between 4-7 g/L (2007-2011). In late 2011, our laboratory acquired a Capillarys2™ analyser (Sebia, France) and we switched over to CZE methodology. In June 2012, we received her first sample since changing methods. No paraprotein was detected by CZE, whereas the IgM Lambda was distinctly visible by immunofixation (IFE). As IgM paraproteins tend to polymerise and precipitate, the sample was pre-treated with a chaotropic agent (Fluidil™) to break down these IgM multimers. Despite pre-treatment, the peak was not detectable by CZE. The sample was re-run using conventional SPE, revealing the IgM Lambda consistent with IFE and previous results.

False negative IgM paraproteins by CZE have previously been reported in the literature. It has been demonstrated that this is due to monoclonal IgM insolubility in CZE alkaline buffer, causing precipitation and failure to migrate through the capillary. This case highlights the importance of laboratory staff awareness of this phenomenon. Our practice is to reflex IFE when turbidimetric measurement of IgM is elevated but no paraprotein is detected by CZE.

W141

Presepsin (sCD14-ST) measurements as a marker for the diagnosis of sepsis compared with C-reactive protein (CRP) and procalcitonin (PCT)

A Enguix-Armada, R Escobar-Conesa, IM Castro-Vega, MV De la Torre-Prados
Hospital Universitario Virgen de la Victoria, Malaga, Spain

Introduction: Presepsin (PSP) or N-terminal sequence of CD14 (sCD14) is the soluble fraction of the receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein (LPBP). PSP has been identified as a protein whose levels increase specifically in the blood of sepsis patients. Presepsin is thought to be a new candidate biomarker for diagnosis of sepsis compared with CRP and PCT.

Objective: To evaluate the diagnostic value of presepsin as a marker for the diagnosis of sepsis compared with CRP and PCT in the blood of patients admitted in the first 24 hours at the intensive care unit (ICU).

Methods: 133 patients consecutively admitted to ICU were enrolled into the study over the period 2013-14, n=81 patients with severe sepsis or septic shock and n=52 patients without sepsis (control patients). Presepsin, PCT and CRP plasma levels were measured in the blood of patients admitted at the first 24 hours at the intensive care unit (ICU), were measured using an automated immunochromoluminescent assay PATHFAST, immunochromoluminescent assay MINIVIDAS and immunoturbidimetric assay DIMENSION RXL respectively.

Result: The areas under the curves (AUC) and 95% Confidence Interval (C.I.) for sensitivity/specificity values for diagnosing sepsis were PCT: AUC 0.986 (95% C.I.:0.947-0.998), PSP: AUC 0.964 (95% C.I.:0.914-0.989) y PCR: AUC 0.922 (95% C.I.:0.860-0.962)

The statistical comparison between the three AUC shows a statistically significant difference between PCT and CRP (p = 0.007), but not with the PSP (p>0.05). The optimal cutoff for the PSP was 1,016 pg /mL with Sensitivity = 85.2% and Specificity=96.1%.

Conclusion: Presepsin levels appears to be a new biomarker for early diagnosis of sepsis and have comparable performance to procalcitonin.

W142

Lactate, procalcitonin, c-reactive protein, and score APACHE II in septic patients' prognosis

A Enguix-Armada, R Escobar-Conesa, IM Castro-Vega, MV De-La-Torre
Hospital Universitario Virgen de la Victoria, Malaga, Spain

Background: APACHE-II is a score, based on several clinical and analytical measurements within 24 hours of admission in Intensive Care Unit (ICU). C-Reactive Protein (CRP), Lactate and recently Procalcitonin (PCT), also are biomarkers for the assessment of septic patients. The
aim of this study was to find out if CRP, lactate and PCT during the first 24 hours from severe sepsis or septic shock onset, improved prediction of the APACHE II in terms of prognosis.

Methods: A prospective, observational study in 162 patients >18 years with severe sepsis or septic shock, was developed in a polivalent ICU of a University Hospital. Demographic, clinical parameters and CRP (determined by immunoturbidimetric assay), lactate (measured by selective electrode RAPIDCOM) and PCT (by immunoluminololuminiscence assay, MINIVIDAS) were studied during one year. Descriptive, comparative statistical analysis and Cox proportional-hazards regression was performed using MedCalc® 9.2.1.0.

Results: We analyzed 162 consecutive episodes of severe sepsis (32%, n=52) or septic shock (68%, n=110) admitted in the UCI, the average age was 64.2 years old (16-85 y.o.), 58.3% were men, 28-day mortality was 28.4% for severe sepsis and 34.5% in septic shock. Cox regression for the total of the patients, showed an increase in the prediction value of the APACHE II, 8.8% per unit and 0.4% per 1 mg/dL for CRP, p=0.0004. If we analyze only patients with septic shock the best predicted mortality was lactate with an increase of 31.0% per 1 mmol/L, p=0.003.

Conclusion: CRP improves the prediction of patients with sepsis used in conjunction with the APACHE II score in severe sepsis and, lactate along with the CRP are the best predictors of survival in the case of septic shock. The PCT did not show any predictive value.

W143

TPMT genotyping in patients exhibiting TPMT deficient activity demonstrates benefit of phenotype screening approach

JL Waldron, J Berg, V Graham, L Eccles
Sandwell and West Birmingham Hospitals NHS Trust, Biochemistry, Birmingham, United Kingdom

Aim: Measurement of Thiopurine S-Methyltransferase (TPMT) activity (phenotyping) is required prior to patients starting thiopurine drugs. Our national TPMT screening service uses genotyping to confirm deficient TPMT results but is not offered as a primary test. Here we re-examine the genotype-phenotype correlation in TPMT deficient patients to identify the level of concordance between the TPMT genotype and phenotype.

Methods: TPMT phenotype (activity) and genotype results for patient samples received between March 2013 and February 2014 were analysed. Data was categorised into the number of patients with high, normal, low and deficient TPMT activity (>150, 68-150, 20-67 and <10 mU/L, respectively). TPMT genotype-phenotype correlation was studied in TPMT deficient patients by comparison of the TPMT genotype result with its paired TPMT activity.

Results: Of 34,642 TPMT requests; 110 (0.3%) exhibited deficient TPMT activity, 4,305 (12.4%) had low activity, 29,714 (85.8%) had normal activity and 513 (1.5%) had high activity. TPMT genotype-phenotype correlation for the deficient group identified 97 patients (88.2%) were homozygote for the TPMT*3 mutation and 6 patients (5.5%) were compound heterozygote for the TPMT*2/TPMT*3 mutations. 1 patient (0.9%) was heterozygote for the TPMT*2 mutation and 6 patients (5.5%) were heterozygote for the TPMT*3 mutation.

Conclusion: The majority of TPMT deficient patients are homozygote for the TPMT *3 mutation. However, a small percentage of these patients are heterozygote for the TPMT*2 or TPMT*3 mutation, which are generally associated with low TPMT activity. The deficient TPMT activity in these patients is likely to be attributed to the presence other rare mutations, since samples are only currently screened for the common mutations, which account for 60-95% of all mutant alleles for deficient TPMT activity. This clearly demonstrates the benefit of TPMT phenotyping as a first line screen with TPMT genotyping used as a confirmatory service in selected patients.

W144

Long-term imprecision performance of SPAplus serum free light chain assays evaluated against goals derived from biological variation

F Braga, A Dolci, M Panteghini
Clinical Pathology Unit, ‘Luigi Sacco’ University Hospital, and Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

Background: Determination of serum immunoglobulin k and λ free light chains (FLC) and k/λ ratio calculation is recommended in diagnosis, risk stratification and monitoring of plasma cell disorders. However, some analytical issues still persist in these measurements, among which a too large lot-to-lot imprecision seems to be the main challenge. Previously published evaluations of available assays reported data on relatively short-term imprecision. Here we report, for the first time, long-term imprecision performance of SPAplus analyzer for FLC determination using Freelite reagents (both from The Binding Site).

Methods: We derived the SPAplus FLC imprecision from Internal Quality Control data of our laboratory over one-year time-span (April 2013-March 2014) using the liquid-frozen Bio-Rad Liquichek Unassayed Chemistry Control Level 2 (lot no. 16672). A total of 83 different runs were performed measuring k and λ FLC using 4 and 6 reagent lots, respectively. Results were evaluated against goals (desirable/minimum quality levels) for imprecision (as CV) derived from biological variation of the analytes, i.e. £4.0%/6.0% for k FLC, £3.5%/5.3% for λ FLC and £2.3%/3.4% for k/λ ratio, respectively.
Results: Overall CVs resulted in 6.1% for k FLC (mean, 13.4 mg/L), 4.9% for λ FLC (mean, 13.8 mg/L) and 6.2% for k/λ ratio (mean, 1.0). Differences in results provided by different reagent lots on the material used for imprecision evaluation along the study were significant for both FLC (ANOVA, \( P < 0.001 \)).

Conclusions: Our study shows that, under routine conditions and over a clinically and analytically relevant time-span, k and λ FLC measurements on SPAplus platform fulfilled the minimum imprecision goal for their clinical application, while the k/λ ratio estimate did not. Although a significant improvement in the lot-to-lot variability of FLC assays was observed, further efforts should be warranted to fulfil the desirable analytical goals and make these tests fully reliable for clinical use.

W145

In search of the ‘Best’ assay for serum and plasma albumin

P McGing1, C Galvin1, C Whelan1, Y O’Meana1, M Fitzgibbon2
1Mater Misericordiae University Hospital, Department of Clinical Chemistry and Diagnostic Endocrinology, Dublin, Ireland, 2Mater Misericordiae University Hospital, Department of Nutrition and Dietetics, Dublin, Ireland

Introduction: Blood albumin concentration is a very important clinical parameter but accurate analysis is not straightforward. We investigated the two albumin assays most commonly used in routine practice, namely Bromocresol Green (BCG) and Bromocresol Purple (BCP), and compared them to a ‘gold standard’ nephelometric assay. The aim of the study was to determine the ‘best’ assay for routine use in our hospital, particularly in respect of haemodialysis patients.

Materials and methods: Albumin was measured on Abbott Architect c16000s using BCG and BCP assays. BCP is our routine method; over a period of 3 days BCG was analysed on all patient samples for which albumin had been requested (\( n = 1674 \)). A representative subset of samples was also analysed for albumin using a nephelometric assay on the Siemens BNII system (\( n = 146 \)).

Results: There was a clear difference between methods, with BCG on average reading 5g/L higher than BCP (\( p < 0.001 \)). This difference was inversely proportional to the BCP albumin level. There was a correlation between markers of inflammation and the magnitude of difference between BCG and BCP albumin (e.g. for CRP \( r^2 = 0.16 \); \( p < 0.001 \)).

In the ‘normal’ cohort BCG was slightly closer to the ‘gold standard’ than BCP (mean 1.6g/L lower versus 2.3g/L higher). BCP was more accurate in the severely hypoalbuminaemic cohort (mean 1.6g/L underestimate) compared to BCG (6.3g/L overestimate). In contrast, for patients on haemodialysis the BCG was more accurate (mean 0.3g/L overestimate) versus BCP (5.1g/L underestimate).

Discussion: The difference between albumin concentration measured by BCG and BCP is often ignored, such as with the use of a single reference range under Pathology Harmony. Our work supports other authors’ findings that this difference is real and is of clinical importance, particularly in renal patients. We conclude that for laboratories servicing dialysis units BCG appears to be the most accurate assay.

W146

Establishment of a threshold for alpha-1-antitrypsin phenotyping studies—the importance of assay-specific validation

K Smith, R Cramb
Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom

In investigation of alpha-1-antitrypsin deficiency (A1AT), measurement of A1AT concentration can be used as a screen before proceeding to A1AT phenotyping. There are guidelines stating the threshold below which phenotyping is warranted (e.g. PRU handbook states < 1.6g/L in adults), however these vary and the analytical method used to validate the cut-off is rarely stated, despite significant differences between assay types. The aim of this study was to revalidate an A1AT threshold to trigger A1AT phenotyping, following a change of analytical platform for A1AT measurement at Queen Elizabeth Hospital Birmingham (Roche Modular to Cobas c700). To revalidate the threshold for A1AT phenotyping, A1AT concentration and phenotyping requests were examined six months before and after the platform change. It was found that post-platform change, the workload for A1AT phenotyping had increased by 61% (an increase from 49% to 62% of A1AT samples that were phenotyped). The median A1AT concentration for each major phenotype group before and after the platform change confirmed the decrease in A1AT assay bias and was as follows; PIM 1.32g/L to 1.27g/L, PIZZ 0.99g/L to 0.84g/L, PIMS 1.24g/L to 1.20g/L. The effect of changing the threshold from 1.5 g/L to different A1AT concentrations ranging from 1.0 to 1.5 g/L was examined. By reducing the threshold for A1AT phenotyping to 1.3g/L we showed; (a) easily allowed identification of all clinically significant phenotypes (Pizz, Pisz, Piss); (b) detected heterozygote deficiency phenotypes with a sensitivity of 91% (PimZ) and 71% (PimS); (c) reduced workload to pre-platform change levels and; (d)
produced cost savings. These data confirm the importance of revalidating A1AT thresholds for phenotyping following changes in A1AT assay bias, with an impact on both clinical effectiveness and cost effectiveness.

W147

Prevalence of bistransferrinemia in Chinese, Indians and Malays

R Hawkins
Tan Tock Seng Hospital, Laboratory Medicine, Singapore, Singapore

Aim: Serum protein electrophoresis is the primary investigation offered by many laboratories for the detection of paraproteinaemia. Genetic variants of transferrin causing split $\beta$-1 bands or bistransferrinemia are a particular problem, especially in the presence of elevated IgA concentrations. This study describes the prevalence of split $\beta$-1 patterns in an Asian population.

Methods: Anonymised details of serum protein electrophoreses (SPE) performed over 18 months were extracted from the laboratory database for review. Tan Tock Seng Hospital Laboratory performs SPE by high resolution agarose electrophoresis (Hydragel 15 HR, acid violet dye) on the Sebia Hydrasys2 automated electrophoresis system (Sebia, France). All samples with a split $\beta$-1 band present underwent serum immunofixation for G, A and M heavy chains, and kappa and lambda light chains using the Hydrasys2 system to exclude the presence of paraprotein.

Results: 128/6310 samples had a split $\beta$-1 band present, all of which were subsequently negative for paraprotein presence by immunofixation. In all cases, the additional $\beta$-1 band ran in an identical position cathodal to the normal $\beta$-1 band. The overall split $\beta$-1 prevalence was 2.0% with a statistically significant (p < 0.05) lower prevalence for Indians of 0.7% (0-1.4) compared to Malays of 3.1% (2.0-4.2) and Chinese of 2.0% (1.6-2.4).

Conclusion: Co-dominant expression of genetic polymorphisms of transferrin variants in heterozygotes results in split $\beta$-1 bands. The TF*DChi allele is seen in East Asian and some Indian populations with prevalences up to 8%. The additional cathodal $\beta$-1 band described here probably represents this allele. Routine inclusion of a known split $\beta$-1 control sample on every gel, re-running of split $\beta$-1 samples alongside such a control or specific transferrin immunofixation are amongst the approaches laboratories could introduce to minimise this problem.

W148

Frequency of pancreatic hyperamylasemia in HIV+ patients in the highly-active antiretroviral therapy (HAART) era

D Szőke1, A Ridolfo2, C Valente1, M Galli2, M Panteghini1
1Clinical Pathology Unit, "Luigi Sacco" University Hospital, University of Milan, Milan, Italy, 2Department of Infectious Diseases, "Luigi Sacco" University Hospital, Milan, Italy

Background: Increased frequency of hyperamylasemia has been reported in patients with HIV infection. However, studies determined total amylase activity and were performed before the introduction of highly-active antiretroviral therapy (HAART). We evaluated the prevalence of pancreatic-specific hyperamylasemia in a large HIV+ population mostly treated with HAART.

Methods: The upper reference limit (URL) for pancreatic amylase (P-AMY) was derived on samples from 299 healthy blood donors according to the CLSI C28-A3 protocol. A cross-sectional study was then performed on samples obtained from 1548 consecutive subjects referred to our infectious disease in-patient clinic to assess serum P-AMY and lipase concentrations (Roche Cobas 6000).

Results: P-AMY URL was 51 U/L. 1456 (94%) of patients were HIV+, in the large majority of cases (92%) on HAART (HIV+Tx+); 92 (6%) were HIV- [HCV or HBV infection (n=58), other liver diseases (n=5), rheumatic disease (n=6), non viral infections (n=13), others (n=10)]. The prevalence of pancreatic hyperamylasemia did not significantly differ between HIV+ and HIV- populations (14.2% vs. 15.2%, P=0.91) nor between HIV+Tx- and HIV+Tx+ patients (8.9% vs. 14.7%, P=0.11). Markedly elevated P-AMY (>3URL) was found in six HIV+ and in one HIV- patients: two had macroamylasemia, one acute pancreatitis, three (including the HIV- patient) chronic pancreatitis and one chronic hyperamylasemia of undefined origin. A small fraction of hyperamylasemic subjects (8% of HIV+ and 14% of HIV-) showed normal lipase indicating a non-pancreatic origin of their P-AMY increase.

Conclusions: Our data evidence a relatively low prevalence of pancreatic hyperamylasemia in a large sample of HIV+ patients tested in the current HAART era. Hyperamylasemia in HIV+ patients was generally mild and only three patients showed a frank pancreatic disease. Further studies are needed to determine factors associated with the risk of pancreatic injury development in HIV+ patients and to assess the setting in which (and the frequency with) pancreatic enzymes should be monitored in this population.
Lipids

W150

A case example of familial hypercholesterolaemia cascade testing within Cwm Taf University Health Board

L Palmer, R Edwards, D Cassidy
Cwm Taf University Health Board, Biochemistry, Merthyr Tydfil, United Kingdom

Familial hypercholesterolaemia (FH) is a common autosomal dominant genetic disorder with a prevalence of \( \sim 1/500 \). It is characterised by hypercholesterolaemia caused by mutations in the LDL-receptor in \( >90\% \) of cases. Due to the elevated cholesterol levels present from birth, patients with FH are at high risk of premature coronary arterial disease (CAD), with untreated FH causing CAD in approximately 50\% of men and 30\% of women before the age of 60 years. This risk can be successfully reduced using high-intensity statin therapy, to reduce LDL cholesterol to \( >50\% \) of baseline levels, and counselling regarding lifestyle. It is estimated that only 15\% of FH cases are diagnosed in UK and therefore in 2010 FH Wales established a coordinated service for the diagnosis and treatment of FH for patients and families. This service enables the genetic diagnosis of an index case to be used to cascade screen family members, who have a 50\% chance of also having the condition. Using a case example here we demonstrate how FH Wales cascade testing has been implemented within Cwm Taf Health Board. Case history: A 48 year old man with a history of hypercholesterolaemia and evidence of CAD was counselled by the FH specialist nurse. The patient’s parents both died prematurely of CAD at 56 years, and of his two sisters, one had been genetically confirmed with heterozygous FH. Biochemistry investigations showed an elevated total cholesterol and LDL-cholesterol despite being on lipid lowering therapy. Secondary causes of hyperlipidaemia were excluded and the patient was consented for predictive genetic testing.

Outcome: Genetics confirmed the patient had the family LDLr mutation (c.1285G>A). The patient is being followed up in the lipid clinic to optimise his therapeutic management and his two children have been referred to genetics for counselling prior to genetic testing.

W151

Pregnancy-induced oxidative stress at various gestational ages and associated lipid peroxidation: a case study among Ghanaian women with normal pregnancy

FA Yeboah1, IAN Awinibuno1, C Obirikorang1, TE Donkor1, B Nkum2
1Kwame Nkrumah University of Science and Technology, School of Medical Sciences, Dept. of Molecular Medicine, Kumasi, Ghana; 2KATH, Dept. of Medicine, Kumasi, Ghana

Objective of study: The study aim was to evaluate the impact and extent of pregnancy-induced oxidative stress on maternal plasma lipids and lipid peroxidation of normal pregnancy at various gestational age among a cross-section of Ghanaian women.

Study design and setting: A cross-sectional study in which convenience sampling technique was adopted to select 239 women with uncomplicated pregnancy. Study subjects were recruited from the Antenatal Clinic of James Town Maternity of the Ussher Polyclinic in the Accra metropolis of Ghana.

Sampling: Recruited subjects were grouped into three according to their gestational age, first trimester\((n=81)\) second trimester\((n=125)\) and third trimester\((n=33)\). Control subjects were non-pregnant women\((n=48)\) and matched for age.

Methodology: Anti-oxidized plasma samples from various grouped subjects were assayed for the levels of Malondialdehyde (MDA), and serum lipid profiles using standardised methods.

Results: Serum Malondialdehyde (MDA) level, the lipid peroxidation marker was significantly raised in women with normal pregnancy \((1.5 \pm 0.3 \mu mol/L) (p < 0.05)\) compared to non-pregnant women \((1.2 \pm 0.2 \mu mol/L) (p < 0.05)\). Serum MDA levels were significantly increased during first, \((1.4 \pm 0.2 \mu mol/L) (p < 0.01)\), second \((1.5 \pm 0.5 \mu mol/L)\) and third \((1.7 \mu mol/L)(p < 0.001)\) trimesters of normal pregnancy compared with nonpregnant \((1.2 \pm 0.2 \mu mol/L) (p < 0.05)\). A significantly positive correlation was seen between MDA levels and total Cholesterol \((p < 0.05)\), triglycerides \((p < 0.05)\) and LDL Cholesterol\((p < 0.05)\) in women with normal pregnancy in all the three trimesters.

Conclusion: MDA a circulating marker of oxidative stress tend to be markedly raised with increasing gestational age of women with normal pregnancy which is characterized by significant changes in serum lipid profile.
Impact of target driven incentivised care on cholesterol levels in patients with diabetes over a 13 year period

K Stuart¹, J Raju¹, S Allen¹, E Clarke¹, A Chithiramohan¹, S Ramachandran²
¹University Hospitals Birmingham NHS Foundation Trust, Clinical Biochemistry, Birmingham, United Kingdom, ²Heart of England Foundation Trust, Clinical Biochemistry, Birmingham, United Kingdom

Aim: We studied the impact of standards set by the National Service Framework (NSF) in 2003 and target-driven incentivised care introduced by the Quality and Outcomes Framework (QOF) in 2004 on the management of total cholesterol (TC) in diabetic patients (target TC < 5 mmol/L).

Method: In preparation for the NSF an audit of 512 primary care diabetic patients in Sutton Coldfield was carried out in 1999/2000. Of the 286 diabetic patients (no selection bias evident: demography, complications, treatment and HbA1c) with a TC result in 2007/2008 and 2012/2013 respectively; mid and end-points of the NSF ten year plan. TC from all 3 audits was compared (paired t-test) using total cohort and quartile data (based on the 1999/2000 TC). Finally, patients meeting the QOF target was estimated.

Results: Compared to 1999/2000 (5.39 (95% CI: 5.17-5.61) mmol/L) a significant decrease (p < 0.001) in mean TC was observed in 2007/8 (4.35 (95% CI: 4.14-4.55) mmol/L) and 2012/3 (4.19 (95% CI: 3.95-4.43) mmol/L). Quartiles 2-4 (Q2 (4.8-5.3 mmol/L), Q3 (5.3-6.0 mmol/L) and Q4 (> 6.0 mmol/L)) showed reductions (p < 0.001) in both 2007/8 and 2012/3 compared to 1999/2000. No significant reduction in Q1 (1999/2000 TC < 4.8 mmol/L) was observed. There was no significant difference in TC between 2007/8 and 2012/3; total cohort and individual quartiles. Lastly, 35.4%, 78.8% and 80.2% of patients achieved target TC in 1999/2000, 2007/2008 and 2012/2013 respectively.

Conclusion: Our audit suggests that the TC target of 5 mmol/l (NSF/QoF) was the main driver in diabetic patients as improvements were not observed in quartile 1 (TC < 4.8 mmol/L). Subsequent guidelines (e.g. JBS2 in 2005) suggesting an optimal TC standard of 4 mmol/l, did not influence TC management.

Lipoprotein(a) levels in people with and without genetically confirmed familial hypercholesterolaemia

SJ Jenks¹, F Musa¹, M Caslake², J Malo¹, J Cooney², SW Walker²
¹Royal Infirmary of Edinburgh, Clinical Biochemistry, Edinburgh, United Kingdom, ²University of Glasgow, Vascular Biochemistry Department, Glasgow, United Kingdom

Introduction: Studies have shown individuals with familial hypercholesterolaemia (FH) have elevated levels of lipoprotein(a) (Lp(a)) in comparison to the general population and that these elevated Lp(a) levels confer an additional increase in the risk of ischaemic heart disease. Many of these studies were carried out prior to genetic testing for FH becoming widely available. Our study is the first to compare Lp(a) levels in people with genetically confirmed FH against Lp(a) levels in people with hypercholesterolaemia sufficient to meet the Simon Broome criteria for FH but without any mutation being found by current genetic screening.

Methods: Lp(a) levels were measured on samples collected from participants recruited at the Royal Infirmary of Edinburgh lipid clinic between August 2010 and August 2012. Lp(a) levels were measured using an isoform independent immunoturbidimetric automated assay (Randox, Crumlin, UK). Lp(a) levels were defined as being elevated if the result was above the European Atherosclerosis Society recommended threshold of >50mg/dl. The Lp(a) results were positively skewed and were log transformed prior to further analysis. Multivariate analysis was adjusted for age, gender, pre-treatment total cholesterol.

Results: 46 (35%) of the 131 samples were from patients with genetically confirmed FH and 85 (65%) were from patients without a mutation. The median Lp(a) results for participants with and without genetically confirmed FH were 31.7 mg/dl (IQR 16.9-98.1) and 25.4 mg/dL (IQR 9.1-93.0) respectively. Similar numbers of participants in each group had elevated Lp(a) levels (35% with genetically confirmed FH Vs 39% without). No significant difference in Lp(a) levels between participants with and without FH was found on either univariate or multivariate analysis (all p values > 0.05).

Conclusions: Our study did not identify any statistically significant difference in Lp(a) levels between people with and without genetically confirmed FH. Additional, larger studies are required to replicate this finding.

Audit of the diagnosis of familial hypercholesterolaemia in primary care

K Shipman¹, S Ganeshamoorthy², M Labib²
¹Russells Hall Hospital, Dept of Clinical Biochemistry, Dudley, United Kingdom, ²General Practice VTS, West Midlands, United Kingdom

Familial hypercholesterolemia (FH) is characterized by high total cholesterol (TC) and/or low-density lipoprotein cholesterol (LDL-C) concentrations in the blood and is associated with high rates of premature atherosclerotic disease. The identification and management of FH is based
on clear guidance published by NICE in 2008 (CG71). The guidance states that FH should be considered in patients with TC>7.5 mmol/L (or LDL-C>4.9 mmol/L), if the levels remain high following a period of lifestyle modification and after excluding secondary causes. The guidance does not specify whether the management should occur in primary or secondary care. We have audited whether NICE guidelines have been followed in a large teaching general practice surgery.

The electronic notes were searched for adults with TC>7.5 mmol/L in 2010. Thirty nine patients were identified and their notes were audited >15 months after the initial test. TC was repeated in 82% with additional tests performed to exclude secondary causes in 59-72%. Dietary advice was given to 38% and repeat lipid profiles were performed in 28% after the advice. Relevant medical history was documented in 64-67%, smoking status in 56%, stigmata of hyperlipidaemia in 8% and family history of ischaemic heart disease and hyperlipidaemia in 56% and 21%, respectively. None of the patients managed exclusively in primary care was given the diagnosis of FH. The various diagnoses given were: pure hypercholesterolaemia (15%), hyperlipidaemia NOS (13%), serum cholesterol raised (8%) and dyslipidaemia (3%).

Our audit showed that FH is poorly identified in primary care. The use of descriptive diagnoses for lipid abnormalities was inappropriate and probably resulted in under-diagnosis of FH and poor compliance with NICE guidance. There is an urgent need to increase the awareness about FH in primary care in order to improve the detection and management of these high risk patients.

**W155**

The impact of statin intolerance in lipid clinic patients

V Mishra, K Williams

Royal Liverpool & Broadgreen University Hospitals NHS Trust, Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom.

**Aim:** Cardiovascular disease is a very common and serious problem in the western world; first line management relates to lifestyle changes and addressing risk factors such as hypercholesterolaemia. Statin drug therapy is used in primary, secondary prevention and familial hypercholesterolaemia. However, these are frequently associated with side effects ranging from muscle problems, elevated liver enzymes and neurological problems; causing poor adherence and thus putting patients at risk for future cardiovascular events. The study aimed to analyse the clinical effectiveness of alternative lipid lowering therapy in achieving NICE (national institute of clinical excellence) lipid targets and reducing cardiovascular risk in patients who were intolerant to statins.

**Methods:** 50 patients attending the Royal Liverpool and Broadgreen University Hospital NHS Trust out patient lipid clinic with statin intolerance were identified. Using a proforma, clinical data on these patients was gathered to see the effectiveness of alternative lipid lowering therapies.

**Results:** The lipid targets required to reduce the cardiovascular risk were not met by the majority of patients; with only 9.5% patients meeting NICE lipid targets. Pravastatin was seen to be the most tolerable statin, with alternative statin plus ezetimibe showing the largest reduction in serum total and LDL cholesterol levels.

**Conclusions:** Pravastatin should be preferred in those patients who are intolerant to one or more statin. A combination of alternative statin plus ezetimibe should be tried, as it appeared to be the most effective therapy in lowering serum total and LDL-cholesterol. The use of ezetimibe, bile acid sequestrant and omacor as monotherapy is ineffective and thus should not be instigated.

**W156**

Fatty acid serum composition and genetic polymorphisms of fatty acid binding protein-2 in elderly subjects with metabolic syndrome

L Feher Turkovic, D Pasalic, I Delas

Applied Health Studies-Zagreb, Zagreb, Croatia, Zagreb University School of Medicine, Zagreb, Croatia

**Background:** Metabolic syndrome (MetS) is a cluster of metabolic abnormalities including dyslipidemia. FABP2 genetic polymorphism might affect serum lipid concentrations and therefore it would be interesting to investigate its association with fatty acid composition.

**Material and methods:** The cross-sectional study included 21 men and 67 women older than 70 years. International Diabetes Federation criteria was used for determination of MetS. Serum lipids were determined with standard enzymatic methods. FABP2 genetic polymorphism Ala54Thr was performed using PCR-RFLP method. The fatty acids (FA) analysis was done after extraction of total lipids from serum with mixture of isopropanol/chloroform(1:5 v/v) and the composition of FA was performed by gas chromatography on SRI 8610C Gas chromatograph.

**Results:** Median concentrations of C14 (myristic acid) was significantly higher in subjects with MetS than in those without MetS (625 vs. 468 mg/mL, p=0.026), while median concentrations of C20:4(n-6) (all-cis-5,8,11,14-eicosatetraenoic acid) was significantly higher in rare allele
carriers than in non-carriers (1719 vs. 516 mg/mL, p=0.014). Weak Spearman rank correlations of C14 with triglyceride and total cholesterol concentrations was also observed (ρ=0.335, p=0.002 and ρ=0.237, P=0.027, respectively.

**Conclusion:** Composition of serum fatty acids might be associated with MetS and FABP2 genetic polymorphism Ala54Thr. Therefore it might be useful to analyses fatty acids for better understanding of MetS etiology.

**W157**

**Identical mutations in exon 7 of the PCSK9 gene with very different phenotypic presentations of familial hypercholesterolaemia in two siblings**

PF Downie¹, GR Bayly¹, M Williams², S O’Shea³

¹University Hospitals of Bristol NHS Foundation Trust, Dept. of Biochemistry, Bristol, United Kingdom, ²Southmead Hospital, Department of Molecular Genetics, Bristol, United Kingdom

Familial hypercholesterolaemia (FH) results from mutations in the low density lipoprotein receptor gene (LDLR), the gene for apolipoprotein B (apoB) or the proprotein convertase subtilisin/Kexin type 9 (PCSK9) gene. Mutations in the PCSK9 gene are unusual in that they are ‘gain of function’ mutations resulting in the disease state. Common mutations in APOB c.10580G > A p.(Arg3527Gln) and PCSK9 c.1120G>T p.(Asp374Tyr) have been reported whereas there are over 1200 different mutations in the LDLR gene.

We present a case of 2 siblings, both seen in the lipid clinic at the Bristol Royal Infirmary. Sequence analysis of the PCSK9 gene in both of these patients identified the heterozygous missense mutation in exon 7; c.1120G>T p.(Asp374Tyr) as the cause of their familial hypercholesterolaemia. Baseline pre-treatment lipid profiles were as follows Sibling A: Total Cholesterol 18mmol/L, HDL-C 1.2mmol/L, LDL-C 15.2mmol/L, Triglycerides 3.5mmol/L. Sibling B Total Cholesterol 7.3mmol/L, HDL-C 0.9mmol/L, LDL-C 6.0mmol/L, Triglycerides 0.9mmol/L.

The specific mutation (genotype) and the subsequent molecular mechanism is a key determinant of the severity of the phenotype in patients with mutations in the LDLR gene. The same is likely to be true for patients with PCSK9 mutations. The c.1120G>T PCSK9 mutation has been widely reported to be associated with a clinically very severe phenotype. However, in this instance the phenotypes of the two siblings are strikingly different despite having both inherited the c.1120G>T mutation.

We conclude that exposure to different environmental factors could influence the phenotype even in individuals with identical mutations. There may also be as yet unknown mutations which moderate the clinical presentation in this family. It has been suggested that even in patients where a mutation is identified a substantial polygenic contribution could lead to variable penetrance of the disease.

**W158**

**Case-finding of severe hyperlipidaemia and the role of the clinical laboratory**

A Gbegbaje¹,²

¹Royal Bolton Hospital NHS Foundation Trust, Farnworth, Bolton, United Kingdom, ²Wrightington, Wigan and Leigh NHS Trust Foundation Trust, Wigan, United Kingdom

Familial hypercholesterolaemia is one of the most common dominantly inherited disorders identified in primary care. Around 1: 500 people are affected by this condition with increased risk of coronary heart disease, but less than 15% of these currently attend lipid clinics, suggesting that the vast majority are unrecognised in other primary and secondary care settings.

The aim of this audit was to determine clinical locations within two acute Trusts and their local General Practices of patients with significant hypercholesterolaemia (greater than 12mmol/L) with or without hypertriglyceridaemia.

Method: Two hospital laboratories based at Crewe and Wigan had qualifying fasting lipid profile results identified for a 3-year and 6-month period respectively using the laboratory computer database.

Results: At Crewe, 142 qualifying lipid profile results were determined for 40 patients with 52 tests from hospital locations and 90 from GP locations. 13 patients had shared-care between primary and secondary locations with 21 patients based solely in primary care and 6 hospital-based. 54% were female and 46% male. Mean age was 53 years (12-84). 4 patients attended the Lipid clinic with 2 patients managed by shared-care with their GP. Other hospital specialities were Gastroenterology (5) and Renal (4). At Wigan, 35 qualifying lipid profile results were determined for 26 patients. 7 patients were at hospital locations, 15 patients at GPs, while 4 patients had shared-care between primary and secondary care. 54% of patients were female and 46% male. The median age was 49 years (5-88 years). Only 2 patients attended the Lipid clinic while the hospital speciality with the highest frequency was Gastroenterology (4 patients). Other hospital locations were Coronary Care and Paediatrics.

In summary, Laboratory medicine has a role with clinical colleagues in identification of patients with severe hyperlipidaemia and/or recognising the relevance of a family history of premature coronary heart disease.
Toxicology/TDM

W159

Thiopurine hypermethylation and clinical outcomes in patients with inflammatory bowel disease

P Blaker1, M Arenas2, E Escuredo2, S Fong1, P Irving1, J Sanderson1, A Marinaki2
1Guy’s and St Thomas’ NHS Foundation Trust, Gastroenterology, London, United Kingdom, 2Viapath, Purine Research Laboratory, London, United Kingdom

Introduction: Azathioprine hypermethylation predicts a lack of efficacy and excess adverse events. Thiopurine hypermethylation (TH) can be circumvented by low dose AZA/MP and allopurinol therapy. The aim of this study is to determine if thiopurine metabolite profiles at week 4 of treatment can predict its occurrence to allow early combination treatment.

Methods: 181/273 patients with average MeMP:TGN ratios < 11 were compared to 92 patients with average MeMP:TGN ratios ≥ 11. Clinical outcomes were recorded for the first 12 months of therapy. Treatment failure was determined by 3 gastroenterologists. Thiopurine metabolite profiles were measured in 139 patients at week 4 of therapy and were compared to average metabolite profiles between 12-52 weeks of treatment.

Results: Patients with TH were more likely to fail AZA/MP monotherapy during the first 12 months of therapy, in comparison with patients with normal methylation profiles (p = 0.0088). The difference in the number of treatment failures at 12 months was 15.7%. There was an excess of hepatotoxicity with TH (p = 0.0006; OR 8.058; 95% CI 2.188-29.670; Fisher’s exact). TH was demonstrated in 84% of cases by 12 weeks of therapy. A MeMP: TGN level > 6.17 at week 4 accurately predicted TH (ratio ≥ 11) after 12 weeks of therapy (AUC = 0.839; p < 0.0001; sensitivity = 75.4%; specificity = 88.4%).

Conclusion: TH is associated with an excess of thiopurine treatment failures as monotherapy immunosuppression during the first 12 months of treatment. We also confirm its association with hepatotoxicity. A MeMP:TGN > 6.17 may be useful in early identification of patients likely to benefit from combination treatment.

W160

A new urine marker for detecting adamantyl type synthetic cannabinoids

L Ford, J Berg
Sandwell & West Birmingham Hospital NHS Trust, Department of Biochemistry, Birmingham, United Kingdom

Cannabinoids (NOIDS) are drugs aiming to mimic the effect of cannabis. They can be purchased as powder, sprayed onto plant material and even as e-cigarette refills. Some newer NOIDS such as AKB-48 and 5F-AKB-48 contain an adamantyl group. These compounds are still legal in the UK, and currently the active compound in many legal high products. Immunological screening methods for cannabis and those targeted for older synthetic NOIDS such as JWH series do not detect adamantyl-NOIDS.

At the start of 2014 we received 10 requests from patients suspected of taking NOIDS. For eight of these cases the name of the suspected legal high taken was known. For three cases we also received a part smoked joint.

Using Waters LC-MSQTof we developed a method to identify the parent NOIDS in legal high products as well as screening for possible metabolites in urine for use as a clinical marker.

Time of flight analysis of Herbal Haze, Sirius and Jolly Joker and the part smoked joint detected AKB-48 and 5f-AKB-48. Ten patient samples were screened for 17 potential metabolites. Adamantyl-A1 was present in all 3 legal highs, the joint and the 10 patient urines.

Retrospective LC-MSQTof analysis of 252 urine samples collected from drug abusers in August 2013 identified 7 Adamantyl-A1 positive patients (2.8%). In the first 2 days of addition of Adamantyl-A1 to our LC-MS/MS routine urine drugs of abuse screening method we identified 5 positive patients out of 202 tested (2.5%), 4 of which were from the same location (9% of requests).

We are the first to report a marker in urine for detecting abuse of the new Adamantyl-NOIDS currently legal in the UK and are gathering evidence of their wide spread use. This marker has now been added to our routine urine and oral fluid drugs of abuse screen.

W161

Systematic approach to legal highs monitoring as part of drugs of abuse screening with LC-MSTof as a foundation

L Ford, J Berg
Sandwell & West Birmingham Hospital NHS Trust, Department of Biochemistry, Birmingham, United Kingdom

With the rise in legal highs and availability of drugs on the internet normally only available on prescription, drug abuse in the UK is changing and increasingly for clinical screening a set panel of classic drugs of abuse is no longer relevant. Using the latest technology such as time of flight it
is possible to screen simultaneously for 1000s of classic drugs of abuse, legal highs and over the counter/prescribed medications making it well suited to monitor changing patterns of drug abuse as well as identify new drugs of abuse that requesting clinicians may not be aware of.

As part of our clinical toxicology vigilance system in November 2013 we analysed 291 routine urine samples received from our local mental health trust using a Waters LC-MSTof (time of flight) analyser.

As well as classic drugs of abuse time of flight identified potential cutting agents. For cocaine positive samples 85% were found to contain the veterinary drug (de-wormer) levamisole, 47% phenacetin (analgesia) and 35% etilefrine (banned sports drug). 10% of the study samples were found to contain legal highs including ethylphenidate a synthetic cocaine mimic, BZP a piperazine stimulant, para-methoxyamphetamine (PMMA) an ecstasy mimic, ethylene, butylene and methylene, all synthetic cathinones. For over the counter medications antihistamines were the most popular drug detected with 15% of patients positive for cetirizine, hydroxyzine, diphenhydramine, chlorpheniramine, promethazine and or fexofenadine. For prescription drugs 4.1% of patients were found to be positive for the anticonvulsants pregabalin and or gabapentin.

Time of flight is a useful tool in our clinical toxicology repertoire. Regular screening forms part of our clinical toxicology vigilance system helping us to tailor our routine drug screening panel to reflect the latest trends in drug abuse as well as best meeting the requirements of our users.

**W162**

**Clinical toxicology screening. What a difference a Tof makes**

*L Ford, J Berg*

*Sandwell & West Birmingham Hospital NHS Trust, Department of Biochemistry, Birmingham, United Kingdom*

Time of flight is a technique capable of accurately measuring the mass of a compound to within 3 decimal places making it highly specific. The ability to create exact mass libraries containing 1000s of different drugs and metabolites together with the ability to use a wide range of sample types makes it particularly well suited for unknown toxicology screening.

We have validated and introduced an unknown screening service using a Waters LC-MSTof analyser. Here we present cases demonstrating the utility of this new method.

**Case 1:** “Miracle Cream” purchased off the internet. The requesting clinician was suspicious it may contain steroids after the patient stopped taking azathioprine for the treatment of eczema. We detected the topical steroid clobetasol propionate and the fungicide Flucanazole. We have since received many more requests to test creams and herbal remedies. All have been found to contain steroids with high levels of fungicides.

**Case 2:** Legal high powder CHING brought from head shop. This patient had been taking this and had a psychotic episode, attacking family members. We identified a new drug to our exact mass library, ethylphenidate, a cocaine mimic. Identification of new legal highs to our exact mass library is now routine and we currently have over 100.

**Case 3:** Unknown tablet. Found in a patients room on a secure ward the concern was they may be dealing. The hand-made tablets were identified as the sweetener aspartame. We receive regular requests to identify tablets that pharmacists cannot recognise. Most turn out to be over the counter vitamins!

Time of flight allows us to screen for multiple drugs in a single analysis reducing turn-around times to hours rather than days allowing us to report results in a clinically relevant time frame, a huge advantage in cases of poisoning and safeguarding where results can be diagnostic.

**W163**

**5 year review of UK 24 hour emergency testing service for plasma ethylene glycol and diethylene glycol**

*L Ford, J Berg*

*Sandwell & West Birmingham Hospital NHS Trust, Department of Biochemistry, Birmingham, United Kingdom*

Ethylene glycol (ETHG) and diethylene glycol (DIEG) can be found in a wide range of products as anti-freezing additives, preservatives, and general purpose cleaner. Poisoning can be the result of accidental or intentional ingestion. Osmolar and anion gap are non-specific clinical indications of glycol poisoning and detection in blood is the only way to make a definitive diagnosis.

In response to concerns that cases of DIEG poisoning were being missed, in 2009 we launched a new GC-FID method for the simultaneous detection of ETHG and DIEG. Here we present 5 year audit of this service from 2009 to 2013.

The total number of requests received was 811 from 93 different UK locations. 56% of requests were for first time screening and 44% repeat analysis. More males were tested 60% compared to females 40%. Only 1 in 3 requests for first time screening was glycol positive. The highest ETHG concentration measured was 8666 mg/L in a 49 year old male who was successfully treated and survived.

This is the first UK audit to include data on the frequency of DIEG poisoning. DIEG was present in ~14% of ETHG positive samples, but was never detected on its own. Typically DIEG increased the total toxic glycol load by ~37%. Our data suggests it is not essential to measure DIEG since its inclusion is unlikely to change patient management. In many cases where the glycol screen was negative methanol screening was requested. None of these requests were found to be methanol positive.

We offer an effective 24 hour service for glycol poisoning. Our data shows a number of glycol poisoned patients appear to be treated without knowledge of ETHG/DIEG levels.
W164

Performance of TDM assays for carbamazepine, phenobarbital, phenytoin, and gentamicin on the Roche cobas c501 analyzer

WL Tsai1, WJ Kuo1, WW Chang1, ST Chiang1, SC Shiesh2
1National Cheng Kung University Hospital, Department of Pathology, Tainan, Taiwan, Republic of China, 2National Cheng Kung University, Department of Medical Laboratory Science and Biotechnology, Tainan, Taiwan, Republic of China

Aim: The present study aimed to evaluate analytical performance of therapeutic drug monitor (TDM) assays for carbamazepine, phenobarbital, phenytoin, and gentamicin on the Roche cobas c501 analyzer.

Methods: Roche TDM assays are based on kinetic interaction of microparticles in a solution (KIMS). Performance characteristics including imprecision, accuracy, linearity, and method comparison with the Abbott AxSYM immunoassay system were evaluated.

Results: For carbamazepine, phenobarbital, phenytoin, and gentamicin, the inter-assay CVs were 2.1%, 2.2%, 3.4%, and 3.8%, respectively, while the intra-assay CVs were 1.5%, 2.0%, 3.0%, and 3.0%, respectively. For the accuracy of the method, the mean measured values of College of American Pathologists (CAP) survey samples at 5 different levels agreed well with the peer group mean with bias ranging between 0.2% and 14.3%. The linear ranges for carbamazepine, phenobarbital, phenytoin, and gentamicin using the CAP survey samples were demonstrated to be between 2.5-18.5 μg/mL, 8.2-70.5 μg/mL, 4.7-34.1 μg/mL, and 1.1-8.8 μg/mL, respectively, verifying the linearity claim of the manufacture. In method comparison, linear regression analysis showed y = 1.101x-0.401 (r² = 0.987) for carbamazepine, y = 0.997x + 1.711 (r² = 0.980) for phenobarbital, y = 1.029x-0.354 (r² = 0.986) for phenytoin, and y = 0.845x + 0.120 (r² = 0.986) for gentamicin.

Conclusions: The TDM assays on Roche cobas c501 provide a rapid, precise and accurate measurement for assessing therapeutic efficacy.

W165

Higher red blood cell methotrexate polyglutamates correlate with increased disease activity, and are useful for assessing adherence

S Fong1, M Arenas2, S Anderson1, P Irving1, J Sanderson1, A Marinaki2
1Guy’s and St Thomas’ NHS Foundation Trust, Gastroenterology, London, United Kingdom, 2Viapath, Purine Research Laboratory, London, United Kingdom

Background: Methotrexate (MTX) is used in patients with inflammatory bowel disease (IBD). Within red blood cells (RBC), MTX is activated by sequential addition of glutamic acid residues to form polyglutamates (MTXPG1-5). In rheumatoid arthritis, low [MTXPG] has been associated with active disease, whereas other studies have demonstrated an inverse relationship, including the only published data in IBD. The aim of this study was to determine if RBC MTXPG reflect clinical response in IBD patients and whether measurement is useful in assessing adherence.

Methods: RBC MTXPG1-5 was measured using high-performance liquid chromatography. Clinical status of the 21 patients recruited (active disease or remission) was assessed by 2 IBD physicians blinded to MTXPG. Pearson correlation coefficient, r was calculated to assess the relationship between MTX dose and PGn concentration. Association between PGn and clinical response was analysed with unpaired t-test.

Results: Four of twenty one (22%) patients (3 of whom admitted non-adherence) had undetectable MTXPGs. MTXPG2-4 were detected in all adherent patients with PG3 accounting for a mean of 43% of total MTXPG. A linear relationship between dose of MTX and PG1-4 was observed. 12/21 (57%) patients were assessed as having active disease. No significant difference in mean [MTXPGn] was observed between those with active disease and remission. For each MTXPGn, a non-significant trend towards a higher concentration was observed in patients with active disease.

Conclusions: In this study, the largest to date in IBD, measuring RBC MTXPG was useful in assessing adherence to MTX. A trend towards higher PG concentrations was associated with active disease confirming the findings in the only other study in IBD. Whether this is confounded by higher doses being used in patients with more active disease warrants further study in larger, prospective trials.

W166

Clinical study of serum trough infliximab concentrations and anti-infliximab antibodies in a cohort of gastroenterology and rheumatology patients

NL Barlow, P Mohammed, JD Berg
Sandwell and West Birmingham Hospitals NHS Trust, Department of Clinical Biochemistry, Birmingham, United Kingdom

Infliximab, a chimeric human-mouse monoclonal antibody directed against tumour necrosis factor, is widely used in the treatment of inflammatory bowel disease and rheumatoid arthritis. The drug is typically administered as an infusion at 8 week intervals. However, its use is
limited by the development of anti-infliximab antibodies, which leads to loss of therapeutic efficacy. Therapeutic drug monitoring of trough serum infliximab concentrations and anti-infliximab antibodies has recently entered into routine clinical practice to guide use of the drug. Trough serum infliximab was measured in 237 samples from 109 gastroenterology and rheumatology patients on infliximab therapy. These data were then correlated with infusion number and markers of disease activity. Anti-infliximab antibodies were also measured in 164 samples with the aim of evaluating a therapeutic cut-off for serum infliximab of 1 μg/mL.

The median (25th-75th percentile) infliximab in maintenance therapy was 3.71 μg/mL (1.22-5.22, n=201), with 23% having a sub-therapeutic concentration of ≤1 μg/mL. No correlation with the number of previous infusions was noted. A significant proportion of samples had positive anti-infliximab antibodies: 84/164 (51%), which subdivided to 39/46 (85%) and 44/155 (28%) with infliximab ≤1 μg/mL and >1 μg/mL respectively.

CRP was found to be significantly higher where infliximab was ≤1 compared to >1 μg/mL (10mg/L (<5-24), n=45 vs < 5mg/L (<5-8), n=158), although there was no direct correlation. A similar relationship was noted for faecal calprotectin; infliximab ≤1 vs >1 μg/mL: 1873 (361-3090) μg/g stool, n=6 vs 249 (105-1048), n=34. However, no association was observed either between the inflammatory markers and antibody status or between Harvey Bradshore Index and infliximab.

The findings support use of the 1 μg/mL cut-off and highlight the importance of trough infliximab levels over anti-infliximab antibodies. Faecal calprotectin may be a more sensitive marker of disease activity than Harvey Bradshore Index and superior for assessing response to infliximab treatment.

W167

Assessment of drug abuse in emergency hospital

RAA Abdeldayem, Toxicology Fellow
Mansoura University, Toxicology Unit-Emergency Hospital, Mansoura, Egypt

Background: Drugs have played a major role in defining the sub cultural and counter cultural influences in society. The pervasive availability of psychotropic chemicals has profoundly altered the cultural environment and can causes a direct physiological and psychological change in the body.

Introduction: The study was conducted on patients (n=390) with acute poisoning by some drugs of abuse. They were admitted to poison unit, emergency hospital, Mansoura University.

Aim of the work: Detect the types of drugs taken by overdose among patients from the laboratory point of view.

Materials and methods: All patients were subjected for detection of drugs of abuse in urine by EMIT system and Gas Chromatography / Mass Spectrometry (GC/MS) for confirmation of the obtained results.

Results: Approximately 75% of patients were encountered in the age group 20-40 years. Also, the study revealed that the majority of patients were of low and moderate social classes. Cannabis was the first abused drug (37.69%) followed by opioids (27.18%). Female patients were likely to abuse benzodiazepines (57.14%). The study revealed that the percentages of positive urine samples by EMIT were; (27.18%, 14.87%, 11.54% 9.74% and 1.79%), for cannabis, opiates, benzodiazepines, barbiturates and ethyl alcohol, respectively and by GC/MS were; 16.15%, 10.25%, 8.97% and 8.46% for cannabis, benzodiazepines, barbiturates and opiates, respectively.

Conclusion: Some of abuse drugs are exist in our life, so adolescents and young adults are really in danger. The analytically and distribution data obtained in this work will be useful for the toxicologists working in this field.

Recommendations: Immunoassay technique should be done on all urine samples of addicts and better to be confirmed using GC/MS. Also, continuous health education and prevention programs concerning health hazards of abuse drugs among adolescents and young adults are highly indicated.

W168

Chronic alcohol abuse diagnosis: comparison between serum Carbohydrate-Deficient Transferrin and hair Ethyl glucuronide

V Bianchi1, S Premaschi1, A Raspagni2, S Secco1, M Vidali3
1Toxicology Laboratory, SS. Antonio e Biagio e C. Arrigo Hospital, Alessandria, Italy, 2School of Medicine, Insubria University, Varese, Italy, 3Clinical Chemistry Unit, Maggiore della Carità Hospital, Novara, Italy

Background: In heavy alcohol consumption laboratory tests represent an objective evidence. To this aim several biomarkers and biological matrices may be evaluated. In this study we compared older and newer biomarkers in blood and in hair.

Methods: Carbohydrate-Deficient Transferrin (CDT), Ethyl glucuronide (EtG), AST, ALT, GGT, MCV were measured in a large sample (n=562). All people declared no alcohol consumption within the last three months. Serum CDT was measured by the candidate HPLC reference method
and expressed as relative amount of disialotransferrin (%DST: cut-off 1.7%). EtG was measured in hair by a validated home-made method in LC-MS/MS (cut-off 30 pg/mg).

**Results:** Respectively, 42 (7.5%) and 76 (13.5%) subjects were positive to CDT and EtG. In particular, 30 (5.3%) subjects were positive to both tests, 12 (2.1%) only to CDT, while 46 (8.2%) only to EtG. The agreement (positive and negative pairs) between CDT and EtG was 89.7%. CDT-positive subjects displayed significantly higher MCV (median 94.7 vs 92.3 fL; p<0.003) and EtG (46 vs 8 pg/mg; p<0.0005), but not AST, ALT and GGT, than CDT-negative subjects. EtG-positive subjects displayed significantly higher MCV (94.0 vs 92.1 fL; p=0.001), GGT (28 vs 19 UI/L; p<0.0005) and CDT (1.4% vs 1.1%; p<0.0005), but not AST and ALT, than EtG-negative subjects. Interestingly, up to 6 out of 12 (50%) CDT-positive subjects had EtG<15 pg/mg, whereas up to 27 out of 46 (59%) EtG-positive subjects had CDT<1.1%. Up to 41 out of 76 (54%) EtG-positive subjects display EtG values within 30-50 pg/mg.

**Conclusion:** Large variability exists between CDT and EtG in detecting chronic alcohol consumption. We suggest to use CDT, or a combination of different biomarkers, to identify alcohol abuse in a forensic context. EtG results close to the cut-off (30-50 pg/mg) should be cautiously considered before any sanction is assigned.

**W169**

**Development of liquid stable commutable material for use in an external quality assessment scheme for therapeutic drug monitoring**

**MA Thomas, J Morris, J Allen, S Jones**
Cardiff and Vale University Health Board, WEQAS Quality Laboratory, Cardiff, United Kingdom

**Introduction:** In June 2013 WEQAS initiated a pilot EQA Scheme for TDM which included: Amikacin, Carbamazepine, Ciclosporin, Digoxin, Gentamicin, Lithium, Lamotrigine, Methotrexate, Phenobarbital, Phenytoin, Sirolimus, Tacrolimus, Teicoplanin, Theophylline, Tobramycin, Valproic acid and Vancomycin. The aim of the study was to develop and assess the stability, commutability and recovery of TDM analytes in a liquid matrix. For the first 3 distributions, a commercial source of lyophilized Quality Control material was used. This provided a means to assess analytical performance and provide a benchmark for the suitability of the “in house” liquid samples.

**Method:** Sterile human serum was used as base material, with each drug gravimetrically added to a target concentration to cover a suitable therapeutic range. Intermediate pools were prepared by mixing a high pool with the base to produce a linear panel. All samples were stored at +4°C until dispatched. The “weighed-in” value of the spiked drug and its known linear dilution was used to calculate the target value. Thirty-five laboratories agreed to take part and 34 samples were distributed in 8 rounds over this period. The inter-laboratory variation for the liquid samples was compared with the commercial QC material. The overall mean of the participants data was used to monitor stability over a 63 day period.

**Results:** Apart from Vancomycin, the stability data showed no significant change at +4°C. For gentamicin, theophylline and tobramycin there was a significant improvement in interlaboratory variation and method agreement for the liquid samples compared with the commercial material. For Amikacin a CV of 2 to 6% (low to high concentration) was observed across the therapeutic range, a CV of 15 to 8% observed for Carbamazepine and Digoxin and a CV of 12 to 4% observed for the majority of other analytes. Good recoveries were observed for the majority of drugs.

**W170**

**Oral fluid drug analysis of a local prison population**

**R Jones, L Ford, J Berg**
Sandwell & West Birmingham Hospital NHS Trust, Department of Biochemistry, Birmingham, United Kingdom

Oral fluid is a convenient non-invasive method for collecting samples for drugs of abuse analysis. With no requirement for toilet facilities or same sex collectors it has become popular in institutions such as prisons. Oral fluid can detect very recent drug abuse 24-48 hours. Here our methods have been used to help in the work up of prisoners who require drug related medical assessment at reception. In 2013 our routine drugs of abuse service changed from immunoassay to an LC-MS/MS method which screens 26 drugs in oral fluid including 4 legal highs. Specimens are collected using a Starstedt Salivette and prepared using an in-house liquid-liquid extraction method. Between April 2013-2014 we analysed 2129 samples received from our local prison population. Requests are mostly from new arrivals including transfers from other institutions. For the classic drugs of abuse 1 in 2.5 prisoners tested were found to be positive for cocaine (42%) and 1 in 3 (35%) the heroin specific marker 6-MAM. Only 43 (2.0%) of prisoners tested positive for amfetamine, metamfetamine or ecstasy. This compares to 35 prisoners (1.7%) that tested positive for the newer synthetic cathinones, mephedrone (meow, meow), 4-MEC (NRG-2) or MDVP (bath salts). These drugs mimic the effect of amfetamines but are not routinely screened in UK clinical laboratories. Thebaine, a poppy seed marker was found in only 2 samples.
Many prisoners require confirmation that they are receiving methadone or buprenorphine. To improve detection we were able to reduce the positive cut off for buprenorphine from 5 to 3ng/mL. 34% of prisoners tested positive for methadone and 13% buprenorphine. This audit of oral fluid drug results has given an insight into current drug abuse amongst prisoners entering a large UK inner city prison and we are currently adding further markers for new ‘legal highs’ to our screen.

W171

Screening method for sulphonylurea use in serum and urine by atmospheric pressure chemical ionisation liquid chromatography mass spectrometry

C McKibbin, I Parkinson, A Armstrong, G Wark
Surrey Pathology Services, Royal Surrey County Hospital NHS Foundation Trust, SAS Peptide Hormones Section, Guildford, United Kingdom

Introduction: Sulphonylurea compounds are insulin-secretagogues and are an important class of oral anti-diabetic medication. Excluding the possibility of sulphonylurea drug use in the hypoglycaemic patient with inappropriately raised insulin and C-peptide is an important step in the diagnosis of insulinoma. Previously these drugs were screened for using an in-house radioimmunoassay (RIA) but identification of specific sulphonylurea compounds was not possible.

Aims: To develop a screening service in a routine clinical laboratory for detection of seven sulphonylurea compounds (chlorpropamide, glibenclamide, gliazide, glimepiride, glipizide, tolazamide and tolbutamide) by atmospheric pressure chemical ionisation (APCI) liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methodology: After salt treatment of urine, protein precipitation of serum and urine with acidified acetonitrile and concentration by solvent vacuum evaporation the extracted sulphonylureas are separated by C18 reverse phase chromatography and identified by at least two specific ion transitions for each compound in multiple reaction monitoring mode. Analyte quantitation is enabled by addition of deuterated trimipramine as an internal standard.

Results: Seven sulphonylurea compounds can be simultaneously identified in serum or urine in a single 8 minute run. The lower limit of detection is shown to range from 5 to 30μg/L with linearity for all analytes to 2000μg/L. Precision profiles demonstrate functional sensitivity at 125μg/L. Positive matrix effects are observed for three sulphonylureas in icteric serum samples. Lithium heparin plasma and boric acidified urine are also suitable for analysis. Concordance was found for all positive sulphonylurea results (N = 30 patient samples, 18 reported positives) as determined by comparison to a validated HPLC-UV wavelength spectrophotometric detection method.

Conclusions: A robust and sensitive APCI LC-MS/MS method for identification of sulphonylureas in serum, plasma and urine has been implemented in a district general hospital clinical laboratory.

W172

Cardiotoxicity and hypothermia in a tricyclic antidepressant and a calcium channel blocker overdose

D Morell-Garcia, IM Bauça, L Quesada, C Yates, A Carrillo, J Paiguriguier, B Barceló
Hospital Universitari Son Espases, Servei d’Anàlisis Clínic, Palma de Mallorca, Spain, Hospital Universitari Son Espases, Servei d’Urgències, Palma de Mallorca, Spain, Hospital Universitari Son Espases, Servei de Cures Intensives, Palma de Mallorca, Spain

Introduction: Some relatively rare intoxications may yield high morbidity-mortality. In such cases, clinical suspicion, along with early analytical-toxicological diagnosis, is essential for effective treatment.

Case report: An undocumented 40-year-old woman was found with low level of consciousness. A first physical examination reported cold skin, bilateral mydriasis, moderate shaking and enolic-like breath smell. She was slightly hypertensive, tachypneic and oxygen saturation was 98%. The case was diagnosed as a severe hypothermia (<35°C) together with probable intoxication, and sexual harassment. Life support measures comprised physical warming and physiological saline infusion. Serum biochemical tests upon admission yielded: creatinine 2.72mg/dL, creatine kinase 793U/L, pH 7.24, bicarbonate 12.4mmol/L, lactate 8.31mmol/L. Other analytes were within normal ranges. Basic qualitative toxicological screening was negative. After conscience recovery, the patient presented hypotension, sinus bradycardia, oligoanuria and tachypnea. A subsequent chest X-ray evidenced a discrete vascular redistribution, so intensive saline therapy was stopped. Dopamine and noradrenaline were even required. After consciousness recovery, the patient presented hypotension, sinus bradycardia, oligoanuria and tachypnea. A subsequent chest X-ray evidenced a discrete vascular redistribution, so intensive saline therapy was stopped. Dopamine and noradrenaline were even required. An expanded toxicological screening using GC-MS and HPLC-UV showed a diltiazem concentration up to 675ng/mL (therap. range 50-200), and amitryptiline+nortryptiline 659ng/mL (therap. range 80-200). The patient was admitted to the ICU with a diagnosis of severe hypothermia with distributive shock, acute kidney failure, rhabdomyolysis, metabolic acidosis and intoxication due to diltiazem and amitryptiline. After an episode of shaking, normothermia and hemodynamic stabilization were achieved (115/65mmHg). Heart rate was 95 bpm, breathing frequency was 25 bpm and oxygen saturation 97%. Acidosis had been
corrected (lactate 0.89 mmol/L), as well as kidney function (creatinine: 0.54mg/dL). Amitryptiline+nortryptiline concentrations had fallen to 366ng/mL, so she was discharged and finally admitted at the Psychiatry ward, where she explained the suicide attempt.

Conclusions: Our patient showed overdose-like symptoms, in good correlation with measured diltiazem levels (675ng/mL). Concentrations between 500-1000ng/mL have been described to induce sinus bradycardia and hypotension. The reported amitryptiline overdose would presumably account for the patient’s confusion and shaking state. The mixed overdose might have synergically contributed to the cardiovascular manifestations and the poor response to the later administered vasopressive drugs.

**EuroLabFocus2014**

**Thursday Posters**

**Oncology**

**Th1**

Diagnostic value of lipopolysaccharide binding protein for infection in cancer patients with febrile neutropenia: comparison with procalcitonin and interleukin 6

_A Hernando Holgado1, E Jiménez Santos1, L García de Guadiana-Romualdo1, P Esteban Torrella1, I Español Morales1, P Cerecuela Fuentes1, M Viqueira González1, J Pedregosa Díaz1, A De-Béjar Almira1, R Carbonell Muñoz1, MD Albaladejo Otón1_

1Hospital General Universitario Santa Lucía, Biochemistry Department, Cartagena, Spain, 2Hospital General Universitario Santa Lucía, Cartagena, Spain, 3Hospital General Universitario Santa Lucía, Hematology Department, Cartagena, Spain, 4Hospital General Universitario Santa Lucía, Oncology Department, Cartagena, Spain, 5Hospital General Universitario Santa Lucía, Microbiology and Parasitology Department, Cartagena, Spain

**Aim:** Early detection of infection is essential for initial management of cancer patients with chemotherapy-associated febrile neutropenia in the Emergency Department. In this study we evaluated lipopolysaccharide binding protein (LBP) as predictor for infection in febrile neutropenia and compared with other biomarkers previously studied: procalcitonin (PCT) and interleukin (IL)-6.

**Methods:**

**Population study:** A total of 61 episodes of chemotherapy-associated febrile neutropenia in 58 adults cancer patients were included. Patients were classified into fever of unknown origin and infection, including microbiologically and clinically documented infection, groups

**Laboratory methods:** From each patient, blood samples were collected on admission for white blood cell, neutrophil count and biochemical analysis, including PCT measurement. Serum samples for IL-6 and LBP were immediately aliquoted, frozen and kept at -80 °C until tested. Besides, blood samples were drawn from each patient for blood cultures before antibiotics were initiated and other biological samples were collected for microbiological studies depending on the suspected focus of infection.

**Statistical analysis:** Statistical analysis was performed with the SPSS, version 18.0. Receiver operating characteristic (ROC) curve analysis was performed for each biomarker for the diagnosis of infection.

**Results:** 32 of the 61 episodes were classified as infection. On admission, PCT, IL-6 and LBP were significantly increased in patients with infection compared to fever of unknown origin group. AUC ROC of PCT, IL-6 and LBP for discriminating both groups were 0.88, 0.82 and 0.82, respectively, without significant difference between them. The combination of IL-6 and PCT or LBP did not lead to a significant improvement of the diagnostic accuracy of PCT or LBP alone.

**Conclusions:** On admission, LBP has a similar diagnostic accuracy than PCT or IL-6 for the diagnosis of infection and might be used as additional diagnostic tool in adult cancer patients with chemotherapy-associated febrile neutropenia.

**Th2**

Evaluation of serum amyloid A, soluble E-selectin and soluble E-cadherin in lung cancer

_V Saini1, C Prashanthi1, J Kaur2, A Jammeja1, S Gupta1, S Jaswal2_

1Govt. Medical College & Hospital Chandigarh, Pulmonary Medicine, Chandigarh, India, 2Govt. Medical College & Hospital Chandigarh, Biochemistry, Chandigarh, India

**Introduction:** Lung cancer is leading cause of cancer deaths worldwide. Symptoms and radiological features of lung cancer overlap with many other respiratory diseases. Interventional investigations like computed tomography guided fine aspiration cytology and bronchoscopy
have many complications. Thus, there is a need for non-invasive diagnostic test before the patients are exposed to invasive procedures for tissue diagnosis. Various researches have been conducted to identify serum biomarkers of lung cancer. The present study evaluates the use of serum amyloid A (SAA), soluble E-selectin (sE-selectin) and soluble E-cadherin (sE-cadherin) in the diagnosis of lung cancer.

**Method:** The study included three groups of 20 subjects each: proven lung cancer patients, other respiratory diseases and apparently healthy individuals. About 5ml of blood sample was collected under aseptic precautions was stored under standard conditions. The serum levels of SAA, sE-selectin and sE-cadherin were measured using ELISA. Statistical analysis was done using SPSS. Cutoff value of the serum biomarkers was calculated by receiver operating characteristic curves. Based on this cut off value and considering demonstration of malignant cells in the pulmonary specimen as gold standard, sensitivity and specificity of each serum marker was calculated.

**Result:** The mean levels of SAA in lung cancer, health and diseased individuals were 24980.5mg/ml, 580.95ng/ml and 11281.05mg/ml respectively. With serum levels of 1068ng/ml as cut off, SAA had sensitivity 80% and specificity of 53% respectively (p< 0.005). Mean sE-selectin levels in the above mentioned three groups were 80.77± 103.67, 103.66 ±60.76 ng/ml and 107.47 ± 68.57ng/ml respectively (P=0.051). Mean sE-cadherin levels were 1042.97ng/ml, 1324.35ng/ml and 1301.92 ng/ml in respectively (p=0.321).

**Conclusions:** SAA was found to be significantly raised in lung cancer patients as compared to patients with other respiratory diseases or healthy controls. SAA could be used as a potential screening tool in diagnosis of lung cancer.

**Th3**

**Early recognition of ovarian cancer symptoms: audit of the use of CA125 in primary care**

*EA Hanon, E Kearney*  
*East Kent Hospitals University Foundation Trust, Department of Laboratory Medicine, Ashford, United Kingdom*

In order to improve early detection of ovarian cancer and its survival rate, the National Institute for Health and Care Excellence (NICE) recommends the measurement of cancer antigen (CA) 125 in primary care in women presenting with symptoms of ovarian cancer. Ultrasound imaging of those women with a CA125 concentration ≥ 35 U/L rules out other causes of elevated CA125 and ensures adequate referral of patients. The impact of these new guidelines was a 92% increase in CA125 requesting within East Kent between 2011 and 2012. This audit was performed to assess the use of CA125 in primary care and to determine the proportion of patients referred for an ultrasound scan.

CA125 results were collected for January to March 2012. For results ≥ 35 U/L, previous CA125 measurements, referral for an abdominal ultrasound scan and outcome were investigated. Patient data was obtained using the Laboratory Information System and the Electronic Patient Record System. Only tests requested by general practitioners were included in the audit.

Out of 540 test requests, 85 results were above 35 U/L. Out of the 48 patients referred for scanning, 36 had no previous CA125 measurement. Eight abnormal results were not followed-up and patients not offered an abdominal ultrasound scan.

The measurement of CA125 in primary care followed by ultrasound scan allowed the identification of one new case of ovarian cancer. Eighty-six percent of patients with CA125 ≥ 35 U/L were referred for abdominal ultrasound scan as per NICE guidelines.

**Th4**

**North West regional audit of CA125 requesting in primary care**

*M Pickersgill1, N Hunt2, S Robinson3, R Alcock2, A Beardsmore2, A Hutchesson4, E Stratta4, L Sandle5, S Hanton6, C Chadwick6*  
1East Lancashire Hospitals NHS Trust, Blood Sciences, Blackburn, United Kingdom, 2Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom, 3Mid Cheshire Hospitals NHS Foundation Trust, Crewe, United Kingdom, 4Royal Bolton Hospital NHS Foundation Trust, Bolton, United Kingdom, 5Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, 6Aintree University Hospital, Liverpool, United Kingdom

The incidence of ovarian cancer is rising with a 2% lifetime risk for women in England and Wales, combined with a poor survival rate as a result of the advanced stage of presentation. In April 2011, NICE published guidelines on ovarian cancer (CG122) recommending the use of CA125 in primary care for women with appropriate symptoms. Guidance regarding appropriate follow up is also detailed.

This audit was carried out to determine compliance with NICE guidance and to assess any changes in adherence from 2012 to 2013. Data for primary care CA125 requests for March 2012 and March 2013 was extracted from the individual laboratory databases and analysed using excel. Electronic patient notes were used to extract further clinical information and imaging results.

In terms of appropriate requests, no single trust achieved 100% compliance with the standard. Compliance averaged at 66% in 2012 and 64% in 2013.

Across the hospital sites there was evidence of simultaneous requesting of CA125 and ultrasound, which negates the use of CA125 to triage patients for ultrasound.
Reassuringly, the majority of patients with a raised CA125 underwent some form of imaging during their investigations. Compliance with this standard was 86.7% for the region but ranged from 67% to 96%. In one trust 33% of patients with a raised CA125 had no evidence of follow up. Clinical outcome data was only available from 3 of the 6 trusts with ovarian cancer being the clinical outcome in 16% of cases for 2012 and 23% of cases in 2013. Feedback of our findings with our colleagues across the region in primary and secondary care in a consistent manner may help to reduce the variation in requesting patterns and improve the appropriateness of CA125 requesting as a tool to select patients for ultrasound imaging in the investigation of ovarian cancer.

Th5

Potential markers for detection of ovarian cancer

MA Milivojevic
Clinical Center of Serbia, Center for Medical Biochemistry, Belgrade, Serbia

Background: In women with pelvic mass, cancer antigen 125 (CA125) had not achieved satisfactory sensitivity and specificity in the detection of ovarian cancer. The objective of this study was to determine the potential of the osteopontin (OPN) and OPN+CA125 combination in differential diagnosis of the ovarian cancers and nonmalignant ovarian disease.

Methods: A prospective cross-sectional study was conducted at the Clinic for Gynecology and Obstetrics, and at the Center of medical biochemistry, Clinical Center of Serbia. Serum samples were obtained preoperatively from 107 women undergoing surgery for pelvic mass; 57 of them had ovarian carcinoma, and 50 had benign cyst. The samples were analyzed for the levels of OPN and CA125 (using ELISA and CMIA methods) and then compared with the final pathologic results. The diagnostic performance of OPN and CA125 was estimated using receiver operating characteristic curve and area under the receiver operating characteristic curve.

Results: The median plasma level of OPN in patients with benign and malignant cysts were 356.33 ng/mL and 865.15 ng/mL, respectively ($p < 0.001$). Receiver operating characteristic (ROC) analysis for plasma OPN revealed the area under the curve of 0.838. At the predefined specificity of 90%, OPN showed sensitivity of 62.5%, whereas the combination of OPN+CA125 reached 74.9% at the same specificity.

Conclusion: OPN showed satisfactory capability of distinguishing benign from malignant ovarian cyst, particularly in combination with CA125.

Th6

Augmenting clinical interpretability of thiopurine methyltransferase (TPMT) laboratory evaluation

M Mrkvicová1,2, R Demlová2,3, J Sterba4, H Bernatikova5, J Stary5, M Sukova5, A Mikuskova2,6, A Chocholova7, B Mladosievicova8, A Soltysova9, D Behulova10, K Pilatova1,2, L Zdrazilova-Dubska1,2,3, D Valík11

1Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic, 2Masaryk Memorial Cancer Institute, Regional Centre for Applied Molecular Oncology, Brno, Czech Republic, 3Masaryk University School of Medicine, Department of Pharmacology, Brno, Czech Republic, 4University Hospital Brno, Department of Paediatric Oncology, Brno, Czech Republic, 52nd Faculty of Medicine, Charles University and University Hospital Motol, Department of Paediatric Hematology and Oncology, Prague, Czech Republic, 6Masaryk University School of Medicine, Department of Laboratory Methods, Brno, Czech Republic, 7Children's University Hospital, Bone Marrow Transplantation Unit, Department of Paediatric Haematology and Oncology, Bratislava, Slovakia, 8Comenius University School of Medicine, Institute of Pathological Physiology, Bratislava, Slovakia, 9Comenius University, Faculty of Natural Sciences, Department of Molecular Biology, Bratislava, Slovakia, 10Children's University Hospital, Department of Laboratory Medicine, Bratislava, Slovakia, 11University Hospital Brno, Department of Paediatric Oncology, Brno, Czech Republic

Objective: Thiopurine methyltransferase (E.C. 2.1.1.67, TPMT) affects the metabolism of important anticancer and immunosuppressive drugs structurally based on 6-mercaptopurine. Individuals with decreased TPMT activity are at risk of adverse effects of administration of thiopurines whereas its increased activity may inactivate drugs faster. We evaluated genotype-phenotype correlations in patients with suspected haematological malignancies and IBD from our region based on finding of nonlinear TPMT enzyme kinetics.

Materials and methods: The study group comprised 371 individuals. They were screened for the most common TPMT low activity variants by real-time PCR using LightSNiP assays (TIB Molbiol) on a LightCycler system (Roche) or using a sequencing method. The PGX-TPMT StripAssay (ViennaLab Diagnostics GmbH) was used as the reference method. TPMT activity was measured in erythrocytes using HPLC rate-blanked method.

Results: Forty patients (10.8%) were heterozygous (30 were TPMT*1/*3A, six TPMT*1/*2, three TPMT*1/*3C) and one was a compound heterozygote (*2/*3A). Normal and low normal TPMT activities substantially overlapped in wt and heterozygous individuals, whereas high
activities were found in 36 wt genotyped patients. Substantial portion of the wildtype patients (16.4%) fell into the low normal activity range. Six non-wildtype patients fell into the normal activity range. Extreme and life-threatening toxicity was observed in the TPMT *2/*3A patient.

Conclusion: Activity measurement performed at diagnosis provides clinicians with information on immediate pharmacokinetic-related adverse events and/or hypermetabolism and genotyping may indicate the rate of pharmacodynamic thioguanine nucleotides accumulation due to slower overall thiopurine metabolism.

This work was supported by MH CZ-DRO (MMCI, 00209805), European Regional Development Fund (RECAMO; CZ.1.05/2.1.00/03.0101) and BBMRI_CZ (LM2010004).

**Th7**

**Salivary gland pathology does not increase prostate-specific antigen concentrations in serum**

*S Ferraro1, G Incarbone2, R Mozzi3, F Gaboardi4, A Peri1, D Schiumarini1, M Panteghini2*

1Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, 2Urology Unit, University Hospital Luigi Sacco, Milan, Italy, 3Otolaryngology Unit, University Hospital Luigi Sacco, Milan, Italy

**Background:** Immunohistochemical detection of prostate-specific antigen (PSA) is used to characterize metastases of unknown primary source and show prostatic origin of the tumor. Some tissues, such as salivary gland ducts, may however be immunoreactive for PSA and thus some parotid diseases have been misinterpreted as metastases in men with prostate cancer. In this prospective study we sought to evaluate if serum concentrations of PSA may increase in patients with parotid calculus.

**Methods:** We enrolled 31 patients (52% males with no history of prostate cancer) with first diagnosis of parotid calculus and 10 age- and gender-matched controls (60% males) admitted to the ambulatory of the Otolaryngology Unit of our hospital. Serum PSA was measured in both groups by Modular Evo (Roche Diagnostics), in cases before treatment with shock wave. A multiple regression analysis was employed to evaluate if the presence of calculus may influence PSA concentrations in serum by adjusting for other covariates.

**Results:** Median age (25-75th percentile) was 57.0 (43.7-65.0) years in patients and 52.5 (43.0-59.0) years in controls, respectively (P=0.44). Median PSA (25-75th percentiles) concentrations in males were 0.81 μg/L (0.55-1.23) in calculus patients and 1.44 μg/L (0.46-2.50) in controls, respectively (P=0.91). All females in both groups showed PSA concentrations < 0.01 μg/L. The multiple regression model (P=0.0001), accounting for age, gender, and presence of calculus, showed that only gender significantly influenced PSA concentrations (P < 0.0001).

**Conclusions:** In studied patients, the presence of parotid calculus did not increase serum PSA concentrations. Monoclonal antibodies used in immunoassays measuring PSA in serum thus exhibit high specificity for the prostate-derived PSA only recognizing the form secreted by the prostatic epithelium.

**Th8**

**The importance of individual biology in the clinical use of serum biomarkers for ovarian cancer**

*F Braga, S Ferraro, R Mozzi, M Panteghini*

Clinical Pathology Unit, ‘Luigi Sacco’ University Hospital, and Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

**Background:** An increased focus on the biological behaviour of serum biomarkers for ovarian cancer, carbohydrate antigen-125 (CA-125) and human epididymis protein 4 (HE4), has been advocated to improve their clinical use. Due to the paucity and poor design of available studies evaluating biological variation (BV) of CA-125 and the lack of BV data for HE4, we evaluated BV of both biomarkers.

**Methods:** We monthly obtained serum samples from 14 pre- (PreM) and 14 post-menopausal (PostM) healthy women for four consecutive months. Specimens were stored at -80 °C and analyzed in a single run in duplicate for CA-125 and HE4 on Roche Modular system. Cochran's test and Reed's criterion were employed for outlier identification, and the Shapiro-Wilk test for checking data distribution. Data were then analyzed by ANOVA.

**Results:** HE4 values in the whole group were normally distributed, while those of CA-125 failed the normality test. Therefore, while HE4 statistics to derive BV components were directly performed on raw data, for CA-125 a log-scale transformation was applied. For both biomarkers no difference in median concentrations was found between PreM and PostM. For CA-125 intra-individual CV was not different between groups (9.1% in both). For HE4 intra-individual CV was higher in PreM (12.1%) than in PostM (6.5%) (P< 0.001). Between-subject CVs were 10.6% for CA-125 and 16.4% for HE4, with no influence by the fertility status. Both biomarkers showed high individuality meaning that the use of population-based reference limits has limited value for their interpretation. Reference change values were 26% for CA-125 (all), 34% for HE4 PreM and 18% for HE4 PostM.

**Conclusions:** Monitoring longitudinal changes in serum concentrations of ovarian cancer biomarkers over time is probably better than using single-threshold rules. According to differences in BV due to the hormonal status, one should differently interpret HE4 changes in PreM and PostM.
Pyrosequencing analysis of PALB2 and RASSF1 DNA methylation in sporadic breast cancer

N Poumpouridou1, A Acha-Sagredo1, N Goutas3, D Vlahodimitropoulos2, S Vasilaros2, ES Lianidou5, T Liloglou2, C Kroupis1
1Attikon University Hospital, University of Athens Medical School, Clinical Biochemistry, Haidari, Greece, 2University of Liverpool, Molecular and Clinical Cancer Medicine, Liverpool, United Kingdom, 3Evgenidio Hospital, University of Athens Medical School, Pathology Laboratory, Athens, Greece, 4Prolipsis Breast Cancer Clinic, Athens, Greece, 5Analytical Chemistry Lab, University of Athens, Chemistry, Athens, Greece

Background-aim of the study: Recent reports have included PALB2 (Partner and localizer of BRCA2) in the growing list of hereditary cancer genes. Its mutations confer a moderate breast cancer risk in heterozygotes and Fanconi anemia in biallelic mutation carriers. PALB2 protein co-localizes with BRCA2 and BRCA1 in nuclear structures and enables error-free homologous recombination DNA repair of double-stranded breaks. This important contribution could be severely diminished if affected by epigenetic mechanisms such as promoter CpG island methylation. The RASSF1A (RAS association domain family protein 1A) gene product interacts with XPA DNA repair protein and its inactivation has been shown in a variety of cancers including breast. The aim of the present study was to evaluate PALB2 and RASSF1A epigenetic inactivation in sporadic breast cancer.

Methods: DNA was extracted with spin column-based purification methods from 96 frozen breast tissues: 92 with sporadic breast cancer and 4 benign controls, all with known histopathological data. Following bisulfite conversion (ZymoResearch), DNA methylation was analyzed by pyrosequencing on a Q96 Pyromark (Qiagen) pyrosequencer with assays designed with PyroMark Assay Design software. Primers for PALB2 promoter cover a region between nt -174 and -288 (related to the ATG start) in the 1402 bp CpG island identified by the CpG Island Searcher software. Primers for RASSF1 promoter cover a region between nt -85 and -201 (related to the ATG start).

Results: The promoter of RASSF1A was methylated in 22 of 92 breast tumors (24%) while all samples were methylation-free in the PALB2 promoter.

Conclusions: While confirming the extent of RASSF1 promoter methylation in our breast tumor cohort, this study demonstrated no evidence of DNA methylation of PALB2 promoter suggesting it as an unlikely mechanism for its expression silencing.

Diagnostic and prognostic potential of differentially expressed piRNA DQ590013 in clear cell renal cell carcinoma

E Trujillo-Arribas1, P Camacho-Martinez2, Z Wotschofsky2, M Jung2, J Busch2, E Kilic3, K Jung2
1Virgen del Rocio University Hospital, Sevilla, Spain, 2University Hospital Charité, Department of Urology, Berlin, Germany, 3University Hospital Charité, Department of Pathology, Berlin, Germany

Background: Piwi-interacting RNAs (piRNAs), a newly identified class of small non-coding RNAs, direct the Piwi dependent transposon silencing, heterochromatin modification and germ cell maintenance.

Piwi protein expression profiles have recently received much attention for their potential functional involvement in a wide variety of human cancers.

The aim of this study is to evaluate the diagnostic and prognostic potential of piRNA DQ590013 in patients suspected of having clear cell renal cell carcinoma (ccRCC).

Material and methods: Tissue samples were obtained from primary tumor after radical nephrectomy. RNA was extracted with the miRNeasy Mini Kit (Qiagen). RT-qPCRs were performed using the miScript PCR System (Qiagen) for piRNA DQ590013 on the Light-Cycler 480 Instrument (Roche Diagnostics GmbH, Mannheim, Germany). TaqMan hsa-miRNA-28 Assay (Applied Biosystems, Foster City, CA) was used for reference gene determination because of the stability in each kind of tissue of the study.

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results: A total of 107 patients with ccRCC either without (n=77) or with metastasis (n=30) at the time of surgery and 13 ccRCC patients with distant metastatic samples were enrolled.

The expression of the piRNA DQ590013 was normalized to the reference gene (hsa-miR-28). Non-parametric statistical test (Kruskal-Wallis, Spearman rank correlation) were used. In comparison to the normal tissue, significant down-regulation was observed in all three kind of tumor samples. No statistical difference of expression was found between primary tumor samples with and without metastasis at nephrectomy (p=0.750). The piRNA expression in META was significantly decreased compared to NC00 tissue (p=0.01).

Conclusion: The present expression study of piRNA DQ590013 proved its potential diagnostic value in clear cell renal cell carcinoma (ccRCC). To evaluate the prognostic value, another study should be made looking for the clinical characteristics of all the patients and its relationship with piRNA DQ590013 expression.
Th11

Identification of novel cancer biomarkers by the Biosystems-Randox-QuantiPlasma-300 monoclonal antibody chip

P Antal-Szalmas1, J Lazar1, Z Steiber1, R Foldesi1, S McGregor2, M Szilasi1, T Flascko1, L Damjanovich1, R Poka1, Z Hernadi7, J Szanto8, M Muller9, J Lammont3, I Kurucz2, J Kappelmayer1, L Takacs2

1University of Debrecen, Department of Laboratory Medicine, Debrecen, Hungary, 2Biosystems International Ltd., Debrecen, Hungary, 3Randox Laboratories Ltd., Crumlin, United Kingdom, 4University of Debrecen, Department of Pulmonology, Debrecen, Hungary, 5University of Debrecen, Department of Urology, Debrecen, Hungary, 6University of Debrecen, Department of Surgery, Debrecen, Hungary, 7University of Debrecen, Department of Obstetrics and Gynecology, Debrecen, Hungary, 8University of Debrecen, Department of Oncology, Debrecen, Hungary, 9Adware Research Ltd., Balatonfured, Hungary

Background: Recently a novel monoclonal antibody based protein chip-QuantiPlasma300 (QP300)-has been introduced by Randox Laboratories. This system uses 290 monoclonal antibodies (mAbs), the QP300 mAb library-developed by BioSystems International-that are immobilized onto 9x9 mm ceramic chips. The QP300 assay recognizes representational changes of several human plasma protein epitopes simultaneously and in this way can identify potential novel plasma markers in a wide variety of diseases.

Materials and methods: Plasma samples and detailed clinical data of 150 patients with prostate, 100 patients with colon, 100 patients with breast, 50 patients with ovary and 150 patients with lung cancer and 300 healthy controls were collected. Individual (lung cancer) and pooled samples (other tumors) of patients and controls were evaluated by the QP300 system. The plasma pools were created from the individual samples based on clinical, histopathological and laboratory data. Other biochemical parameters and the classical tumormarkers were also measured. To find the most valuable predictive parameters random forest, binary logistic regression and ROC analyses were performed beside the classical statistics.

Results: In case of lung cancer results of 4 mAbs could be incorporated into the final model providing a new parameter with ROC-AUC>0.940, while adding Cyfra 21-1 further increased its power. Measuring pooled samples of other cancer patients 8 to 10 mAbs could effectively discriminate the patients from controls.

Conclusions: The QP300 kit can be an effective tool in biomarkers’ search and discovery.

This work was supported by the National Office for Research and Technology of Hungary (TECH-09-A1-2009-0113; mAB-CHIC).

Th12

Detection of genital human papillomavirus genotypes in self-collected cervical swabs

FA Yeboah, EK Wiredu, F Agyeman-Yeboah, E Timmy-Donkoh, RH Asmah

Kwame Nkrumah University of Science and Technology, School of Medical Sciences, Dept. of Molecular Medicine, Kumasi, Ghana

Background: Cervical cancer disproportionately affects women in developing countries owing to existing difficulties facing cytology-based screening programs. Human Papillomavirus (HPV) testing also plays an important role in the primary prevention of cervical cancer and in the triage of patients with atypical squamous cells of undetermined significance (ASCUS) and low-grade cervical smears.

Aim & methods: To examine the feasibility of HPV testing and genotyping in resource-challenged settings, we evaluated a previously validated nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers for the detection and typing of eighteen (6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68) human papillomavirus (HPV) genotypes using self-collected cervical swabs. In all, self-collected cervical swabs were available for 200 previously unscreened women recruited from May to August 2011 in a suburb of Kumasi, Ghana. Cervical samples were obtained with Pap Pak® cytology mail-kit (Medical Packaging Corporation, Camarillo, CA, USA) according to the manufacturer’s instructions and held in DNA Guard® at room temperature until DNA purification, amplification and HPV genotyping at a separate location.

Results: Out of the total of eighteen HPV genotypes screened for, four low-risk and thirteen high-risk HPV genotypes were identified. Seventy-one women tested positive for HPV infection representing 35.5% of all women. In all, 42.0% of infections involve more than one HPV type. The commonest types were, HPV-42 (low-risk, 20 women), HPV-52 (high-risk, 13 women) and HPV-66 (high-risk, 12 women). Vaccine-preventable HPV types -16 (3 women) and -18 (10 women) were also detected.

Conclusion: A nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers is sensitive for HPV DNA detection, especially when exact HPV genotyping is required in developing healthcare systems.
The frequency and appropriateness of requests for multiple tumour markers

KJ Langford-Smith, AM Milan, LM Bailey
1Aintree University Hospital, Clinical Biochemistry, Liverpool, United Kingdom, 2Royal Liverpool & Broadgreen University Hospitals NHS Trust, Clinical Biochemistry, Liverpool, United Kingdom

Tumour markers are valuable tools in the diagnosis, prognosis and monitoring of certain cancers; however due to relatively low clinical specificity and sensitivity they should only be measured in appropriate clinical situations. An audit of requests for multiple tumour markers was performed at Royal Liverpool University Hospital, to determine whether multiple requests should be reviewed by clinical scientists, and whether departments required additional education. All requests for more than one tumour marker, received over 6 months, were classified as appropriate or inappropriate, based on strict and lenient criteria. These criteria were defined using guidelines on tumour marker requesting from the Association for Biochemists in Ireland (ACBI) and Pathology Harmony. Of the 13,632 requests, 94.2% were for a single marker, whilst 5.8% were for 2, 3, 4, or 5 markers. Of the multiple marker requests, 33.5% (strict criteria) or 13.1% (lenient criteria) were classified as inappropriate. The more markers that were requested, the less likely the request was to be considered appropriate. Most requesting locations had fewer than 10 inappropriate requests each; only 4 locations had a larger number. In many cases, patients had clinical indications for some of the markers, but additional non-indicated markers were also included. Less than 1% of requests were used inappropriately for opportunistic screening or identification of unknown primary tumours. Overall, inappropriate requests account for 2% (strict) or 0.8% (lenient) of all tumour marker requests, assuming that all single marker requests are appropriate. To tackle inappropriate requests, the audit recommendations are that requests for 3 or more tumour markers will be reviewed by clinical scientists, who will liaise with the requesting clinician to determine which markers should be measured, and the audit results will be communicated to the 4 locations with high numbers of inappropriate requests in order to provide additional education regarding tumour marker requesting.

Evaluation of novel biomarkers to identify subclinical anthracycline-mediated effects on organs

M Jones, J Dinneen, P O’Gorman, M Fitzgibbon
1Mater Misericordiae University Hospital, Department of Clinical Chemistry and Endocrinology, Dublin, Ireland, 2Mater Misericordiae University Hospital, Department of Haematology, Dublin, Ireland

Introduction: Anthracycline chemotherapeutics are successfully utilised in the treatment of oncological and haematological malignancies. However, treatment with anthracyclines can result in myocyte damage leading to ventricular dysfunction and renal injury.

Aims: To assess the clinical utility of novel cardiac biomarkers high sensitive troponin-I (HsTn-I), galectin-3 (GAL-3) and renal biomarkers cystatin C and neutrophil gelatinase associated lipocalin (NGAL) in identifying subclinical anthracycline-mediated organ damage.

Methods: Eighty five patients were recruited (40 anthracyline and 45 controls on non-anthracyline regimes). Serum and urine samples were obtained prior to each cycle of treatment. Biomarkers were analysed using Abbott immunoassay. Wilcoxon tests were used for statistical analysis with interim results reported here.

Results: There was a statistically significant increase in HsTn-I among the anthracycline cohort following the third (p=0.0256) and fourth (p=0.0078) cycles of chemotherapy with median increases in HsTn-I from 1.9 pg/ml to 12.35pg/ml after 4 cycles. There was a statistically significant decrease in GAL-3 (p=0.0108) among the anthracycline cohort following 3 cycles of chemotherapy with median decreases from 14 ng/ml to 11.5 ng/ml. Median cystatin C remained constant for the anthracycline cohort following the first 3 cycles with higher concentrations observed for the non-anthracycline cohort (P= 0.0117). NGAL analysis revealed no difference between cohorts when stratified for age and gender.

Conclusions: This study highlights the diagnostic benefits of the novel HsTn-I, with levels as low as 5.0 pg/ml (CV < 10%) analytically achievable. The changes observed in the anthracycline cohort are suggestive of subclinical cardiac damage. The anti-neoplastic effect of anthracyclines may explain the decrease of GAL-3 among this cohort as it is also a marker of cancer progression. Cystatin C concentrations revealed stable renal function. Cancer heterogeneity may explain higher non-anthracycline cystatin C concentrations. NGAL has not provided additional diagnostic value at present.

The role of vascular endothelial cell growth factor (VEGF) in breast cancer

R Findeisen, A Frister, S Albrecht
1Oberlausitz-Kliniken gGmbH, Institute for Laboratory Diagnostic, Microbiology and Hospital Hygiene, Bautzen, Germany, 2Technical University Dresden, Dept. Gynaecology and Obstetrics, Dresden, Germany

We investigated the role of Vascular Endothelial Cell Growth Factor (VEGF) in breast cancer. VEGF, CA 15-3, CEA and TPA were determined over a period of 18 month in the department of Gynaecology and Obstetrics at the Technical University Dresden, Dresden, Germany. The
investigation was conducted on 314 sera from patients with previously diagnosed breast cancer. They undergone surgery and were classified in stages 0-IV according the TNM-classification. The control group was composed of 58 sera from healthy women aged 23 to 84 years old. VEGF was measured according to the sandwich principle with a monoclonal EIA from R&D Systems Minneapolis MN, USA and CA 15-3, CEA and TPA were measured with a monoclonal ILMA from DiaSorin Deutschland GmbH, Dietzenbach, Germany.

**Results and discussion:** The median of all 4 parameters increases with the tumour stages. Only VEGF has a high decrease between stage 3 and 4. The differences of the values between the control group and stages 0-3 were only significant for VEGF and TPA. The combination of TPA and VEGF bring the highest sensitivities for stages 0-3. It can be suggested that VEGF plays crucial roles in the promotion of angiogenesis in breast cancer. But this investigation shows too, that tumour markers are not used for definitive diagnosis, they are used as aids to help physicians make decisions, after combining other clinical and diagnostic data.

**References:**

**Th16**

Readit of GP CA125 requests: follow-up of raised results

**SJ Glover**
Bradford Teaching Hospital NHS Foundation Trust, Blood Sciences, Bradford, United Kingdom

**Background:** In the UK the National Institute for Health and Care Excellence (NICE) provides guidance and sets the standards for high quality healthcare. In April 2011 NICE released Clinical Guideline 122: 'The recognition and initial management of ovarian cancer'. In response to this guideline, on 4/4/11, Bradford Royal Infirmary Blood Sciences Department, starting offering CA125 measurement to GP patients. In May 2012 a NICE Quality Standard for Ovarian Cancer was published, stating 'Women with raised CA125 should have an ultrasound of their abdomen and pelvis within 2 weeks of receiving the CA125 test results'. At the Departmental Clinical meeting 13th June 2012 it was agreed all first time CA125 results ≥35 ku/L from GP patients would be phoned to the GP surgery.

**Aim:** To assess the follow up of raised CA125 results (≥35 ku/L) originating from GP requests.

**Method:** All CA125 results originating from GP patients between 4/4/12 and 3/4/13 were gathered. GP patients with CA125 ≥35 ku/L were selected for further analysis.

**Results:** Between 4/4/12 and 3/4/13 the laboratory reported 1164 (1110 patients) CA125 results on GP samples. 55 patients had CA125 ≥35 ku/L, 46 of which were a first time raised result. Radiology follow up was indicated in 73% of patients. 70% (n=28) of patients, in whom radiology was indicated, had subsequent pelvic radiology. There was a small reduction from 2011/12 to 2012/13 in the percentage of patients with no known follow up or with a repeat CA125 only. 50% (n=14) of patients referred for radiology, had this within 2 weeks of the CA125 result being reported. The mean time between reporting of CA125 results and radiology was 21 days. The number of 1st time raised CA125 results phoned to the GP surgery and the number of patients undergoing pelvic radiology has risen steeply from 2011-12 to 2012-13.

**Th17**

Purification and characterization of Momordica balsamina seed lectin and testing its modulating effects on gastric cancer commercial cell lines

**MA Ibrahim1, SM Ahmed1, ME Mohamed1, EH Konozy2, BO Saeed3**
1College of Medical Laboratory Sciences, Sudan University of Science and Technology, Department of Clinical Chemistry, Khartoum, Sudan,
2Taibah University, College of Applied Medical Sciences, Almadeena Almonawarah, Saudi Arabia, 3University of Khartoum, Khartoum, Sudan,
4Libyan International Medical University, Department of Biochemistry, Benghazi, Libyan Arab Jamahiriya, 5Sudan International University, Department Pathology, Khartoum, Sudan

**Aim:** The aim of this study is to isolate, purify and characterize *Momordica balsamina* seeds lectin (MbSL) and study the possible modulating effects of the purified lectin on four types of commercial gastric cancer cell lines.

**Methods and results:** MbSL agglutinated all types of human red blood cells, as well as mouse, donkey and cow red blood cells. Lactose was the most potent inhibitor of MbSL hemagglutinating activity, with minimal inhibitory concentration (MIC) = 25mM, followed by galactose, MIC = 50 mM, and then arabinose; MIC = 100mM.

Using SDS-PAGE, the lectin was found to be composed of a single protein of molecular mass around 30 kDa.
The search for sequential identities of the purified lectin was carried out using BLAST, and the N-terminal of the lectin shared major similarities with that of *Momordica charantia* lectin I(MCL I) and was found to be one of ribosome inactivating proteins type II (RIP II). The hemagglutinating activity of the lectin remained stable in the pH range 2-12. Lectin activity was gradually lost above 50 °C and it was totally inactivated at 90 °C. MbSL activity decreased slightly with increasing urea concentrations, with a significant drop in activity at the urea concentration of 3M. The purified lectin showed no inhibitory effect on the growth of these commercial gastric cancer cell lines, using the MTT Assay: AGS (Human Gastric Adenocarcinoma), MKN65 (Human Gastric Cancer), U87-MG (Human Glioblastoma) and ECV-304 (Human Urinary Bladder Carcinoma).

**Conclusions:** A lactose-binding lectin from the seeds of *Momordica balsamina* medicinal plant shares a high degree of similarity with other Cucurbitaceae family lectins in physicochemical features, including sugar specificity, effect of pH, temperature and urea on lectin stability. MbSL was found to be one of RIP II. It had no effect on the growth of four types of commercial gastric cancer cell lines.

**Th18**

**Association study of cytochrome P450 1A1*2A polymorphism with prostate cancer risk and aggressiveness in Croatians**

S Mandić1, V Horvat1, S Martzi2, J Galić1

1University Hospital Centre Osijek, Department of Clinical Laboratory Diagnostics, Osijek, Croatia, 2University Hospital Centre Osijek, Department of Molecular Diagnostics and Tissue Typing, Osijek, Croatia

**Background:** Cytochrome P450 1A1 (CYP1A1) is an enzyme participating in the bioactivation of various endogenous and environmental reactive compounds that can bind to DNA and thus induce cancerogenesis. Gene encoding the enzyme is expressed in the prostate tissue and is polymorphic. CYP1A1*2A gene polymorphism is associated with elevated enzyme activity and/or inducibility which can lead to accumulation of genotoxic compounds and consequently to cancerogenesis. The aim of this study was to examine the association of this polymorphism with prostate cancer (PCa) risk and aggressiveness.

**Methods:** The case-control study consisted of 120 PCa patients and 120 benign prostatic hyperplasia (BPH) controls, in Croatian population. Regarding aggressiveness, PCa patients were grouped according to the Gleason score (GS), tumor stage (T) and existence of distant metastasis (M). The polymorphism was analyzed using real-time polymerase chain reaction (PCR).

**Results:** We did not observe association of mutated allele with PCa risk, neither with PCa aggressiveness. Furthermore, frequency of polymorphic genotype was slightly higher in BPH group (16.6% vs. 14.2%, respectively) and also in less aggressive form of PCa (20.4% vs. 9.6% for GS<7; 15.6% vs. 9.1% for T<3; 16.7% vs. 10.0% for no distant metastasis).

**Conclusion:** Comparing our findings with other published results, we can assume that the ethnicity influence the genotype distribution and thus may affect the etiology of PCa, even possibly in the way to cause an opposite effect among different ethnic groups. Given the small number of participants, results should be validated on the larger sample size.

**Th19**

**Critical electrolyte disturbances in a mixed chemotherapy population**

SJ Costelloe1, M Purkis1, M Seymour1, G Hall1, T Perren1, M Bosomworth1

1Leeds Teaching Hospitals NHS Trust, Department of Blood Sciences, Leeds, United Kingdom, 2Leeds Teaching Hospitals NHS Trust, Department of Pharmacy, Leeds, United Kingdom

**Introduction:** The incidence of electrolyte dysfunction is not described in mixed cancer populations undergoing complex regimens. This study describes the incidence of critical electrolyte abnormalities both at baseline and during a 3 month follow up period in new chemotherapy patients being treated in a tertiary referral centre over a 1 year period.

**Methods:** New presenters were retrospectively identified, characterised, and monitored for 90 days using data from pharmacy and pathology databases (for the period 1st July 2012 to 30th June 2013). Analytes considered, along with critical limits applied, were: sodium (≤120 or ≥160 mmol/L); potassium (≤2.5 or ≥2.6 mmol/L), urea (≥30 mmol/L), creatinine (≥500 μmol/L), adjusted calcium (≤1.8 or ≥3.5 mmol/L), phosphate (≤0.3 mmol/L), magnesium (≤0.4 or ≥2.5 mmol/L) and glucose (≤2.5 or ≥20 mmol/L). Critical limits are based on RCPath advice and local practice. The most recent electrolyte measurements taken in the 14 days up to commencement of therapy were considered the baseline measurement.

**Results:** Over the period 12 months, 2186 new consecutive patients were included. No patient had critical hypernatraemia, hypermagnesaemia, or hypoglycaemia at either baseline or follow up. The frequency of critical results at baseline (B) and follow-up (F) was as follows:
Laboratory Medicine at the Clinical Interface, United Kingdom, 7th–10th October, 2014

**hyponatraemia (B:0.00%/F:0.57%); hyperkalaemia (B:0.05%/F:0.48%); hypokalaemia (B:0.00%/F:1.16%); hyperuraemia (B:0.19%/F:0.16%); hypocalcemia (B:0.00%/F:1.10%); hypophosphataemia (B:0.00%/F:0.12%); hyperglycaemia (B:0.45%/F:3.30%).

**Conclusion:** Electrolyte disturbance is common in chemotherapy populations. Instances of severe hyponatraemia, hyperkalaemia, hypokalaemia, hyperuricaemia, hypercreatininaemia, hypomagnesaemia, hyper- and hypo-calcaemia, hypophosphataemia, and hyperglycaemia occur with significantly higher frequency during chemotherapy than at baseline. However, for all analytes examined except glucose, critical disturbances occur with a frequency < 2%. Future consideration of the baseline characteristics may allow identification of patients prone to electrolyte dysfunction.

**Methods, Instrumentation**

**Th20**

**Development of a LC-MS/MS method for the measurement of total fractionated urine metadrenalines and determination of a healthy population reference range**

*A Banks1, JJ Dutton1, K Phillipson2, AS Davison1*

1Royal Liverpool and Broadgreen University Hospitals Trust, Clinical Biochemistry & Metabolic Medicine, Liverpool, United Kingdom,
2Newcastle upon Tyne NHS Foundation Trusts, Clinical Biochemistry, Newcastle upon Tyne, United Kingdom

**Aims:** Develop and validate a LC-MS/MS assay for measurement of total urinary normetadrenaline (NMA), metadrenaline (MA) and 3-methoxytyramine (3-MT) and to establish a healthy population reference range.

**Method:** Samples underwent solid phase extraction (Evolute-SCX), 30mg. 1mL of urine was hydrolysed (5M-HCl) at 100°C for 30 minutes. 50 μL of hydrolysate was diluted in 2mL of deionised water containing deuterated NMA, MA and 3-MT internal standards and loaded onto an SPE plate. Eluted extracts were injected onto a Phenomenex C18 phenyl-hexyl column (2.6 μ, 4.6 x 100mm) using a UPLC separations module coupled to a Xevo TQS tandem mass spectrometer.

A preliminary reference range was established from samples obtained from patients that had phaeochromocytoma excluded.

**Results:** Measurement of NMA, MA and 3-MT was linear to 13.0 μmol/L, intra-assay CV’s were < 6% across the linear range of the assay. Recovery of NMA (n=4, 106-111%) and MA (n=83-102%) were acceptable. Comparison between the validated LC-MS/MS assay and an established LC-MS/MS assay was acceptable for NMA (n=84, y=0.97x-0.02) and MA (n=87, y=1.01x-0.05). 3-MT results were compared to a HPLC-ECD method and were acceptable (n=67, y=1.04x-0.08). Analysis of five distributions of EQA samples showed close agreement with the LC-MS/MS method mean. Reference ranges determined for total NMA (n=84), MA (n=87) and 3-MT (n=64) were < 3.0, < 1.3 and < 1.4 μmol/L, respectively.

**Conclusions:** A robust method has been developed for the measurement of urinary metadrenalines and a preliminary reference range established.

**Th21**

**Glucose interference in creatinine measurement**

*KM Stepien1, J Adaway2,3, L Owen2,3*

1Salford Royal Foundation Hospital, Clinical Biochemistry Department, Manchester, United Kingdom, 2University Hospital of South Manchester, Clinical Biochemistry Department, Wythenshawe, United Kingdom, 3University of Manchester, Manchester, United Kingdom

**Background:** Many analytical factors affecting creatinine results are well recognised. It is difficult to make creatinine measurement by Jaffe method free from interfering substances and a significant one in the current Abbott Architect analytical system is glucose. A recent EQA return highlighted an unexpected result of the creatinine assay in EQA.

**Methods:** We retrieved anonymised serum samples with a spread of concentrations for creatinine (50 to 1000 μmol/L). 11 pools of the serum samples had baseline creatinine concentrations established (50, 100, 150, 200, 300, 400, 500, 600, 700, 1000 μmol/L) and were subsequently spiked with 7 different glucose concentrations of 5, 10, 15, 20, 25, 30, 35 mmol/L. Prior to the experiment, the baseline glucose concentration was measured in all glucose solutions that were prepared from 2 mol/L solution of D-glucose.

**Results:** The study confirmed that glucose at high concentrations may result in a false increase in the creatinine result. Creatinine concentrations within or below normal reference ranges (< 150 μmol/L) may be artefactually increased by up to 20% at glucose concentration ≥ 25 mmol/L. The interference in the creatinine assay is proportional to the glucose above 25 mmol/L, hence the comment was added to all
reported creatinine results: ‘Glucose ≥ 25.0 mmol/L may cause positive interference in creatinine results, especially at low or normal creatinine concentrations.’

The higher the glucose, the higher the creatinine interference. However, creatinine at concentrations above 150 μmol/L may still be affected but to a lesser extent.

**Conclusions:** In conclusion, the performance of the Abbott creatinine assay in the presence of glucose was superior to what was expected after reviewing the eGFR return (UKNEQAS distribution 86). The EQA samples were spiked with glucose of 16 mmol/L, whereas our experiment showed the positive interference with glucose concentrations of 25 mmol/L and above.

Th22

**Validation of an assay for the simultaneous quantitation of the urinary metabolites glycolate, glycerate and 4-hydroxy-2-oxoglutarate in primary hyperoxaluria**

O Clifford-Mohley, L Hewitt, G Rumsby

1University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

**Introduction:** The primary hyperoxalurias (PH) are inborn errors of glyoxylate metabolism causing overproduction of oxalate. Further metabolites are also excreted in elevated amounts by patients with PH: glycolate in PH1, glycerate in PH2 and 4-hydroxy-2-oxoglutarate (HOG) in PH3. The aims of this study were to set up and validate an assay for these metabolites using GCMS, to use it to replace a current enzymatic assay for glycolate, and to investigate the stability of HOG in urine.

**Methods:** Samples were prepared by addition of internal standards followed by sequential oximation, extraction and silylation. A Shimadzu GC2010S instrument was used in single ion monitoring mode. Statistical analysis of the results was performed using Microsoft Excel.

**Results:** Large matrix effects required the preparation of calibrators in blank urine. The method was linear up to 760 μmol/L for glycolate (R^2=0.999), 350 μmol/L for glycerate (R^2=0.997) and 200 μmol/L for HOG (R^2=0.995). Recovery of the analytes spiked into normal urine was 97-101% for glycolate, 88-95% for glycerate and 99-103% for HOG. Total imprecision was 1.4-1.5% for glycolate, 5.0-7.3% for glycerate and 8.2-12.5% for HOG across the assay measuring range. The lower limit of quantitation (defined as CV≤20%) was 20 μmol/L for glycolate and glycerate and 10 μmol/L for HOG. Glycolate results had a mean bias of +9% compared to the enzymatic method. HOG was unstable in plain urine at room temperature (reduction of 40% over 3 days) but was stable in acidified urine for 29 days.

**Conclusions:** This method performed well for all 3 metabolites and provides a suitable replacement for the enzymatic measurement of glycolate. It is of value for further investigation of patients with hyperoxaluria of unknown aetiology. Samples should be acidified prior to transport to prevent loss of HOG.

Th23

**Preserved in vitro functional characteristics of double dose Buffy-coat pooled platelet concentrates prepared with amotosalen and ultraviolet A light for pathogen inactivation (INTERCEPT blood system™)**

P Sandgren, Agneta Wikman Research Group
Karolinska University Hospital, Clinical Immunology and Transfusion Medicine, Stockholm, Sweden

**Background:** The INTERCEPT Blood System™ for Platelets (PLTs) utilizes amotosalen-HCl (S-59) in combination with ultraviolet A (UVA) light to inactivate viruses, bacteria, protozoa and leucocytes that may contaminate platelet concentrates (PCs). Platelets undergo a number of events during collection, processing, and storage which may affect their structure and function, resulting in reduced post transfusion recovery and survival.

**Aim:** The objective of this study was to evaluate potential in vitro effects on PLTs of the INTERCEPT treatment.

**Material and methods:** Buffy-coats (BCs) were separated from routinely collected 450 mL whole blood donations. Seven ABO matched BCs were selected and manually pooled to compare treated and non-treated DD PLTs. Platelets were aliquoted and suspended in 65% platelet additive solution (PAS) and 35% plasma (n=8). Metabolic and cellular in vitro parameters were measured as an index of PLT quality over a 7-day storage period.

**Results:** Throughout storage, slightly significant extracellular differences were observed regarding PLT count (p<0.05), pH, pCO2, glucose consumption and calculated bicarbonate levels (p<0.05) between treated and untreated units. Throughout storage, no significant differences were observed in ATP levels, ESC, HSR reactivity and CD62P expression. Similarly, no differences were observed in the expression of the conformational epitope on the GpIIb/IIIa, determined by using PAC-1, or in the expression of CD42b and PECAM-1 at any time points. Maintenance of mitochondrial membrane potential (MMP) expressed as JC-1 positive PLTs remains unaffected by means of >90% maintenance.
of mitochondrial function until day 7 in all treated and untreated units. The release of sCD40L increased over time (p< 0.01) in all units but without any significant differences between treated and untreated PLTs.

**Conclusion:** Our data demonstrate that pathogen inactivation with INTERCEPT Blood System™ has no influence on the PLT *in vitro* functional, phenotypic and mitochondrial properties over 7 day of storage.

**Th24**

**Development of an LC-MS/MS method for the measurement of serum dehydroepiandrosterone-sulphate**

*OEE Kaye, AE Armstrong, PR Cook*

*University Hospital Southampton, Clinical Biochemistry, Southampton, United Kingdom*

**Background:** Dehydroepiandrosterone sulphate (DHEAS) is a steroid hormone produced in the adrenal cortex. Serum DHEAS levels aid the investigation of disorders of androgen excess including congenital adrenal hyperplasia (CAH). DHEAS is most often measured using immunoassay which is subject to characteristic analytical limitations. Liquid chromatography tandem mass-spectrometry (LC-MS/MS) provides an alternative method that is renowned for its enhanced specificity and capability for measuring multiple analytes simultaneously. An LC-MS/MS method has been developed and validated for the quantification of serum DHEAS with the determination of age and sex-specific reference ranges.

**Methods:** Analysis was performed on the Waters Acquity UPLC instrument with BEH C18 column and TQD mass spectrometer. DHEAS-d2 was used as an internal standard with a DHEAS standard curve for quantification. The sample preparation procedure employed a simple protein precipitation. Assay validation was in line with recognised criteria. A method comparison was performed with Deming Regression for mathematical transference of the existing in-use reference ranges.

**Results:** DHEAS and DHEAS-d2 had mass transitions of 397->97 and 369->97 respectively. Gradient elution produced consistent Gaussian shaped peaks with both DHEAS and DHEAS-d2 eluting at 1.27 minutes, and a total run time of 4 minutes. The lower limit of quantitation and detection were 0.06umol/L and 0.04umol/L respectively, and a mean percentage coefficient of variation of 10% for precision was achieved. The assay has a mean recovery of 105%. The regression equation (LC-MS/MS=0.6533×Immulite+0.176umol/L) derived from comparison with the Siemens Immulite 2000 immunoassay was used to derive LC-MS/MS reference ranges.

**Conclusion:** A specific and sensitive LC-MS/MS method has been developed and validated for the measurement of serum DHEAS. Age specific reference ranges have been determined. The assay is cost-effective and time saving, without the interference issues associated with immunoassay and will facilitate the investigation of CAH and other adrenal disorders.

**Th25**

**Are vitamin D immunoassays fit for all patients?**

*L Evans, KJ Hedges, J Wadsworth, LM Bailey, AS Davison, JJ Dutton, AM Milan*

*Royal Liverpool & Broadgreen University Hospitals NHS Trust, Clinical Biochemistry, Liverpool, United Kingdom*

**Background:** Increased awareness of the roles 25-hydroxyvitamin D plays in both classical processes (calcium homeostasis and bone metabolism) and non-classical conditions (cardiovascular disease, cancer, diabetes) has led to a surge in requesting. Measurement is a balance between cost, convenience and turn-around-time, with quality of results always taken into consideration. DEQAS data (October 2013) demonstrated that 84% of laboratories utilised immunoassay for analysis with 12% using tandem mass spectrometry (LC-MS/MS). Despite improved standardisation of assays, variation still exists.

**Method:** Roche Elecsys total vitamin D competitive automated immunoassay was compared with an in-house LC-MS/MS vitamin D assay (NIST aligned standards, tri-deuterated D2 and D3 internal standards). Specific groups of patients were chosen, those 1) on vitamin D2 supplementation, 2) with established liver disease, 3) with renal failure and 4) pregnant.

**Results:** Analysis of EQA samples (n=15) showed good correlation and recovery when compared against own method group (LC-MS/MS R²=0.96, Immunoassay R²=0.91). Samples with measurable 25(OH)D, all exhibited under-recovery on immunoassay, (average 47%, range -9 to -74%; R²=0.36); recovery in liver disease patients ranged from -71% to +22% (R²=0.83); renal patients recovery ranged from -58 to +60%, (R²=0.76) and pregnant patients had variable recoveries (R²=0.77). Analysis of independent IQC material on both assays confirmed a significant under-recovery on immunoassay, with results an average of 42% lower than target.

**Conclusion:** Using classifications of insufficient (>30 to ≤50nmol/L), deficient (>15 to ≤30nmol/L) and severely deficient (<15nmol/L), 48% of the patient samples (n=123) would be classified differently when assayed by immunoassay compared to LC-MS/MS. Furthermore 34% of the samples differed by two or more categories. (e.g. insufficient by LC-MS/MS would be severely deficient on immunoassay). Caution should be used when interpreting vitamin D results generated by immunoassay in patients who are on 25(OH)D, (5% of UK) or who may have altered vitamin D binding protein concentrations.
Th26

An evaluation of quantitative faecal immunochemical tests for haemoglobin (FIT)

MR Carroll¹, C Piggott¹, S Pearson¹, HE Seaman¹,², H Bruce¹, SC Benton¹, SP Halloran¹,²
¹NHS Bowel Cancer Screening Southern Programme Hub, Guildford, United Kingdom, ²University of Surrey, Guildford, United Kingdom

Introduction: The NHS Bowel Cancer Screening Programme (BCSP) in England provides biennial screening using a guaiac faecal occult blood test (gFOBT) to people aged 60-74 years. The 2010 European guidelines recommend use of a quantitative faecal immunochemical test for haemoglobin (FIT) in population screening and the BCSP plans to replace gFOBT with FIT from 2016. The BCSP Southern Programme Hub Research Team (allied with the Guildford Medical Device Evaluation Centre) has evaluated FIT systems to guide future BCSP procurement. Four quantitative FIT systems were evaluated: HM-JACKarc (Kyowa Medex Co. Ltd.), NS-PLUS C15 Hb (Alfresa Pharma Corp., Japan), OC SENSOR DIANA (Eiken Chemical Co. Ltd., Japan) and FOB Gold NG (Sentinel CH. SpA, Italy; analysed on a general chemistry analyser, BioMajesty, Jeol, Japan).

Methods: Each system was assessed using the manufacturers’ recommended sample collection tube loaded with haemoglobin (Hb)-spiked faecal samples or Hb in buffer. The evaluation included an assessment of imprecision, linearity, analytical sensitivity and carryover.

Results: The most analytically sensitive platforms were found to be HM-JACKarc and NS-PLUS. Measured analytical sensitivity was superior to manufacturers’ claims in each instance. Imprecision with NS-PLUS was found to be higher than manufacturers’ claims. All analysers except BioMajesty demonstrated good linearity. Automated or semi-automated dilution of highly concentrated samples was available with all analysers, except HM-JACKarc, which has a limited measurement range. No evidence of carry-over was found on any of the systems. The NS-PLUS and BioMajesty did not alert the user to a hook/prozone effect. Sample stability over a range of temperatures was similar to manufacturers’ claims for all analysers and much improved from previous studies. Fewer but more highly trained laboratory staff will be required to process FIT samples.

Conclusion: This evaluation provides essential information to guide the BCSP through the usual tendering procedure.

Th27

Traceability of electrolyte assays in the UK (sodium, potassium, calcium, magnesium, lithium)-comparison with JCTLM listed reference methods

DH Ducroq, SE Thompson, MA Thomas
Cardiff and Vale University Health Board, WEQAS Quality Labs, Cardiff, United Kingdom

With the implementation of ISO15189 accreditation, labs are becoming aware of the need for traceability of their routine methods. Traceability of results to the SI unit utilising reference target values is the preferred method of comparison of returned EQA results, ensuring the transfer of accuracy from definitive methods to routine methods.

Eight samples encompassing the analytical range for electrolytes were distributed over an eight month period with additional samples for serum indices (icterus and lipaemia). All samples were analysed by validated reference methods utilising JCTLM flame atomic absorption/emission reference methods. Deviations from the ‘true’ result (the reference method) for main analyser groups were plotted in the form of bias plots (Bland-Altman plots).

Sodium: The overall mean, heavily influenced by the dominant Indirect ISE group, showed a positive bias at low concentrations, a crossover at approximately 125mmol/L, with a 2% negative bias at higher concentrations.

Potassium: The overall mean was heavily influenced by the dominant Indirect ISE group. This showed good agreement with reference target across the analytical range. The Vitros showed a marked positive bias at concentrations above 3mmol/L.

Calcium: The majority of methods showed a crossover at approximately 2mmol/L with approximately -3% bias at lower concentrations and 1% positive bias at higher concentrations. Both Beckman ISE and Abaxis Picolo showed a negative bias across the analytical range.

Magnesium: The majority of methods showed a crossover at approximately 0.8mmol/L with a positive bias at low concentrations and negative bias at higher concentrations. The enzyme group showed an overall agreement with reference target but high degree of scatter between distributions.

Lithium: The dominant colourimetric (trace) method showed good agreement across the range.

Peer review of performance against method mean and overall mean data cannot identify true errors in accuracy. This can only be achieved by comparison with traceable reference methods.

Th28

Audit on osmolality requesting

SS Min, N Vithanage, R Vincent
King’s College Hospital, Chemical Pathology, London, United Kingdom

The serum and urine osmolality are manual tests performed in the laboratory. They are done to investigate hyponatraemia, to monitor osmolality active drug therapies such as mannitol used in cerebral oedema and to diagnose the presence of toxins including methanol and ethylene
glycol. Serum osmolality is best performed in conjunction with urine osmolality to assess the appropriateness of the renal response. This audit assessed the appropriateness of the request for serum osmolality received by the laboratory.

Our lab received in total 567 serum (315) and urine (252) osmolality requests in the audit month. Out of 315 serum osmolality requests, 126 were randomly selected for the final analysis. Majority (38%) had been ordered for pre or post-pituitary surgery, and 34% for hyponatraemia. Other indications included: hypernatraemia (8), polyuria (9), mannitol administration (9) and hyperglycaemia (4). No clinical information was given on 2 requests. Out of 126 requests, 25 (20%) were unpaired of which 12 (50%) were appropriate; Mannitol administration (5), Hyperglycaemia (4), Renal failure (2) and the rest was for pituitary surgery. The other 13 (50%) was for hyponatremia thus, inappropriate. The overall appropriateness of serum osmolality requesting was 88% (111 out of 126). Based on the findings, we have implemented a change in the requesting practice on our electronic patient record system. This alters the requester to consider if a paired serum and or urine osmolality is required. We will re-audit in 6 months to assess the effectiveness to a target of at least 98%. There is also a need to improve the training of junior doctors on appropriate requesting of osmolality.

Th29

Derivation and cross-site comparison of albumin-adjusted calcium equations

LE Rashid1, C Clarke1, RJ Pattenden1, C Thomson2, JP Ashby1
1Western General Hospital, Clinical Biochemistry, Edinburgh, United Kingdom, 2Royal Infirmary of Edinburgh, Combined Laboratories, Edinburgh, United Kingdom

Introduction: It is recommended that individual laboratories evaluate their equations for calculating albumin-adjusted calcium. The aim of this study was to re-assess the equation currently in use and determine whether a single equation could be applied across three sites, all employing the same equipment and methodologies-calcium:Arsenazo-III dye; albumin:BCP (Abbott).

Methods: Data was retrospectively obtained from the laboratory computer database for patients with a normal serum phosphate and no biochemical evidence of abnormalities in renal or liver function. Results were separated according to hospital site and linear regression analysis performed to derive the site specific albumin-adjusted calcium equations. The results for each hospital site were then combined to generate a single equation.

Results: The albumin adjusted calcium was normalised to a mean calcium level of 2.4 mmol/L and expressed by the following relationships for the three different sites: adjusted \( [\text{Ca}] = \text{total} [\text{Ca}] + 0.018 \times (43-\text{albumin}) \), \( n=6064 \); adjusted \( [\text{Ca}] = \text{total} [\text{Ca}] + 0.017 \times (44-\text{albumin}) \), \( n=4758 \); adjusted \( [\text{Ca}] = \text{total} [\text{Ca}] + 0.019 \times (40-\text{albumin}) \), \( n=1987 \). The appropriate site specific equation was used to calculate the 2.5th-97.5th percentiles for distribution of albumin-adjusted calcium for patient populations on individual sites, yielding ranges of 2.20-2.64, 2.23-2.63 and 2.20-2.61 mmol/L respectively. The use of a single equation (adjusted \( [\text{Ca}] = \text{total} [\text{Ca}] + 0.018 \times (42-\text{albumin}) \)) derived using data from all three sites, generated an adjusted calcium range of 2.20-2.62 mmol/L for the combined patient population.

Conclusion: While there were small differences in the equations derived on the different laboratory sites, the 2.5th to 97.5th percentile ranges were comparable. It is unlikely that the use of a single combined equation across sites will result in clinically significant errors.

Th30

Development of a fully automated method for determination of methylmalonic acid in human serum/plasma by MPS-LC-MS/MS

R Gorska1, DJ Harrington1, PH Roberts2,3
1Viapath, Human Nutristasis Unit, London, United Kingdom, 2Anatune Ltd, Girton, Cambridgeshire, United Kingdom, 3Biotage GB Ltd, Ystrad Mynach, Hengoed, United Kingdom

Measurement of methylmalonic acid (MMA) in serum/plasma has been used as a biochemical marker for vitamin B12 deficiency for over 20 years. In our laboratory, improvements in sample preparation and the ability to analyze MMA without derivatization have simplified the analysis in comparison to the previous methods used.

We have developed a GERSTEL Multi Purpose Sampler coupled directly to liquid chromatography tandem mass spectrometry (MPS-LC-MS/MS) method. Extraction of MMA is achieved through a simple protein precipitation with 800 μl of acetonitrile containing 0.5% acetic acid and the internal standard MMA-d3. Each sample is then moved using the MPS to the Anatune CF-100 robotic centrifuge, whereby the contents are thoroughly vortexed for 30 seconds to assist in the protein precipitation. The vial is then centrifuged for 1 minute to separate the proteins from the supernatant. 20ul of extract is injected on the system and separated using hydrophilic interaction liquid chromatography (HILIC). The chromatographic mobile phases consisted of (A) acetonitrile and (B) 100 mM ammonium acetate in water adjusted to pH 4.5 with formic acid. Data was gathered in multiple reaction monitoring mode measuring the carbonyl loss of MMA and MMA-d3 respectively. Calibration curves exhibit linearity in the range of 60-2000nmol/L. Intra- and inter-assay CVs were < 10%. The recoveries of MMA from “spike” experiments were comparable.
between 96-100%. Approximately 190 MMA samples can be analyzed on a single MPS-LC-MS/MS system. Although chromatographic separation of MMA from the isobaric interference succinic acid can be achieved within 1.2 min, the total run time is 8 mins because of the washing and reconditioning steps that are an important consideration when performing HILIC on biological samples. We developed a fully automated, robust assay which allows the analyses of MMA in human serum/plasma without the need for the derivatization. The assay is suitable for a high-throughput clinical laboratory.

Th31

A simple algorithm to eliminate interferences due to UV absorbing substances in capillary electrophoresis

L Seaux, S Van Houcke, E Dumoulin, T Fiers, E Lecocq, J Delanghe
Ghent University Hospital, Department of Clinical Chemistry, Microbiology and Immunology, Ghent, Belgium

Background: Iodine-based contrast agents and various antibiotics have been reported to interfere with interpretation of serum protein phero-
grams, resulting in false diagnosis of paraproteinemia. Most potentially interfering substances show UV absorption spectra distinct from those of serum proteins

Aim: This study explored the possibility of measuring UV absorbance at two distinct wavelengths to eliminate interferences.

Design: Pherograms showing suspect peaks were recorded at 2 wavelengths (the conventional peptide bond wavelength 210 nm and the absorbance maximum for contrast media 246 nm) to distinguish between true and false paraproteins on a Helena V8 clinical electrophoresis instrument (Helena Biosciences, Newcastle). A query for all serum protein electrophoresis requests (Jan 2013-March 2014) showed it is not uncommon to find iodine-based contrast media interfering with serum protein electrophoresis in hospitalised patients. This interference was reported in 54 cases out of 13237 analyses (0.4%) while 1631 true paraproteins (12.3%) were detected.

Results: Comparing pherograms recorded at both wavelengths enabled to distinguish paraproteins from UV absorbing substances. In case of a true paraprotein, the peak in the gamma-region virtually decreased. Contrast media and antibiotics showed an increased absorption.

Conclusions: Implementation of the proposed algorithm may significantly improve the interpretation of routine electrophoresis results. However, attention should be paid to possible interference due to presence of atypical proteins fractions (e.g. tumour markers, C3).

Th32

Integrated targeted quantitation method for insulin and its therapeutic analogs

R Chaudhari1,2
1Thermo Fisher Scientifc, Hemel Hempstead, United Kingdom, 2Thermo Fisher Scientific, Tempe, United States

Introduction: The need to detect and quantify insulin and its analogs has become paramount for medical and athletic doping. Conventional insulin assays have an inability to differentiate endogenous insulin from exogenous insulin analogs. The use of LC-MS can overcome this, however, methods to-date lack the analytical sensitivity demanded.

Objectives: Development of a Mass Spectrometric Immunoassay (MSIA) workflow for the high-throughput, analytically-sensitive quantification of insulin and its analogs from human plasma.

Methods: Samples were prepared neat and in donor plasma using human insulin and 5 insulin analogs. The analogs were prepared independently and mixed to test selectivity and sensitivity of the enrichment method. Enrichment was performed using MSIA tips derivatized with pan-anti-insulin antibody which recognizes an epitope in the beta chain conserved across all variants. Detection/quantitation was performed using LC-MS on a a Thermo Scientific™ Ultimate™ 3000 LC system coupled to a Thermo Scientific™ Q Exactive™ mass spectrometer.

Results: The insulin pan-antibody allows capture and detection of all variants from the sample. Full scan MS mode in the analysis stage enables simultaneous detection of multiple insulin analogs and can screen for unsuspected analogs post-acquisition. Accurate intact mass and fragmentation of the analogs confirmed the identity of each variant. With the MSIA workflow, the LLOQ was 15 pM (87 pg/mL) and LOD was < 7.5pM (47 pg/mL). Reproducibility studies demonstrated inter- and intra-day CV’s of < 3%. Spike and recovery resulted in recoveries of 96-100%. Additionally, the MSIA workflow significantly reduces the background matrix affording shorter LC gradients and LC-MS analysis times.

Conclusion: Incorporation of a pan-antibody, used to capture human insulin and 5 commercially available insulin analogues, into a LC-MS detection/quantitation assay.
**Th33**

Development of a new immunoassay for the accurate determination of anti-infliximab antibodies in inflammatory bowel disease

*J Semmler*, A Pilch*, FP Armbruster*, A Dignass†, W Krüsi†, J Stein†

1Immundiagnostik AG, Bensheim, Germany, 2Interdisziplinäres Crohn Colitis Centrum Rhein-Main, Frankfurt, Germany, 3Agaplesion Markus Krankenhaus, Frankfurt, Germany, 4Evangelisches Krankenhaus Kalk, Cologne, Germany, 5Krankenhaus Sachsenhausen, Frankfurt, Germany

**Introduction:** The formation of antibodies to infliximab (ATIs) is closely associated with loss of response to infliximab in patients with inflammatory bowel disease (IBD). Therapeutic drug monitoring strategies have been based mainly on assessment of serum ATI levels by immunoassays based on the double-antigen format, using immobilized infliximab or protein A to capture ATIs on a carrier. In these assays, however, the presence of infliximab can interfere with the binding of labeled infliximab to the captured ATIs, leading to false-negative results. In this study, we developed a novel immunoassay for ATIs to enable their measurement in the presence of infliximab.

**Methods:** The new assay is based on the dissociation of immune complexes between infliximab and ATI at low pH-values and the use of biotinylated and peroxidase-labeled infliximab to prevent re-formation of immune complexes. Measurement of ATI levels was performed in 29 patients with Crohn’s disease on infliximab maintenance therapy using both the novel immunoassay and the conventional method. Infliximab trough levels were determined by ELISA.

**Results:** Whereas ATIs were detected in 7 out of 29 patients (24.1%) using the new method, they were detected by the conventional method in only 1 patient (3.4%), for whom the two highest ATI titers were determined by the new assay. The addition of infliximab to the samples dose-dependently inhibited the detection of ATIs in the conventional method but not in the new method. Patients positive for ATIs had significantly lower serum trough levels of infliximab (p < 0.01) and significantly higher clinical activity scores (p < 0.001) in comparison to patients negative for ATIs.

**Conclusion:** The novel assay allows the measurement of serum ATI levels independent of the presence of infliximab. The new method is therefore a useful tool in strategy selection for the optimal management of IBD patients showing loss of response to TNFalpha-antibodies.

**Th34**

Investigation of icteric interference in the Roche enzymatic creatinine assay

*T Keys, E Burgess, S Ibrahim

Gloucestershire Hospitals NHS Foundation Trust, Chemical Pathology, Gloucester, United Kingdom

**Background:** The Roche (CREP2) enzymatic creatinine method sheet states that there is significant interference when icterus exceeds 15 mg/dl for conjugated bilirubin and 20 mg/dl for unconjugated bilirubin. Our laboratory therefore employs an icterus threshold of 15 for reporting results. It was desirable to test this interference because of the large number of samples affected.

**Methods:** Icteric samples with low creatinine concentrations were selected, along with non-icteric samples of varying creatinine concentrations. These samples were mixed at various ratios to achieve ‘low creatinine’ (icterus 18, creatinine 70 μmol/L), ‘medium creatinine’ (icterus 22, creatinine 321 μmol/L) and ‘high creatinine’ (icterus 25, creatinine 609 μmol/L) pools. These pools were then serially diluted with 0.9% saline and analysed for creatinine, icterus, direct and total bilirubin levels.

**Results:** The dilution experiments showed that all creatinine results were within 12% of the expected value. That is to say, up to an icterus level of 32, significant interference was not observed in the Roche enzymatic creatinine assay. Additionally, audit data showed that the Roche serum index method overestimates the icterus level in the sample when compared to the expected icterus for the measured bilirubin level. A brief laboratory audit showed that during January and February 2014, creatinine results were not reported in 146 samples because the icterus index exceeded 15.

**Discussion:** The icterus interference in the creatinine assay appears to be less significant than suggested by Roche. The serum index method also appears to overestimate icterus. These findings have given our laboratory confidence to increase the icterus threshold for reporting creatinine. This allows us to reduce the number of samples where a result is either not reported or where the report is delayed by the need to dilute the sample before analysis. The bias in the icterus method should also be checked periodically.

**Th35**

Development of a high-resolution mass spectrometric immunoassay (MSIA) for human hepcidin

*SA Handley*, L Couchman*, P Sharp*, EE Niederkofler*, CF Moniz*

1King’s College Hospital, London, United Kingdom, 2King’s College London, London, United Kingdom, 3Thermo Fisher Scientific, Tempe, United States

Hepcidin, a 25-amino acid polypeptide, is the master regulator of systemic iron homeostasis, and may be a promising marker for the diagnosis of iron related disorders. Measurement may help in the diagnosis of hereditary haemochromatosis, and guide treatment with erythropoietin or
intravenous iron. Truncated isoforms of hepcidin-25 (hepcidin-20,-22) have been identified, but their role has not yet been elucidated. The aim of this study is to develop a MSIA method for the measurement of hepcidin isoforms. Calibration solutions (N=7) over the range; 100 μg/L were prepared in phosphate buffered saline (PBS) containing 10 g/L bovine serum albumin. A recovery solution (all analytes, 100 μg/L) was prepared in PBS only. Samples (200 μL) were diluted with internal standard solution (hepcidin-25-C<sub>18</sub>-N<sub>3</sub>, in HBS-EP buffer, 500 μL), and extracted by immunocapture onto MSIA tips pre-bound with anti-hepcidin antibody fragments. The tips were washed with (i) PBS, and (ii) deionised water before elution with acetonitrile:deionised water (33+67, v/v), containing 0.4% (v/v) trifluoroacetic acid. Prepared samples (100 μL) were injected onto an ACE C18 analytical column (100 x 2.1 mm, 3 μm). Detection was by positive heated-electrospray-ionisation, in full-scan mode (400-1000 m/z). The 3+, 4+, and 5+ charge states were monitored to produce an extracted ion chromatogram. Retention times for hepcidin-20, -22, -25, and internal standard were 3.57, 3.86, 3.78, and 3.78 minutes, respectively. Mean (N=7) recovery for hepcidin-20, -22, -25, and internal standard was; 56, 60, 73, and 64% respectively. Calibration was linear for all analytes (R<sup>2</sup> > 0.98) over the concentrations studied and imprecision (% CV) at 100 μg/L was < 1%. The proposed method is simple, and easily automated. MSIA is an ideal workflow solution for analytes which are present at low concentrations, and where interference from similar compounds is likely with conventional immunoassays.

**Th36**

**Development and validation of a semi-automated liquid chromatography tandem mass spectrometry serum steroid profile**

*C Syme*

*Glasgow Royal Infirmary, Glasgow, United Kingdom*

The Clinical Biochemistry laboratory at Glasgow Royal Infirmary provides a number of specialist steroid analyses. The aim of this study was to replace three labour intensive, manual assays with one partly automated assay. A liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of multiple steroids has been developed. The analytes included are testosterone, androstenedione (A4), 17-hydroxyprogesterone (17OHP), 11-deoxycortisol (11DOC) and 21-deoxycortisol (21DOC). The new method has replaced the extraction radio-immunoassays for A4 and 17OHP and the manual testosterone extraction, used as a second line test for female samples with high testosterone concentrations.

Samples (100 μL serum/plasma) are extracted using supported liquid extraction (isolute® SLE+ Biotage) on an automated platform (CTC PAL, Presearch), followed by dichloromethane elution. The extracted sample is then injected onto a UPLC BEH C18 column for chromatographic separation, followed by detection using the Waters XEVO-TQS. Deuterated internal standards are used for each steroid.

The five steroids demonstrated a linear response up to 50 nmol/L and 17OHP up to 151 nmol/L. The limits of detection were: testosterone 0.18 nmol/L, A4 0.09 nmol/L, 17OHP 0.15 nmol/L, 11DOC 0.03 nmol/L and 21DOC 0.29 nmol/L. The inter- and intra-assay coefficients of variation were < 10% for all analytes, across the analytical range. Testosterone, A4, and 17OHP results compared well with other LC-MS/MS users (data from UK NEQAS for Steroid Hormones). Interference studies demonstrated that the LC-MS/MS method is very specific.

The new technique provides an accurate, robust method for analysis of multiple steroids simultaneously. The partly automated method has been a significant improvement for a busy clinical laboratory as the time required for analysis has been reduced from 4 days to just 1 day. Also, the addition of 11DOC and 21DOC will assist with the diagnosis of rarer forms of congenital adrenal hyperplasia and other rare adrenal pathologies.

**Th37**

**The effect of haemolysis on insulin and c-peptide measurements by Mercodia ELISA**

*EA Willis, A Pickett, A Dodd, S Neale, C Evans*

*University Hospital of Wales, Biochemistry, Cardiff, United Kingdom*

Insulin and C-peptide are central to investigation of hypoglycaemia. Samples received in the laboratory are often haemolysed and haemolysis is known to cause degradation of insulin due to release of insulin-degrading enzyme from erythrocytes.

The original Mercodia Iso-Insulin ELISA instructions stated that haemolysed samples do not interfere in the assay, however they now warn of a negative interference which is dependant upon time, temperature and haemoglobin concentration. Our aim was to characterise the effects of haemolysis on the Mercodia assays for insulin and C-peptide and generate a practical guide for our laboratory on how to process haemolysed samples and clinically validate the results.

A postprandial heparinised sample was split into aliquots which were subjected to differing degrees of mechanical stress to achieve a range of haemolysis. The extent of haemolysis was quantified using the haemolysis index on the Abbott Architect. Aliquots were then exposed to different storage conditions prior to separation and freezing. Samples were thawed and analysed immediately or after two hours.
Our results confirmed haemolysis does cause a significant decrease in the insulin measured (gross haemolysis caused 57.6% decrease) and that C-peptide is unaffected. Insulin is more sensitive to a delay in separation, especially at room temperature (13.7% decrease at 4 hours in non-haemolysed sample). This effect is reduced by storing samples at 4°C prior to separation (5.1% decrease at 4 hours). Samples thawed for 2 hours prior to analysis showed a decrease in the values obtained for insulin (12.2% decrease in non-haemolysed sample) but not for C-peptide. Based on these findings we will visually screen samples for haemolysis prior to assay. Haemolysed samples will be noted on our computer system to ensure an appropriate comment is made at clinical validation. Ideally samples will be separated within 2 hours and frozen, then analysed promptly after thawing.

Th38

A liquid chromatographic tandem mass spectrometry assay for serum total thyroxine

DJ Halsall1, KP Taylor1, N McArdle2, C Moran3, VK Chatterjee3

1The Pathology Partnership, Biochemistry Department, Cambridge, United Kingdom, 2Norfolk and Norwich University Hospitals, Clinical Biochemistry, Norwich, United Kingdom, 3Institute of Metabolic Sciences, Metabolic Research Laboratories, Cambridge, United Kingdom

Whilst the estimation of serum total thyroxine (TT4) has largely been replaced as a marker of thyroid function by methods which attempt to measure the non-protein bound or free hormone (fT4), TT4 methods remain useful in the investigation of aberrant thyroid function test results. In particular anti-T4 antibodies and heterophilic antibodies directed against immunoassay reagents are a well described cause of interference in many fT4 assays in current use. Discordance between the measured fT4 result and a calculated fT4 value, computed from TT4 and thyroxine binding globulin (TBG, the principal T4 binding protein) measurements, can be used to expose this type of interference. However TT4 immunoassay can also be confounded by antibody interference.

For this reason we have developed a mass spectrometry (MS) based TT4 assay. Serum proteins were precipitated in methanol including a 13C(12)-labelled Thyroxine internal standard, with further sample clean-up using an on-line C8 solid-phase extraction (‘trap’) column. Chromatographic separation was performed using a C18 analytical column. An API 5500 triple quadrupole mass spectrometer was used in positive ionization electrospray mode.

The limit of quantitation of the method, based on a signal/noise ratio greater than 10 and a coefficient of variance of < 20%, was 0.8 nmol/L, which is appropriate for the biological range of this analyte (reference range ∼ 50 - 160 nmol/L). Recovery, inter-assay imprecision and accuracy (based on NEQAS ALTM) were all acceptable at 80-120%, less than 10% and ±/− 15% respectively. Method comparison with a commercially available TT4 immunoassay (PE-Delfia) using 81 patient samples with no suspicion of assay interference based on TSH and clinical criteria gave a Passing and Bablock fit of $MS(nmol/L) = 1.01*immunoassay(nmol/L) - 2.91$ ($R^2 = 0.9844$). Significant discrepancies were observed in patient samples with proven anti-iodothyronine antibodies and probable heterophile antibody interference.

Th39

The development of a liquid chromatography-tandem mass spectrometry method for the detection of four laxatives in urine

H Wilshaw, E Hinchliffe, P Reed, JJ Scargill, G Waite

Salford Royal Foundation NHS Trust, Biochemistry, Salford, United Kingdom

Aim: Chronic diarrhoea caused by laxative abuse is believed to be a widespread yet generally underreported problem. Guidelines state that if laxative abuse is suspected, a urine laxative screen should be performed. The aim of this project was to develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to detect rhein, phenolphthalein, desacetyl bisacodyl and dantron in urine and then compare the developed method to the high-performance thin layer chromatography (HPTLC) method currently in routine use at Salford Royal Foundation Trust.

Methods: Suitable sample extraction techniques were evaluated and chromatography and mass spectrometry parameters optimised. The final method utilised diethyl ether liquid-liquid extraction and reverse-phase ultra-performance liquid chromatography with a Thermo Scientific UPLC Synchroni C18 1.7 μm 2.1 x 50 mm column. Detection was performed using a Waters TQD mass spectrometer that cycled between positive and negative ion modes. Final run time was 13 minutes. The method was validated against FDA and EMA guidelines.

Results: The LC-MS/MS method demonstrated acceptable intra- and inter-assay precision and accuracy. Limits of detection were determined for both HPTLC and LC-MS/MS methods, with the LC-MS/MS method demonstrating greater sensitivity. Cut-off values were assigned as concentrations above the LLOQ.
47 patient samples submitted for laxative screening were analysed using both HPTLC and LC-MS/MS methods. Of these, 37 screened negative using both methods, 7 screened positive using both methods and 3 screened positive using LC-MS/MS but negative using HPTLC.

**Conclusion:** A sensitive LC-MS/MS method for the detection of four urine laxatives has been developed and validated and has several advantages over the current method including greater sensitivity, shorter analysis times and more easily interpretable results. Further work needs to be performed to determine clinically relevant cut-off concentrations.

**Th40**

**Accuracy and random errors in immunological tests on Siemens high automation platform**

**S Rapi, B Salvadori, A Ognibene, A Fanelli**  
Careggi Hospital, Laboratory, Florence, Italy

**Background:** An accurate overview of the performance and characteristics of laboratory systems and the estimation of random errors in laboratory working flow is a significant target in laboratory setting. A new highly automated platform based on a sample track, 4 Dimension Vista 1500 analysers, decappers and a refrigerated storage system (Siemens Healthcare Diagnostics USA) was acquired in Careggi Hospital (Florence, Italy) at the end of 2013. Aim of this report is the investigation of analytical errors that may occur in the measurement of some of the most important immunological tests (Trop I, TSH, hCG) during the daily working flow in the first 3 months after the system introduction.

**Material and methods:** After setting the system, random biological samples stored for no more than 24h at 4-8 °C were selected and the tests were repeated on different instruments. The resulting data was investigated using Bland Altman plotting methods. A difference between two repetitions >/= 10% from the mean value of the two results, for values higher than 0.04 ug/L for Trop I, 0.005 mUI/L for TSH and 1 mUI/mL for hCG, was considered an analytical error. The number of samples included in the investigation was related to the number of instruments daily available for single test (4,3,2 for Trop, TSH and hCG respectively).

**Results:** 730 repetitions were performed and 23 errors (3.15%) were registered. Error distribution was significantly different between methods: Trop 12 out of 330 (3.6%); TSH 10 out of 240 (4.2%) and 1 out of 160 for hCG (0.62%) (p< 0.01). No systematic bias was observed between the different instruments.

**Discussion:** The incidence of casual errors in immunological tests during daily working flow remains significant even in high automation. The use of biological samples appears a fruitful tool for inter-instrument imprecision evaluation and system control.

**Th41**

**Interference in routine chemistry assays due to the formation of precipitates during analysis**

**J Jeffery, RM Ayling**  
Clinical Biochemistry, Derriford Combined Laboratory, Plymouth, United Kingdom

**Introduction:** Routine chemistry spectrophotometric assays rely on changes in the absorbance of light to measure the concentration of analytes. These assays are susceptible to interference from the formation of precipitates which reduce the passage of light through a solution. Two examples of this problem are described, affecting the Roche total bilirubin and HDL cholesterol assays. The precipitate in the bilirubin assay was likely to have contained a paraprotein, but the precipitate in the HDL cholesterol assay was related to another cause.

**Methods:** Total bilirubin (diazonium) and 3rd generation homogeneous HDL cholesterol (dextran sulphate/Mg) Roche COBAS assays. 

**Results:** Two cases are presented which illustrate assay interference due to the formation of precipitates; the first case was a sample from a myeloma patient with an erroneously high bilirubin result of 198 umol/L and an icteric index of 17.1 umol/L. A precipitate was formed when bilirubin reagent was added to the sample. It was possible to remove this interference in the bilirubin assay by pre-treating the sample with polyethylene glycol. The second case was a sample from a patient with an erroneously low HDL cholesterol result of 0.13 mmol/L where an insoluble precipitate was formed when dextran sulphate/magnesium reagent which limited the ability of the analyser to subsequently detect the change in absorbance produced by the enzymes used to measure cholesterol. It may be possible to remove this interference in the HDL cholesterol assay by diluting the sample prior to analysis.

**Conclusions:** Reviewing the reaction profiles obtained for routine chemistry spectrophotometric assays may help to identify the formation of precipitates during the course of a reaction which may be interfering in the analysis. This can then be confirmed by manually adding patient sample to the reagents and visually examining the reaction product for the presence of a precipitate.
Th42

Analytical evaluation of a new capillary electrophoresis method for carbohydrate-deficient transferrin (CDT) measurement

T Woolley
Synergy Health Laboratory Services Ltd, Pathology, Abergavenny, United Kingdom

Background: Carbohydrate-Deficient Transferrin (CDT) measurement is currently used within the UK for monitoring chronic intake of excessive amounts of alcohol and plays an essential role reissuing driving licences and determining custody battles. The aim of this study is to report the results obtained from the evaluation of a new CDT quantification assay, when compared to a method used currently in routine use.

Methods: We assessed the analytical performance of two automated capillary electrophoresis (CE) systems, the Capillarys 2 from Sebia (Surrey, UK) and V8 E-Class from Helena Biosciences (Tyne and Wear, UK) for the measurement of Carbohydrate Deficient Transferrin (CDT).

Results: Within laboratory imprecision CV% using four commercially available quality control materials with different CDT concentrations, spanning the clinical cut points for CDT were <7.5% for the V8 and using only two commercially available quality controls, <9.1% for the Capillarys 2. The comparison between the two methods gave a Pearson coefficient of r = 0.985. The accuracy of both systems was highly satisfactory.

Conclusions: The findings of this study indicate that the new capillary electrophoresis CDT method from Helena Biosciences is comparable to the established Sebia method. The new method is acceptable for routine use in clinical laboratories and has a high level precision, accuracy and is robust, with a high throughput.

Th43

Development of an LC-MS/MS assay for the measurement of the serotonin metabolite 5-hydroxyindole acetic acid in whole blood samples

S Armitage, J Adaway, B Keevil
University Hospital of South Manchester, Biochemistry, Manchester, United Kingdom

Background: 5-hydroxyindole acetic acid (5-HIAA), a metabolite of serotonin, is used as a marker for patients with serotonin-secreting neuroendocrine tumours (NET). Currently, the majority of laboratories measure 5-HIAA excretion in 24 hour urine samples. Given the practical and analytic problems of these samples, our laboratory successfully developed a LC-MS/MS method for the analysis of 5-HIAA in serum samples. Further to this, we have now developed a method to measure 5-HIAA in whole blood samples.

Methods: We developed a method for measurement of 5-HIAA in whole blood samples using a simple protein precipitation step prior to LC-MS/MS analysis. To validate the method, ion suppression, recovery from spiked whole blood, linearity, inter- and intra-assay imprecision, lower limit of quantitation (LLOQ) and lower limit of detection (LLOD) was assessed. The analysis was performed in positive ion mode using a Waters® Acquity Premier™; the ion transitions were m/z 192.9 > 145.9 and 199.1 > 149.8 for 5-HIAA and d5-5-HIAA respectively.

Results: Using post-column infusion of 5-HIAA, ion suppression was deemed to be negligible. Mean recovery was 93% (range 85-101%), and the method was linear up to 100,000 nmol/L. Within and between batch imprecision (CV), assessed over the normal and pathological range, was <9% and <7%, respectively. LLOQ and LLOD was 12.5 and 11.2 nmol/L, respectively. Samples were deemed stable for up to 12 hours at room temperature and 4°C and up to 6 hours at 37°C. A reference range for the assay was determined as <408 nmol/L.

Conclusion: We have successfully developed and validated a LC-MS/MS method for the measurement of 5-HIAA in whole blood samples. It is proposed that this method could be further developed to enable measurement of 5-HIAA in fingerprick or dried blood samples.

Th44

The impact of specimen haemolysis on the measurement of total and direct bilirubin-a regional study by the Yorkshire Laboratory Medicine Discussion Group

CM Lippiatt1, N Jassam2, H Delaney3, S Slack4, J Spencer5, C Evans6, S Glover7, D Carless8, P Craddock9, P Walker1
1Mid Yorkshire Hospitals NHS Trust, Clinical Biochemistry, Wakefield, United Kingdom, 2Harrogate and District NHS Foundation Trust, Clinical Biochemistry, Harrogate, United Kingdom, 3Sheffield Teaching Hospitals NHS Trust, Clinical Chemistry, Sheffield, United Kingdom, 4York Teaching Hospitals, Clinical Biochemistry, York, United Kingdom, 5Leeds Teaching Hospitals NHS Trust, Clinical Biochemistry, Leeds, United Kingdom, 6Airedale NHS Foundation Trust, Clinical Biochemistry, Airedale, United Kingdom, 7Bradford Teaching Hospital NHS Foundation Trust, Blood Sciences, Bradford, United Kingdom, 8Calderdale & Huddersfield NHS Foundation Trust, Clinical Biochemistry, Huddersfield, United Kingdom, 9Sheffield Children’s NHS Foundation Trust, Clinical Chemistry, Sheffield, United Kingdom

Introduction: Jaundice affects approximately 60% of term babies and up to 80% of pre-term (gestation < 37/40) babies. As stated in NICE guideline 98 (2010), a conjugated bilirubin level greater than 25umol/L indicates possible serious liver disease. Accurate measurement of total...
and direct bilirubin is crucial to identify those babies that need urgent medical attention. A recent UKNEQAS study revealed that significant negative interference from haemolysis was evident in many conjugated bilirubin assays. Our aim was to investigate whether interference from haemolysis affected the ability of laboratories in our region to report total and direct bilirubin.

**Methods:** A serum pool with a high direct bilirubin concentration was prepared and split into ten sub-pools to which were added increasing amounts of fresh haemolysate to represent samples with increasing degrees of haemolysis. These haemolysate-spiked pools were distributed to nine laboratories within the Yorkshire region for measurement of haemolysis index, total and direct bilirubin.

**Results:** Haemolysis index values varied considerably between the five analytical platforms. All automated methods for measuring direct bilirubin were affected to some degree by haemolysis. Each of the laboratories in this study had a different policy for reporting total and direct bilirubin results on haemolysed samples and the haemolysis index threshold at which the policy was invoked differed from lab to lab, even among laboratories that used the same analytical platforms.

**Conclusion:** There are several limitations to the study protocol- the samples that were distributed were not generated from paediatric samples and haemolysis was simulated by spiking the pooled serum with haemolysed whole blood. Nevertheless, our findings highlight the importance of recognising the limitations of the analytical methods we use and the benefits that arise from communication with colleagues using similar methodologies for laboratory tests.

**Th45**

**Identification of a Regan ALP isoenzyme on the Sebia HYDRASYS® electrophoresis platform**

B McDonald¹, R Olufadi², A Armstrong²

¹University Hospital Southampton, Department of Laboratory Medicine, Southampton, United Kingdom, ²Dorset County Hospital, Clinical Pathology, Dorchester, United Kingdom

Alkaline phosphatase (AP) is expressed by most organs of the body and in humans is encoded by at least 4 loci. Three isoenzymes are expressed in a tissue specific manner -intestinal (I), placental (P) and germ cell, whilst the fourth isoenzyme is ubiquitous but especially abundant in liver (L), bone and kidney. The isoforms of AP can be differentiated by a number of techniques enabling the tissue of origin to be identified when increases in AP are observed.

At UHSNFT samples with an elevated total AP undergo electrophoresis using the Sebia HYDRASYS® system using a HYDRAGEL ISO-PAL kit. Samples are run neat and after treatment with lectin which preferentially binds to the bone isoform. This gives good separation of the main isoenzymes which can be quantified by densitometry.

Mrs E A was a 70 year old woman who had previously been diagnosed and treated for ovarian cancer. Earlier this year she re-presented to her GP following a recurrence of symptoms. She was found to have a raised AP(407 IU/L). Subsequently a serum sample from DCH underwent AP isoenzyme electrophoresis revealing an unusual pattern with diffuse bands between L1 and L2 and I1 and I2. To investigate this further samples were heated at 56°C for 30 minutes prior to electrophoresis. This denatures the liver and bone isoenzymes confirming the presence of a placental isoenzyme. The age of the patient made placental isoenzyme improbable suggesting a Regan isoenzyme as the most likely cause.

Regan isoenzymes of AP are a rare finding demonstrated in patients with a number of tumours such as ovary and breast and bronchogenic carcinoma. This is the first Regan isoenzyme identified at UHS during the 4 years we have been using the HYDRASYS® platform and one of the first reports in the literature.

**Th46**

**Maximising analyte recovery using the Phenomenex Phree clean up plate with an established LC-MS/MS method to quantify 25-OH vitamin D2 and D3**

L Evans¹, R Pruden¹, JJ Dutton¹, JB Rudge¹, AS Davison¹

¹Royal Liverpool and Broadgreen University Hospitals Trust, Department of Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom, Phenomenex, Melville House, Macclesfield, Cheshire, United Kingdom

**Aims:** To improve sensitivity in an established LC-MS/MS method for the quantification of 25-OH vitamin D2 (D2) and 25-OH vitamin D3 (D3).

**Method:** Samples underwent clean up using a Phenomenex Phree® phospholipids removal plate. A 20 μL extract was injected onto a Phenomenex Kinetex® C18 column using a Waters Acquity UPLC system, coupled to a Waters Xevo TQS mass spectrometer. Samples were also assayed following a simple protein precipitation protocol, using zinc sulphate and acetonitrile containing deuterated d3 labelled internal standards. Confirmation of phospholipid removal was achieved by monitoring the 184 to 184 m/z transition.

**Results:** Protein precipitation: D3 calibrators at 24.0, 68.4 and 183nmol/L gave typical areas of 21 500, 54 500 and 133 000, respectively, with internal standard areas, typically 54 000.D2 calibrators at 17.7, 56.5 and 154.0nmol/L gave typical areas of 10 000, 28 000 and 73 500, respectively, with internal standard areas, typically of 27 000.Phree clean up: D3 calibrators gave typical areas of 30 500, 87 000 and 229 000.
respectively, with internal standard areas typically 109,000. D2 calibrators gave typical areas of 11,000, 30,000 and 71,000, respectively, with internal standard area of 28,000.

**Conclusion:** Phospholipids are known to have negative effects in LC-MS/MS analysis, such as ion suppression, reduced column lifetime and analyte sensitivity. Use of the Phenomenex Phree clean up plate significantly reduced ion suppression as it successfully removed phospholipids present in the samples analysed.

**Th47**

**Bone specific alkaline phosphatase: immunoassay versus electrophoresis**

*F Ahmed, S Gibbons*
Nottingham University Hospitals, Clinical Pathology, Nottingham, United Kingdom

**Background:** Measurement of bone specific alkaline phosphatase (BALP) may be useful in diagnosing and monitoring metabolic bone disease. This study aimed to evaluate the BALP immunoassay and compare it with electrophoresis (densitometry) for the quantitation of BALP.

**Methods:** Metra BALP immunoassay kits (Quidel®, USA) were used for the method evaluation. BALP was also quantitated by electrophoresis (densitometry). 7 patient samples with active Paget’s disease, 5 samples from the United Kingdom National External Quality Assessment Service (UKNEQAS) bone scheme, 4 independent BALP quality control (QC) material and 5 patient samples with raised liver, placental and intestinal isofrm of ALP were used in this study.

**Results:** Immunoassay results did not correlate well with densitometrically quantitated BALP; there was a statistically significant (p<0.01), negative bias (23%) for results obtained by immunoassay compared to those derived by densitometry. Possible interference with other isoforms of alkaline phosphatase (ALP) such as liver, placental and intestinal was also observed. The Metra BALP immunoassay is quoted as having an upper limit of detection of 140U/L, we observed inconsistent results upon dilution of samples below this level.

**Conclusions:** Immunoassay and electrophoresis did not correlate well for BALP quantitation. Possible interference with other isoforms of ALP was observed with the BALP immunoassay. The accuracy of the BALP immunoassay is questionable at higher concentrations. Larger studies are required to confirm these observations.

**Th48**

**Comparison of a new commercial kit for immunosuppressant analysis with established assays**

*D Blake, F Fung, D Leigh*
ABSCIEX, Warrington, United Kingdom

We present instrument performance and expected results obtainable from the SCIEX IVD-MS™ Immunosuppressants Kit for the analysis of Cyclosporin A, Tacrolimus, Sirolimus and Everolimus in whole blood when using the SCIEX IVD-MS™ Analyzer. This reagent kit is an in vitro diagnostic product to be used in clinical laboratories for the quantification of these immunosuppressant drugs in human whole blood samples by LC-MS/MS (liquid chromatography-tandem mass spectrometry). We present here results and discussion demonstrating the favourable performance achieved from the kit when compared to traditional analytical techniques for these compounds

**Performance results:** For all compounds (Cyclosporin, Tacrolimus, Everolimus and Sirolimus) except where indicated:
- LOQ 0.5ng/ml, (Cyclosporin 5ng/ml)
- Intra-assay CV at lowest calibrator <5%.
- Inter-assay CV at lowest calibrator <8%
- Recovery 96-104%

**Th49**

**Early detection in the monitoring of multiple myeloma (MM) by Hevylite® chain (HLC) ratio**

*KJ Jenkins, D Elks, J Forsyth*
Derby Hospitals NHS Foundation Trust, Pathology, Derby, United Kingdom

During multiple myeloma (MM), when a paraprotein is co-migrating and therefore obscured by another protein, there is difficulty in quantification by protein electrophoresis which can affect patient monitoring. Here we looked at the relevance of using the HLC ratio in the monitoring of an IgGκ MM patient with a co-migrating monoclonal immunoglobulin (M-Ig) overlapping with β2-proteins. HLC analysis provides a
numerical result of both the involved and the uninvolved HLC which can be used to generate a ratio. This allows the quantification of the suppressed polyclonal Immunoglobulin of the same isotope as the tumour as well as accurate measurement of the paraprotein that is obscured by co-migration with other serum components. The ratio allows determination of monoclonality.

HLC ratio was measured retrospectively over a 16 month period using the Hevylite assay (The Binding Site SPA PLUS) and then compared with standard methods for monitoring MM (capillary zone electrophoresis (CZE), total Immunoglobulins and serum free light chains [sFLC]). The HLC ratio became abnormal and continued to rise indicative of a monoclonal progression. At this time there was no evidence of a relapse on the CZE. The HLC ratio indicated changes in M-Ig three months before detection by CZE was possible. The rise in M-Ig by CZE was accompanied by an increase in the sFLC ratio and total IgG.

In this case study the monitoring of HLC ratio would not have effected the management of the patient. However the HLC ratio can be a useful addition to standard techniques in the monitoring and early relapse detection of patients with MM, especially where the M-Ig co-migrates or is too small to quantify.

**Th50**

Development and validation of a candidate reference method for the measurement of serum cortisol using supported liquid extraction and UPLC-MS/MS

**J Hawley, L Owen, B Keevil**

*University Hospital South Manchester, Clinical Sciences-Biochemistry, Manchester, United Kingdom*

**Background:** Accurate and precise measurements of cortisol are required to better diagnose and treat disorders of adrenal function. Cortisol measurements have been reported to be inaccurate and show high variability, especially in patients with high concentrations of cortisol binding globulin. We present a candidate reference method for the measurement of serum cortisol that utilises certified reference material to provide results that are traceable to the International System of Units.

**Method:** An isotopically labelled internal standard, cortisol-d4, was added to serum and allowed to equilibrate prior to supported liquid extraction (SLE). Extracted samples were reconstituted and analysed using ultra-performance liquid chromatography coupled to a tandem mass spectrometer with electrospray ionisation in positive ion mode. Multiple reaction monitoring was used to detect a cortisol quantifier (m/z 363>97), qualifier (m/z 363>121) and the corresponding internal standard transitions (m/z 367>97 and 367>121 respectively). Each sample was measured in triplicate over three days and a mean result calculated.

**Results:** The lower limit of quantification was determined to be 15 nmol/L and the recovery of spiked cortisol ranged from 99-104%. A mean within-batch CV of 1.77% and between-batch CV of 2.13% was achieved. Analysis of structural analogues of cortisol and ion suppression studies confirmed no interferences. The accuracy of the method was determined through the measurement of 34 certified European Reference Materials (83-764 nmol/L) provided by the Institute for Reference Materials and Measurements. The results obtained all agreed to within <4.2% of the certified values.

**Conclusion:** The high accuracy and precision of this method qualify it as a candidate reference method that can serve to provide measurement traceability. It has been applied locally to target-set calibration and internal quality control materials and can be used to underpin external quality assessment schemes to provide samples with a traceable target value.

**Th51**

Method verification study and transfer of serum kappa and lambda Freelite assay from Roche Cobas c502 to SPA-PLUS analyser

**SK Moore, AS Everitt, S Madhusudhanan, S Whiting**

*Basildon Hospital, Clinical Biochemistry, Essex, United Kingdom*

**Introduction:** The Binding Site (TBS) Freelite assay was run on the Roche Cobas c502 (Cobas) analyser at Basildon. In 2013, four patients were found to have inconsistent results. Subsequent investigations were unable to explain these flyers, and a decision was made to transfer the assay to SPA-PLUS analyser (developed exclusively for TBS specialist protein assays to ensure optimal assay performance).

**Aims:** To undertake a method verification of Freelite assay on the SPA-PLUS and to compare results with Cobas.

**Method:** Method verification included assessment of imprecision using low and high liquid quality control (LQC) levels from TBS and Randox, assessment of bias against Cobas using 48 patient serum samples, NEQAS & TBS EQA material and Freelite Panel with spiked samples, and recovery studies.

**Results:** Within-run and between-run imprecision (CV) for kappa low (16.2 mg/L) and high (31.0 mg/L) LQC were 2.10% and 1.40%, and 3.20% and 2.41% respectively. Within-run and between-run imprecision for the lambda low (31.2 mg/L) and high (60.0 mg/L) LQC were 1.00% and 1.00%, and 5.00% and 5.41% respectively. Patient studies showed no significant bias at levels ≤50 mg/L for kappa and ≤70 mg/L for lambda.
However, significant differences between methods were found at higher values due to differences in dilution protocols. EQA and spiked serum sample analysis showed very good correlation with target means for SPA-PLUS user group. Recovery studies showed an almost linear recovery for both Kappa and Lambda on SPA-PLUS.

Conclusions: Method verification of SPA-PLUS showed good precision and agreement with EQA. However, patient results at higher values showed significant differences between the two methods. Therefore, in previously monitored patients, with a serum Kappa of >50 mg/L or a Lambda of >70 mg/L as measured on the new SPA-PLUS, samples will also be analysed by Cobas to provide dual reporting for six months.

Th52

Development of a novel microbial biosensor system modified by single wall carbon nanotube-gelatin-glutaraldehyde immobilization method and its adaptation to the determination of dopamine

E Bayram, E Canbay, C Güvenç, I Yaşa, E Dinçkaya, E Aksylimaz
Ege University, İzmir, Turkey

In this project, a novel single walled carbon nanotube (SWCNT) -gelatin-glutaraldehyde (GA) modified Candida tropicalis based microbial biosensor was developed which brings a new and original perspective to biosensor technology intended for dopamine determination. The microbial biosensor was formed with SWCNT and gelatin on a glassy carbon electrode (GCE) surface. The non-covalently gelatin-carbon nanotube (CNT) possessed and improved solubility in aqueous solution and showed an enhanced biocompatibility with Candida tropicalis. GCE/gelatin-CNTs/Candida tropicalis modified system was formed by dropping a mixture of SWCNT-gelatin aqueous solution on surface of GCE and then Candida tropicalis was immobilized with dropping modified GCE and cross-linking with GA. Dopamine measurements were done at between (-0.2)-(0.5) V potentials with differential pulse (DP) method by the developed biosensor system. The measurements were carried out with the determination of increasing current values directly proportional to dopamine concentration using DP method. In the optimization studies of the biosensor, firstly bioactive layer components such as optimum SWCNT-gelatin rational concentration, optimum Candida tropicalis amount and optimum GA concentration, were carried out. In the optimization of working conditions; optimum pH and optimum temperature were also investigated. Afterwards, in the characterization studies of the biosensor some parameters such as linearity, reproducibility, substrate specificity, interferent effects of some compounds and storage stability were determined. From the result of the optimization studies, the SWCNT-gelatin rational concentration, optimum Candida tropicalis amount and optimum glutaraldehyde concentration were determined to be 1:2 ratios, 10 mg/ml, and 0.5%, respectively. From the experiments, the phosphate buffer (PBS) (50 mM, pH 7.0) and 35°C were chosen to be optimum working conditions for the microbial biosensor. The characterization studies on the biosensor revealed linear curves between the ranges of 2.5 μM to 250 μM dopamine concentration.

Th53

Intralaboratory competition of immunological test in clinical chemistry

R Findeisen1, A Frister1, J Steglich1, B Pätzcke1, S Neumann1, C Günther1, K Borgwardt1, C Hintze1, H Welle-Scharmann1
1Oberlausitz-Kliniken gGmbH, Institute for Laboratory Diagnostic, Microbiology and Hospital Hygiene, Bautzen, Germany; 2Hochschule Zittau-Görlitz, Zittau, Germany; 3DiaSorin Deutschland GmbH, Dietzenbach, Germany

Introduction: Quantitative automated luminometric immunoassays for the determination of Cancer Antigen 125 (CA125), Alpha-Fetoproteine (AFP), Total Prostate-Stimulating Antigen (t-PSA), Free Prostate-Stimulating Antigen (f-PSA), Ferritin, Prolactine, Parathormone (PTH), Luteinizing hormone (LH), Follicle Stimulating hormone (FSH) were compared with a Architect i2000SR (Company: ABBOTT GmbH & Co. KG Max-Planck-Ring 2, Wiesbaden, Germany) and a LIAISON XL (Company: DiaSorin Deutschland GmbH Von-Hesey-Str. 3, Dietzenbach, Germany).

Material and methods: The commercially available luminometric immunoassays were performed as described by the manufacturer. The luminometric immunoassays are monoclonal two-site immunoluminometric methods (sandwich principle). Antibody-coated tubes serve as solid phase. The light signal is directly proportional to the concentration of the analyte of interest. The whole measuring procedure consists of pipeting of samples, incubation periods, washing cycles, and measurements. Twenty patient samples for each method have been measured parallel at ARCHITECT i2000SR [Comp.: ABBOTT GmbH & Co. KG Max-Planck-Ring 2, Wiesbaden, Germany] and at LIAISON XL [Comp.: DiaSorin Deutschland GmbH Von-Hesey-Str. 3, Dietzenbach, Germany]. Pearson’s Correlation Coefficient and Passing-Bablok-Correlation were used to evaluate the results mathematically.

Results: Passing-Bablok-Regression and Pearsons Correlation Coefficient (R) were calculated for the following methods: t-PSA (Y = 0.9489x + 0.034; R=0.99997); fPSA (Y = 0.6225x + 0.1835; R=0.9745); AFP (Y = 1.1307x + 0.1626; R=0.99423); Ferritin (Y = 0.842x + 53.124; R=0.93395); Prolactine (Y = 0.7017x + 1.3913; R=0.99978); PTH (Y=0.6225x + 0.1835, R=0.9915); LH (Y=1.2019x + 0.679; R=0.9824); FSH (Y=1.265x + 1.2344, R=0.8875); CA125 (Y=1.1379x + 1.6201; R=0.999624).

Conclusions: The results showed a high degree of correlation and regression. The immunoassays at LIAISON XL can be accurately measured using the automated method which can be recommended.
Th54

25 hydroxy vitamin D (25(OH)D): comparing methodologies

AT Scacchetti, C Rota, E Cariani, D Debbia, E Baraldi, T Trenti
Azienda USL, NOCSAE, Dipartimento di Medicina di Laboratorio, Modena, Italy

Background: As we seek to understand the importance of newly discovered roles for vitamin D in health and disease, the accurate and precise measurement of 25(OH)D in blood is essential. Given the wide range of methodologies available, the choice of which one is the best is difficult especially given the dramatic increase, over the last few years, of both routine clinical and research uses of the measurement. Lack of standardization between the different methods can lead to different results.

For this reasons, we measured total 25(OH)D levels by 3 different immunoassays analyzers and compared them with HPLC method.

Methods: The total 25(OH)D levels of 126 healthy subjects were analyzed simultaneously with: Liaison from DiaSorin (Italy), Architect ci8200 from Abbott (IL, USA) and Access DxI 600 Unicel (Beckman Coulter, USA). Determination of both 25(OH)D2-D3 was assayed by HPLC UV employing a commercially available kit.

2-tailed test was employed for statistical analysis, correlation between different assays was assessed by Bland-Altman plot.

Results: The total 25(OH)D mean value obtained by the immunoassay methods vs HPLC was significantly different (p<0.0001); also the immunoassay methods showed significant differences: Abbott vs DiaSorin (p<0.0011); Abbott vs Beckman Coulter (p<0.0464). We did not find any significant difference between Beckman Coulter and DiaSorin.

Bland-Altman plot showed: Abbott vs Beckman: Bias=-1.120, SD of Bias=6.52; Abbott vs DiaSorin: Bias=1.313, SD of Bias=3.330; Abbott vs HPLC Beckman: Bias=1.837, SD of Bias=4.224; Beckman vs DiaSorin: Bias=-0.1922, SD of Bias=5.178; Beckman vs HPLC: Beckman: Bias=-2.957, SD of Bias=5.119; DiaSorin vs HPLC Beckman: Bias=-3.349, SD of Bias=3.600.

Conclusions: There are significant differences in the specificity of 3 immunoassays methods. It would be interesting to study the possible impact of these differences in patients.

The total 25(OH)D level immunoassays method by Architect ci 8200 correlates very well with HPLC Beckman.

Th55

Does the separator gel contained in blood collection tubes interfere with mass-spectrometry quantification of steroids?

L Roli1, S Belli2, MC De Santis1, E Baraldi1
1AUSL Modena, Dipartimento Interaziendale Integrato Medicina di Laboratorio ed Anatomia Patologica, Modena, Italy, 2AUSL Modena, Dipartimento Integrato di Medicina, Endocrinologia, Metabolismo e Geriatria, Modena, Italy

Background: Many scientific evidences demonstrated the interfering effects of different blood collection tubes on a variety of laboratory assays, but only few authors investigated the interferences on LC-MS/MS-based laboratory tests; recently the negative effects on measurement of testosterone have been described.

Objective: To evaluate if blood collections tubes used in our hospital generate interference on LC-MS/MS steroid assay.

Methods: Waters TQD LC-MS/MS; Perkin Elmer Multiplexing LC-MSMS Steroid Kit. MS/MS conditions: Source: ESI; Polarity (+) ion mode, MRM mode. 11 samples were double collected with (G) and without gel (NoG) separating tubes (Vacutest KIMA s.r.l. Italy), after separation serum samples were immediately stored at -20°C. Statistical analysis: ordinary least squares analysis (LF) at the 1% significance level.

Results: The LF analysis accepted the equivalence at the 1% significance level of the serum concentrations of Androstenedione, Cortisol, DHEAS, Progesterone and 17OHP in samples collected with and without gel separating collection tubes, while the null hypothesis was rejected in favour of the alternative one for Testosterone [Measuring Interval: G=0.15-8.64 ng/ml; NoG=0.13-6.00 ng/ml; r=0.969. LF analysis: intercept=0.25 (95%CI=-0.09-0.59) p=0.135; slope=0.77 (95%CI=0.65-0.89) p=0.001].

Conclusions: The results of this experience, in agreement with other evidences recently published, suggest that the separator gel may affect the mass transitions used in testosterone quantification, accordingly the validation of any LC-MS/MS assay used in the clinical diagnostic laboratory should assess the potential interference of blood collection tubes.

Th56

Effect of serum-clot contact time on acyl carnitines and amino acids detected by mass spectrometry

A Angiolillo, A Cristofano, N Sapere, A Di Costanzo
University of Molise, Department of Medicine and Health Sciences, Campobasso, Italy

Variation in serum biochemical values can be due to preanalytical, analytical or biological factors. Thanks to significant advances in technology, the analytic variability has been reduced to acceptable levels, which do not cause any impact on the clinical interpretation of the results. The contribution of preanalytical variability is nowadays the major issue for the reliability of the
whole testing process: collection and handling of samples, as well as their transport and storage, can have an impact on the outcome of the test. To identify real pathophysiologic results in patients, artefacts should be reduced to minimal levels.

Changes in serum biochemical values as a function of serum-clot contact time have already been investigated for several analytes commonly used in routine laboratory tests.

Metabolomics is increasingly being used in a variety of health applications, including pharmacology, pre-clinical drug trials, toxicology, transplant monitoring, newborn screening, clinical chemistry. This approach enables a specific quantitative description of the low molecular-weight endogenous metabolites, providing a metabolic “fingerprint” of organisms.

In this study we investigated serum variation of 57 metabolites, using mass spectrometry. Venous blood was collected from fourteen healthy people using vacutainer tubes and centrifuged at different times. Serum samples were spotted on filter paper after centrifugation of the blood at 0h, 2h and 6h. The metabolites (36 acyl carnitine and 21 amino acids) were measured by electrospray tandem mass spectrometry, according to conventional validated method, using an API 4000 AB Sciex triple quadrupole. The analyte concentrations were calculated automatically using the software Chemoview. Results showed that the concentration of some metabolites, especially amino acids, is affected by a prolonged contact with the clot. Particularly aspartate, glutamate, phenylalanine, methionine, glycine and ornithine showed a linear increase of concentration with time. This finding suggests that a maximum of two hours from collection to separation would be desirable.

**Point of Care Testing**

**Th57**

Can the glucose tolerance test be performed by point of care testing? A comparison in cystic fibrosis patients

J Donovan1, JC Burgess2, H Berry1, KM Gyi1, S Collins2, D Bilton2, NJ Simmonds1

1Royal Brompton Hospital, Clinical Biochemistry, London, United Kingdom, 2Royal Brompton Hospital, Department of Cystic Fibrosis, London, United Kingdom

**Background:** Annual assessment of glucose tolerance in cystic fibrosis (CF) by the oral glucose tolerance test (oGTT) is required by the European CF registry. Additional venepuncture is distressing for some CF patients, and immediate access to results could expedite changes to patient treatment and ease anxiety. A point of care testing system for measuring glucose in capillary samples was evaluated.

**Methods:** A prospective study of consecutive clinically stable patients undergoing OGTT as part of their annual review was performed (June 2013-April 2014). All patients had simultaneous venous (fluoride oxalate) and capillary blood sampling before (fasting = 0 min) and 2 hours (120 min) after a glucose 75g solution drink. Capillary glucose was analysed immediately using the HemoCue 201 RT™ system and venous plasma glucose was analysed on the Beckman DxC600 autoanalyser (Beckman Coulter, High Wycombe, Buckinghamshire, UK).

**Results:** 70 patients agreed to participate. The HemoCue showed good reproducibility on control samples at 3 concentrations (n= 70, CV < 5%). However correlation of capillary and plasma glucose at 0 and 120 minutes was poor. Fasting capillary and plasma glucose are expected to be equivalent but differences of between -1.5 and 0.8mmol/L were found, with no correlation with concentration (r=0.17); at 120 minutes, capillary glucose would be expected to be up to 1 mmol/L higher than plasma, however the differences ranged between -2.9 and 0.7 mmol/L with no correlation with concentration (r = 0.03). Sensitivity and specificity for diabetes were 87.5% and 98.4% respectively, with 7 of the 8 patients with diabetes detected by the HemoCue results.

**Conclusion:** The Hemocue 201 RT system is not suitable for the diagnosis of diabetes by capillary blood sampling in CF.

**Th58**

Evaluation of three point-of-care cardiac troponin assays for use in an emergency department low-risk chest pain pathway

JR Bailey1, T Harris2, B Bloom3, SC Benton1

1Barts Health NHS Trust, Clinical Biochemistry, London, United Kingdom, 2Barts Health NHS Trust, Emergency Department, London, United Kingdom, 3Surrey Pathology Services, Royal Surrey County Hospital NHS Foundation Trust, Guildford, United Kingdom

We set out to evaluate the analytical performance of 3 point-of-care-testing (POCT) cardiac Troponin (cTn) assays. We aimed to develop a chest pain assessment pathway for our 3 Emergency Departments (ED’s) using history, examination, ECG and cTn. This would consolidate 3 previous pathways that included myoglobin, creatine kinase and cTn (POCT and laboratory).

Two POCT devices covering three cTn assays (Abbott i-STAT cTnI, Radiometer AQT90 FLEX (AQT90) cTnI and AQT90 cTnT) were evaluated. Emphasis was placed on imprecision (%CV) around the reference population 99th-percentiles to ensure compliance with European Society of Cardiology Guidelines (ESCG) for acute coronary syndrome (< 20% acceptable; < 10% good).
The 99th-percentiles are 17ng/L (AQT90 cTnT), 23ng/L (AQT90 cTnI) and 80ng/L (Abbott i-STAT). Imprecision at these levels was 17% and 12% respectively for the AQT90 assays. More samples are required to confirm i-STAT 99th-percentile imprecision; current results indicate it is < 20%. Imprecision, determined across the analytical ranges using patient samples (n=19) measured 10 times, was < 10% at higher concentrations for all assays. All assays demonstrated linearity (r²>0.98). Accuracy was confirmed measuring EQA samples (n=6). Patient results were compared with Roche’s hs-cTnT to evaluate clinical status concordance with the current laboratory method. Relative sensitivities and specificities, using 99th-percentile values, were 67% and 100% respectively for AQT90 cTnT; 39% and 100% for AQT90 cTnI; 80% and 100% for i-STAT cTnI.

All assays were acceptable for use according to ESCG requirements. Sensitivities and specificities suggest all are suited to ruling-out rather than ruling-in ACS. A clinical pathway has been introduced using AQT90 cTnI. cTnI is measured upon arrival in ED. A second sample is drawn for i-STAT cTnI. All assays were acceptable for use according to ESCG requirements.

We conclude that the availability of results is not a rate limiting step influencing LOS, and suggest that significant patient pathway re-design needs to be carried out to improve this measure; POCT should only be used if this is essential to facilitate such re-design.

**Th59**

**Point of care testing has no effect on patient length of stay in an accident and emergency department**

*L Jama¹, M Piracha¹, L Jones², PJ Waller³*

¹Kingston University, School of Life Sciences, Faculty of Science Engineering and Computing, Kingston upon Thames, United Kingdom, ²Kingston University, Faculty of Science Engineering and Computing, Kingston upon Thames, United Kingdom

The use of point of care testing (POCT) has increased over recent years due to the availability of robust and portable analytical equipment. POCT can usually provide diagnostic test results more rapidly than central laboratories, and it is generally assumed that this will improve patient outcomes. This project aimed to implement a point of care service for the analysis of full blood count, urea and electrolytes in an Accident and Emergency department, and to evaluate the impact of this service on patients’ length of stay (LOS).

The study was conducted over a 6-month period, during which 1106 patients had investigations carried out by the POCT service. Patient LOS was compared with that seen for 6035 patients seen during the same period whose investigations were carried out by the central laboratory. Patient LOS was also calculated for patients with three common presenting complaints; chest pain, abdominal pain and shortness of breath. The POCT analysers (Sysmex XS-1000i, and Abbott i-STAT) were linked to the hospital network to ensure immediate availability of validated results. Mean test turnaround time was significantly shorter for point of care devices compared to the central laboratory [24 minutes vs 63 ± 24 minutes, p = 0.000]. The LOS for patients receiving POCT was not significantly different from those receiving central laboratory testing (median(interquartile range); 208 minutes (161-233) vs 223 minutes (173-138), p = 0.439). There was also no significant difference in LOS for patients with different presenting complaints.

We conclude that the availability of results is not a rate limiting step influencing LOS, and suggest that significant patient pathway re-design needs to be carried out to improve this measure; POCT should only be used if this is essential to facilitate such re-design.

**Th60**

**Analytical evaluation of POCT HbA1c instruments**

*SG Phillips, Y Nwagbo, K Ashton*

Royal Liverpool & Broadgreen University Hospitals NHS Trust, Biochemistry, Liverpool, United Kingdom

**Background:** The performance of POCT HbA1c instruments was investigated to assess their suitability for use in monitoring HbA1c in known diabetics. The following POCT instruments were included in the study:

1. *Quo-Test A1c* (boronate affinity), BHR, Warwickshire, UK.
3. *Afinion™ AS100* (boronate affinity), Alere, Cheshire, UK.

**Method:** Routine laboratory and diabetic clinic patient samples (N = 39-80) were analysed once on the routine laboratory method (Menarini, HPLC ion exchange) and once on the POCT instruments. The results from each POCT instrument were compared to the result from the laboratory method. Within-run precision was evaluated using repeat analysis (N=5) of patient samples at three different HbA1c levels. EQA samples at two different levels were also analysed on each instrument and the results compared to the target reference value. The % total error (TE =%bias + 1.96x%CV) was calculated for each instrument.

**Results:** All instruments displayed acceptable correlation the laboratory method (r = >0.97). The Hemocue showed an overall slightly positive bias (4.4 mmol/mol 95% limits of agreement -7.3 to 16.2) and the B Analyst an overall slight negative bias (-3.4 mmol/mol 95% limits of agreement -11.1 to 4.3)
The Affinion and Quo Test displayed no significant bias (0.6 mmol/mol and 0.7 mmol/mol respectively). Precision for the Affinion and Hemocue was < 5%, the Quo Test was < 3% and the B analyser was > 5% across the range tested (~36-100 mmol/mol).

**Conclusion:** Both the Affinion and Quo Test displayed acceptable analytical performance for monitoring HbA1c in known diabetic patients (TE = ~5% at 50 mmol/mol and 90 mmol/mol for both instruments). However, any difference in performance between different reagent lot numbers and the effect of haemoglobin variants was not investigated.

**Th61**

An external quality assessment scheme for pre-term labour markers

**MA Thomas, J Morris, S Jones**
Cardiff and Vale University Health Board, WEQAS Quality Laboratory, Cardiff, United Kingdom

**Introduction:** Preterm delivery is a serious complication of pregnancy and is responsible for the majority of non chromosomal prenatal morbidity and mortality. New Point of Care tests are now able to aid in assessing the risk of preterm delivery in symptomatic and asymptomatic patients with a previous history of premature delivery. Foetal Fibronectin (fFn) is not normally present in amniotic fluid between 22 and 35 weeks of gestation and an increased fFn of >50 ng/ml indicates an increased risk of likelihood of a preterm birth within the following 14 days. The negative predictive value (NPV) of the test at 10ng/ml is quoted as 100%, with a NPV at 50ng/ml of 99.2%.

The aim of the study was to develop stable material suitable for assessing the analytical performance of the Point of Care fFn test.

**Pilot design:** Forty two sites agreed to take part in the pilot. The samples were prepared from a source of human fFn added in a stable protein matrix and stored at -20 °C until dispatch. Twenty two samples were distributed from January 2013 to March 2014. For the short term stability study, a positive pool, mean 143 ng/ml was stored at +20 °C and +4 °C and assayed in duplicate over a period of 14 days. Long term stability was assessed from the reported overall mean data for 3 pools, mean 52, 146 and 310 ng/mls over a 8 month period.

**Results:** There was no significant deterioration in the results for both short and long term stability at the three temperatures. The inter-site variation (CV) of 8.4%, 11% and 16% was observed at concentrations of 57.7ng/ml, 56.8ng/ml and 323.4ng/ml respectively. The study is now being extended to include other pre-term labour markers such as ph IGFBP-1 and offered to all users.

**Th62**

Point of care ketone testing in a multi-site teaching hospital: use and misuse

**MG Flenley, R Allcock, J Armer**
Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom

Point of Care ketone tests are widely used as a convenient and rapid means of β-hydroxybutyrate quantitation in patients with suspected or resolving ketoacidosis. Measurements are carried out on hand-held blood glucose meters, using test strips containing β-hydroxybutyrate dehydrogenase, which typically cost between 5-10 times more than glucose test strips. Judicious use is required to prevent unnecessary expenditure and ensure accurate interpretation of ketone results.

Following the release of a new diabetic ketoacidosis protocol in our trust, we audited the use of ketone test strips with Abbott Precision Xceed Pro meters over two months (Jan-Feb 2014), using data extracted from the Abbott PrecisionWeb database. A total of 1626 ketone measurements performed across 17 locations were analysed. Of these, 921 were controls and 705 were performed on patients. The following standards were applied:

1. NHS Number should be entered for all patient tests. This was entered for only 38% which was attributed to poor entry of patient ID in emergency departments, where 61% of all ketone tests are performed.
2. Control Tests must be performed as stated in the protocol. Some areas were found to be performing a four-fold excess of control tests.
3. Testing should be limited to patients at risk of developing ketoacidosis with a blood glucose >11mmol/L. 32% of patients were non-insulin-dependent and 15% of tests were performed in patients with a blood glucose < 11mmol/L.

Following the audit, we liaised with specialist nurses to educate staff about risk factors for ketoacidosis and adjusted instrument settings to reduce unnecessary control testing.

This study highlights the risk of test misuse in a point-of-care setting where the cost of a test and indications for testing can be forgotten. The role of vigilant service-management in ensuring effective and efficient use of tests beyond the confines of the hospital laboratory is emphasised.
Clinical audit of urine dipstick practice at Salford Royal Foundation Hospital

C Portsmouth, D Darby
Salford Royal Foundation Hospital, Clinical Biochemistry, Salford, United Kingdom

Introduction: Current national guidelines recommend the use of urine dipsticks to measure a number of parameters in a variety of clinical situations. However, there must be a high level of training for those manually reading urine dipsticks to avoid false positives and negatives. For this reason we decided to investigate the performance of those carrying out manual urine dipstick analyses here is Salford Royal Foundation Trust (SRFT).

Methods: In total, 25 individuals across 14 different wards at SRFT recorded the values they assigned different parameters when manually reading Siemens Multistix 8SG urine dipsticks following immersion in negative and positive quality control solutions (Siemens Chek-Stix). Results were then compared with results generated using an automated urine dipstick reader (Siemens Clinitek Status+).

Results: There was strong agreement between both manual and automated analysis of most analytes for the negative QC material, with the exception of pH and SG. With the positive QC material it was observed that there was poor manual reading of glucose, protein and leukocytes, pH and SG. It was also observed that none of the users adhered to the timings at which different parameters should be read; with all the users reading the results off the dipstick in the wrong order.

Conclusions: These findings demonstrate that many of the results currently generated by manually reading urine dipstick are unreliable. This has the potential to result in either unnecessary investigations or a false reassurance and subsequent lack of necessary investigations, both of which have a negative impact on the care of patients.

Recommendations: It was recommended that mandatory training and competency assessment on the use of urine dipsticks must be implemented as a priority and consideration given to the introduction of automated analysers for high volume users.

Evaluation of the Abbott i-STAT for use in monitoring of peritoneal dialysis patients

ND Elkin1, E Leach1, S Fan2, SC Benton3
1Barts Health NHS Trust, Clinical Biochemistry, London, United Kingdom, 2Barts Health NHS Trust, Nephrology, London, United Kingdom, 3Royal Surrey County Hospital NHS Trust, Surrey Pathology Services, Guildford, United Kingdom

Peritoneal equilibration testing (PET) is used for monitoring peritoneal dialysis (PD) patients to assess solute clearance and ultrafiltration. Sodium, urea, creatinine and glucose are measured in PD fluid at 0, 2 and 4 hours following instillation of dialysis fluid, with a paired blood sample at 2 hours. Samples are currently analysed in the laboratory with patients attending hospital for the entire 4 hours. Point of care testing (POCT) in the community for PD monitoring would improve the patient pathway, reducing the time patients spend at hospital. To our awareness there are currently no POCT devices that are validated for analysis of PD fluid. Our aim was to evaluate the Abbott i-STAT handheld POCT device for measurement of sodium, urea, creatinine, and glucose in PD fluid to assess its potential for future use in POCT PD monitoring. Patient PD fluid samples were used for the evaluation. A preliminary method comparison with laboratory methods on the Roche Cobas analyser demonstrated acceptable correlation for urea, and sodium (n=56); the bias was -0.9, and 0.71 respectively. Further work is being undertaken to investigate glucose and creatinine. Urea, sodium, creatinine, and glucose methods demonstrated good between day precision (CV < 5%) and within day precision of < 10%. Analyte stability was assessed by repeat measurement over 5 days with a final result obtained on day 8. PD fluid showed no clinically significant variation in creatinine or urea measurement at 3 different levels across 8 days following storage at room temperature and in the fridge (4-8°C). Further work is being carried out to assess glucose and sodium stability. This preliminary work suggests that the Abbott i-STAT has potential to be used for PD fluid analysis and to improve the PD monitoring patient pathway. Further studies, including a clinical evaluation, will be carried out to assess this.

Comparison of point of care haemoglobin A1c meters

H Berry, G Hall, J Donovan
Royal Brompton and Harefield NHS Trust, Clinical Biochemistry, London, United Kingdom

Background: Our point of care (POC) committee was approached by 2 separate clinics on our Royal Brompton site about the possibility of having Haemoglobin A1c (HbA1c) measurement at the point of care. Firstly the pre-admissions clinic uses HbA1c as part of their ‘fit for surgery’ algorithm. A result whilst still at the hospital would allow the patient to be informed of the outcome before they leave. Secondly the Cystic
Fibrosis clinic uses HbA1c to guide dietary advice given to patients. A result in the presence of the patient would allow appropriate advice to be given immediately.

**Methods:** We evaluated the POC HbA1c meters currently available on the market, for ease of use in the clinic setting, time to result and a comparison against our current laboratory method an ion-exchange high-performance liquid chromatography method on the D10 analyser (Biorad, Hemel Hempstead, UK).

The devices compared were:
- Cobas b101 (Roche, Burgess Hill, UK)
- HbA1c 501 System (HemoCue, Angelholm, Sweden)
- DCA Vantage (Siemens, Camberley, Surrey)
- Affinion (Alere Ltd, Cheshire)
- In2It (Biorad, Hemel Hempstead, Hertfordshire)

**Results:** Samples in the concentration range 29-131mmol/mol were used for the comparison. Altman Bland graphs were plotted. Roche, Hemocue, Siemens and Biorad devices all showed acceptable agreement with the lab. The average differences (device-lab method) were Roche =$-1.5$mmol/mol (n =17), Hemocue = $1.5$mmol/mol (n=49), Siemens = $2.2$mmol/mol (n=45) and Biorad = $-1.2$mmol/mol (n=14). The Alere device showed a negative bias in comparison to the laboratory method with mean difference $= -4.2$mmol/mol (n=49). The Siemens device came highest in user evaluation taking into ease of use, time to result, reagent storage and method of user identification.

**Conclusion:** In conclusion the Siemens DCA is the most suitable for us as it showed acceptable agreement with our laboratory method and scored highest in our user evaluation.

**Th66**

The impact of introducing point of care testing for renal function on the time patients spend in the emergency department?

**PR Jarvis**1,2, **KL Mitchell**3, **M Davies**4

1Calderdale & Huddersfield NHS Foundation Trust, Department of Emergency Medicine, Halifax, United Kingdom, 2Clinical Associate of the Lean Enterprise Academy, Goodrich, United Kingdom, 3Calderdale & Huddersfield NHS Foundation Trust, Blood Sciences Department, Huddersfield, United Kingdom, 4Calderdale & Huddersfield NHS Foundation Trust, Department of Emergency Medicine, Huddersfield, United Kingdom

**Background:** Overcrowding in the Emergency Department (ED) is a common phenomenon. The aim of this study is to quantify the impact of introducing Point of Care Testing (POCT) for renal function on the length of time patients spend in the ED.

**Methods:** A prospective, observational study was conducted in an Emergency Department in a District General Hospitals in the United Kingdom. The study consisted of two phases. Patients attending the ED during Phase 1 had their renal function (Na+, K+, Urea and Creatinine) investigated using the hospital’s centralised laboratory analyser (Advia® 2400 Chemistry System (Siemens Healthcare Diagnostics Ltd, Camberley, UK)). Phase 2 patients requiring renal function analysis were investigated in the ED using the’CHEM 8+’ cartridge (Na+, K+, Urea, Creatinine, Glucose, Chloride & Calcium) and the Abbott iSTAT POCT device. The time from patient arrival in the ED to the time their care was complete and the patient was ready to move onto the next destination of care was recorded.

**Results:** 10868 patients attended the ED during the whole study period. Phase 1 contained 3835 patients. 7033 patients were recruited into Phase 2. Not all patients attending the ED during the trial period had their renal function analysed. The median time for patients to be declared ready to leave the ED in phase one was 129 minutes (Interquartile range [IQR 110-139]) compared to 109 minutes (Interquartile range [IQR 86-128]) for phase two (p = 0.0025).

**Conclusions:** This study demonstrates that using POCT for renal function in the ED was significantly quicker than using a centralised hospital laboratory. The use of a bedside POCT device enables clinicians to make informed clinical decisions in a more timely manner. This research was supported by a grant from Abbott Point of Care.

**Th67**

How does the Abbott i-STAT CHEM 8+ cartridge compare to the laboratory Siemens ADVIA 2400 assays?

**L Tooth**1, **F Cossor**2, **G Griffiths**2, **P Collinson**1, **F Boa**1

1St George's Hospital, London, United Kingdom, 2Abbott Laboratories, London, United Kingdom

**Introduction:** The Abbott i-STAT is a portable handheld device that uses cartridge systems to quantify numerous analytes in whole blood. This study compared the Abbott i-STAT CHEM8+ cartridge (LOT:J13322) to the Siemens ADVIA 2400 assays. The aim was to introduce i-STAT devices into the Emergency Department at St George’s Hospital to improve the rapid assessment pathway for patients.
Th68

A comparison between POCT and laboratory urine drugs of abuse testing

J Armer, J Taylor, R Allcock, M Myers
Lancashire Teaching Hospitals NHS Foundation Trust, Clinical Biochemistry, Preston, United Kingdom

The community Drug and Alcohol Recovery Service Teams (DARST) perform urine drugs of abuse screens on their clients using the Concateno Drug Screen Test Cup (cocaine, amphetamine, methadone, morphine, benzodiazepines and buprenorphine). They also send urine samples to Clinical Biochemistry for drugs of abuse testing by immunoassay (opiates, methadone metabolite, benzodiazepines, cocaine and amphetamine) followed by confirmatory analysis using GCMS.

To investigate uncertainty in the point of care testing strategy, a preliminary study on 31 urine samples was undertaken to compare POCT and laboratory results.

Of the 31 samples, 23 were positive and 8 negative for morphine by POCT. The 8 negatives were confirmed by the Laboratory. However only 21 were confirmed as positive by the lab. 19 of the 31 samples were positive and 12 were negative for methadone. The 12 negatives were confirmed by the laboratory. Methadone and methadone metabolite were detected in only 17 samples by GCMS, and methadone only was detected in the other 2 indicating that methadone had been spiked into both samples. All positive and negative cocaine and amphetamine samples by POCT were confirmed in the laboratory. 6 of the 31 samples were positive and 25 were negative for benzodiazepines by POCT. The 25 negatives were confirmed by the Laboratory. However only 5 were confirmed as positive by the lab.

All POCT negative results were confirmed by the laboratory providing confidence that a negative POCT result reflects a true negative. However, there were false positive results for opiates and benzodiazepines and spiked methadone was missed using the POCT. These results indicate that positive opiates and benzodiazepines must be confirmed by the laboratory and POCT for methadone is not recommended as spiked samples are not detected. A more extensive study is being undertaken and changes to the diagnostic pathway will be recommended.

Th69

How does the Abbott i-STAT CG4+ cartridge compare to the radiometer ABL800 blood gas analyser?

L Tooth1, F Cossor2, G Griffiths2, P Collinson1, F Boa1
1St George’s Hospital, London, United Kingdom, 2Abbott Laboratories, London, United Kingdom

Introduction: The Abbott i-STAT is a portable handheld device that uses cartridge systems to quantify numerous analytes in whole blood. This study compared the Abbott i-STAT CG4+ cartridge (LOT: M13328) to the Radiometer ABL800 blood gas analyser assays. The aim was to introduce i-STAT devices into the Emergency Department at St George’s Hospital to improve the rapid assessment pathway for patients.

Method: The comparison included pH, pCO2, PO2 and Lactate. Preliminarily 25 arterial blood gas samples were run in duplicate on the i-STAT following analysis on the ABL800 blood gas analyser. A further 25 samples are to follow. Quality Control material at level 1 and level 3 was run 20 times for assay imprecision.

Results: Overall the i-STAT compared well with the Radiometer ABL800, although a significant bias was noted for the Lactate. Bland Altman plots showed a mean relative bias for pH (0.0%), pCO2 (-4.2%), pO2 (+2.2%), Lactate (+9.2%). Imprecision studies showed a CV below 10% for all assays.
**Conclusion:** Use of an i-STAT in the Emergency Department would allow results to be available within minutes. This would enable patients to be triaged more efficiently. Other issues to consider are connectivity to ensure the results are incorporated into the patient records and the cost of the device and cartridges.

**Quality Assurance**

**Th70**

**Determining measurement uncertainty and defining performance requirements in accordance with ISO15189:2012; an example methodology**

*MC Sleeman*

*Royal Berkshire NHS Foundation Trust, Clinical Biochemistry, Reading, United Kingdom*

Section 5.5.14 of ISO15189:2012 requires that the laboratory determines measurement uncertainty (MU) for the examination phase of procedures used for reporting measured quantity values. It also requires that the performance requirements for these uncertainty measurements are defined and regularly reviewed. A procedure was developed to routinely and simply meet these requirements for all assays which output a measured value.

Internal quality control (IQC) results were extracted from the laboratory quality control database (Biorad Unity) for an appropriate period to meet the requirements of intermediate precision conditions. For each analyte up to 200 results were included covering 3-4 months minimum (longer for less regularly run assays) covering changes in reagent and calibrator lots, operators and routine service / maintenance. The results were combined (taking into account any changes in IQC material lot) to provide an overall MU for each QC level. The most clinically relevant IQC level was chosen and, where appropriate, the effects of known and clinically relevant bias were incorporated using the RSSu (Phillips et al., J Res Natl Inst Stand Technol 1997) or Ue (Synek, Talanta 2005) methods to generate the 95% confidence interval for the result (expanded MU with coverage factor of 2).

Performance requirements were defined using biological variation goals based on minimum, desirable and optimum performance following the methodology of Fraser et al. (Ann Clin Biochem 1997). Where biological variation data was unavailable, the Royal College of Pathologists of Australasia allowable limits of performance were used. Laboratory determined performance criteria were used for remaining analytes. All data was incorporated into a heavily customised Microsoft Excel spreadsheet facilitating regular update, review and communication to users while protecting key data from inadvertent modification.

**Th71**

**Evaluation of technopath controls on the ARCHITECT family of instruments**

*D Brault*, A Croce*, L Lemnartz*, M Orth*, J Shih*

*1Hopital Tenon, Service de Biochemie, Paris, France, 2Ospedale Civile Sondrio, Sondrio, Italy, 3Abbott Laboratories, Delkenheim, Germany, 4Vinzenz von Paul Kranken, Marienhospital, Institut für Laboratoriumsmadizin, Stuttgart, Germany, 5Abbott Laboratories, Global Scientific Affairs, Abbott Park, United States*

**Introduction:** Quality controls ensure that results generated by laboratories meet the required quality in regard to accuracy and precision. The use of multi-constituent controls is an important trend in laboratories to simplify QC testing by consolidation of controls.

**Objective:** The goal was to evaluate the performance of the Technopath Multichem S Plus, Multichem IA Plus and Multichem U controls on the ARCHITECT instruments. Precision and accuracy compared to the target value were evaluated.

**Methods:** Three European sites evaluated the controls in parallel with their routine QC on the ARCHITECT instruments. Data are from the following clinical chemistry analytes: ALT, AST, total bilirubin, chloride, total cholesterol, creatinine, glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassays: CA 19-9, CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, testosterone, estradiol and FSH; and on the following urine assays: chloride, creatinine, glucose, potassium, sodium and urea. Means, standard deviations and ranges were calculated for all controls.

**Results:** The results from the clinical chemistry assays were evaluated. %CV with the Multichem S Plus control ranged from 0.42 to 4.71% at the individual sites. The %CV for the 6 assays with the Multichem U control ranged from 0.50 to 5.24% at the individual sites. For both controls, the majority of the CVs were less than 2%. The results from the 11 immunoassays were analyzed and the %CV with the Multichem IA Plus control

Unauthenticated
Download Date | 9/23/18 7:27 PM
ranged from 1.82 to 14.94% at the individual sites; however the majority of the CVs were less than 5%. Overall little variation was seen instrument to instrument, site to site or reagent lot to reagent lot.

Conclusions: The use of these MCCs reduced the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise on quality.

Th72

Don’t monkey around with paper, bring your competency assessments into the 21st century

PJ Smith, SL Reynolds
ABMU Health Board, Laboratory Medicine, Swansea, United Kingdom

Competence assessments are used in the Laboratory Medicine Department to assess whether Biomedical Scientists/Associate Practitioners possess the skills and knowledge that are required to perform analytical tasks or processes to minimum standards.

Competence assessments have historically always been administered in a paper format. The main disadvantage to this method is that everyone receives the same assessment; this can lead to plagiarism and therefore mask the need for further training.

In August 2013 it was decided to use “Survey monkey” to deliver this new form of competence assessment. This was trialled in the automated immunoassay section which uses a Roche Diagnostics E-170 analyser. Using Survey Monkey, the assessment can be altered to reflect each individual’s strengths and weaknesses. The assessment is sent via a unique web link; this, along with modification of individual assessments reduces the chance of staff being made aware of the questions and answers.

All areas of automated immunoassay can be tested: maintenance, calibration, internal quality control, external quality assessment, analytical process, interference issues, health and safety, outstanding work list and troubleshooting.

The aims of tailor made competence assessments using Survey Monkey are to give individuals confidence in their job, improve efficiency of processing work flow and identify areas of weakness to aid further training.

Since this scheme was introduced, six members of staff have been tested, with an average mark of 84%.

Advantages include
- Tailor made to each individual allowing specific areas to be targeted.
- Further training can be given in areas of weakness post assessment.
- New questions can easily be added.
- Reports are downloaded via an adobe file for CPD.

Disadvantages:
- Time is required to change questions within Survey Monkey.

To conclude, online assessments are the way forward to assess competence in Laboratory Medicine.

Th73

The performance of two high sensitivity troponin methods with low level external quality assessment material

A Reid
UK NEQAS-Cardiac Markers, Southern General Hospital, Biochemistry Department, Glasgow, United Kingdom

Introduction: Troponin assays with increased analytical sensitivity allowing lower diagnostic thresholds are now in routine use. As an EQA provider there is a requirement for us to issue materials to assess performance at these lower levels.

Method: Four EQA batches were produced (base matrix is pooled female serum) to cover cTn levels < 20 ng/L. This material was issued as a forth sample to participants using the Roche hs cTnT assay and Abbott Architect hs cTnI assay.

Results: Data for CMSHS01 and CMSHS02 is derived from two distributions of these samples. Data for CMHS03 and CMHS04 is derived from one distribution.

Data returned gave the following:
CMSHS01 (Roche cTnT (n=193): 9.6 ng/L (CV=8.6%)) (Abbott cTnI (n=14): 8.6 ng/L (CV=14.0%)). CMSHS02 (Roche cTnT (n=225): 13.2 ng/L (CV=6.5%)) (Abbott cTnI (n=21): 12.7 ng/L (CV=7.7%)). CMHS03 (Roche cTnT (n=107): 14.7 ng/L (CV=8.6%)) (Abbott cTnI (n=13): 12.5 ng/L (CV=11.4%)). CMHS04 (Roche cTnT (n=112): 19.6 ng/L (CV=6.0%)) (Abbott cTnI (n=12): 16.9 ng/L (CV=8.6%)).

Conclusion: Both methods show good between laboratory performance at these low levels. Increasing numbers of participants using the Abbott hs cTnI will allow better assessment of this method’s performance in the future.
Th74

Implementation of NICE guidelines on the recognition and initial management of ovarian cancer

DB Nice
Central Manchester University Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, Manchester, United Kingdom

CA125 is used as a first line investigation for the diagnosis of ovarian cancer. Cancer antigen 125 (CA125) is a glycoprotein that lines the female reproductive tract, and although its function has not been fully determined, it is thought to provide a lubricating barrier against particles and infectious agents at mucosal surfaces. Levels are found to be greatly elevated in ovarian cancer.

NICE guideline CG122 was published in April 2011, and stated that CA125 should be measured as a first line test in all women presenting with symptoms of ovarian cancer. This differed from previous guidelines that suggested simultaneous CA125 measurement and ultrasound scanning of the pelvis. Ovarian cancer presents with non-specific symptoms, so CA125 screening provides a triage before patients are referred to secondary care.

The aim of the audit was to assess compliance in primary and secondary care with the clinical guideline; ensuring patients had the initial CA125 measurement before being referred for further testing. A further aim was to assess if the Trust reference range, which differed from the NICE guideline, prompted referral at an earlier stage.

The audit found that all patients in primary care that should be referred for additional testing were referred appropriately. In both primary and secondary care reasons for request were poorly defined, with compliance with only requesting CA125 on women with appropriate symptoms was 66% in primary care and 53% in secondary care. Poor compliance was found in secondary care when investigating women under 40 with only 38% compliance with the guideline. Standards were taken from audit support material for CG122.

From this audit, the results are to be disseminated to the GPs, with the aim to improve knowledge of the test and reasons for requesting.

Th75

Performance summary icons for EQA reports ~ breathe a psi of relief

F MacKenzie, A Robins, J French
Birmingham Quality, UK NEQAS, QEHB, Birmingham, United Kingdom

EQA Schemes have moved from providing hard copy tabulated data, through personalised on-line reports, and have been using traffic light colour coding for some time. The Birmingham Quality UK NEQAS reports now operate a new feature to further simplify handling of EQA data. Although having interactive links in web or pdf pages is hardly a new concept, we feel that any feature which eases and supports the often inexperienced QA/QC officer is to be welcomed. Feedback suggests that it indeed does succeed in its aim of simplifying the often complex route through the EQA report and it does allow better targeting of effort.

All our reports are accessed online via a secure website with password protection. The method we adopted was to turn the familiar penalty box plot into a colour-coded icon that, when clicked, would take the user to the analyte selected. The icon itself would allow users to ascertain whether the performance was in keeping with the user’s peer group and whether the B score (Bias) and C score (Consistency of Bias) were Green, Yellow or Red. It was our intention that most Laboratories would be primarily interested in analytes where the rolling time-window performance summarising six months’ worth of data was outside the National Quality Assurance Advisory Panel’s Limits of Acceptable Performance. These would be coloured Red.

We have successfully rolled out this initiative for all Schemes and those with multiple analytes are benefitted the most. We can accommodate 32 plots per page.

The conclusion is that far from dumbing down EQA comprehension, the new approach allows a much more focussed way of allowing better use of time and resources.

Th76

Implementation of ISO 15189-particular requirements for quality and competence-in medical laboratory: does it improve performance?

VVK Hemraj, F Hemraj
Omnimed Laboratory Services, Biochemistry, Vacoas, Mauritius

The present study aimed at assessing the performance of a Medical laboratory through implementation of ISO 15189. Quality indicators were compared in Laboratory 1, with ISO 15189 being implemented, and Laboratory 2 as control with no implementation. Laboratory 1 had
translated the requirements of ISO 15189 to a checklist of 258 conformities. Six monthly periodic internal audits indicated a progressive increase in number of conformities achieved 63, 98, 155, and 177 respectively between January 2008 to June 2011 i.e. an increase from 24.4% to 68.6%. Six main quality indicators were quantified to a sigma metric value to establish any gradual change in performance of both laboratories. The number of conformities was correlated with sigma metric of each indicator with following R2 values: Missing clinical diagnosis, R2 = 0.944; Patient identification inaccuracy, R2 = 0.948; Glucose measurement, R2 = 0.784; Cholesterol measurement, R2 = 0.168; Critical value and interpretation reporting, R2 = 0.857; Turnaround Time, R2 = 0.975. The mean performance indicator sigma metric score increased from 1.4 ± 1.2 (2008) to 3.9 ± 2.2 (2011). A paired t test (t (5) = 4.756, N = 6) showed that the increase in mean performance sigma metric by 2.5 ± 1.3 was significant at p < 0.05. This indicates a significant increase in performance in laboratory 1. An independent t test (t (6.1) = 2.837) showed that the laboratory 1 with a 68.6% completion of ISO 15189, had a statistically higher performance sigma metric score (3.9 ± 2.2) than laboratory 2 (1.2 ± 0.7), significant at p < 0.05. This indicated that implementation of ISO 15189 was increasing performance of laboratory 1. However, clause to clause implementation of the Standard rather than a process based approach may have clustered negative effects on analytical aspects, which warrant further studies.

Th77

Hierarchy of target values and acceptance limits in external quality assessment

K Goossens1, D Stöckl2, K Van Uytfanghe1, L Thienpont1

1University Ghent, Gent, Belgium, 2STT-Consulting, Horebeke, Belgium

Many external quality assessment (EQA) schemes face the dilemma of choosing between limits subjectively defined state-of-the-art, or objectively derived, e.g., from biological variation. This leads to different acceptance limits and hinders comparison of performance. Here we describe a method comparison study, using 20 single-donation sera and measurement with random access platforms (≥ 8 labs per platform), in which we used different approaches to target setting and selection of acceptance limits.

The quality was estimated from the $S_0$, bias and total error. Firstly, we combined peer group target setting with variable limits calculated with peer values. Secondly, the peer group targets were combined with fixed limits based on state-of-the-art performance. Finally, the target was set by the “all method trimmed mean” (AMTM) or a reference method (REF) and combined with fixed limits, however, the bias was also assessed against limits derived from biological variation.

Distinction was made between laboratory and assay quality. This was important because performance is very much influenced by the quality of the assay/platform. In total 5% and 11% of laboratories were identified as poorly performing by the frequency of peer failures against peer-group and fixed limits. Laboratory performance versus the AMTM/REF target and fixed limits showed huge between laboratories differences for all estimates, especially influenced by assay biases. Assay performance at the peer group level and against fixed limits showed excellent within-run imprecision; in contrast considerable biases were observed when assessed against the AMTM/REF and fixed limits.

In our study we choose for a hierarchy of target values and acceptance limits and found that different strategies give complementary information. For example, deviation of a laboratory from its peer group is a signal to contact the manufacturer, whilst violation of the biologically derived bias limits might be a signal for the manufacturer for improving the calibration stability.

Th78

A plea to improve performances of faecal immunological tests for Haemoglobin: 1- standardization of Sampling and pre-analytical phase: 2-Revision of procedures for methods comparison

S Rapi1, T Rubeca2, CG Fraser3

1Careggi Hospital, Laboratory, Florence, Italy, 2Cancer Prevention and Research Institute (ISPO), Florence, Italy, 3University of Dundee, Centre for Research into Cancer Prevention and Screening, Dundee, United Kingdom

Background: Quantitative faecal immunochemical tests for Hb (FIT-Hb) appear to be the best non-invasive approach for colorectal cancer screening.

Since the lack of reference materials and standard procedures in both pre-analytical and analytical phase on faecal test, manufacturers have developed different sampling procedures and report units for quantitative FIT-Hb. This does not allow the comparison of clinical outcomes obtained with different systems. Moreover, the physical characteristics of the faecal specimen and the designs of collection devices do not allow the analysis of samples on different analytical systems, as the faecal tests cannot be compared using standard evaluation protocols.

Methods: To improve the harmonization of results and the overall performances of tests on faecal materials, we propose the introduction of standard procedures for sampling and pre-analytical phase, as well as the implementation of specific methods comparison protocols, based on the use of artificial biological samples (ABS). The use of a standard design for pickers and the use of a standard ratio between sample and buffer represents a mandatory step in the roadmap for harmonization of clinical laboratory measurement on faecal materials.
The introduction of specific protocols to investigate the analytical performances of faecal tests based on ABS, allows to compare data supported by different instrument, avoiding sampling errors and to can help to discriminate differences due either to sampling or to analytical phase.

In the first protocol we propose the investigation of a large series of ABS samples collected with both sampling systems and run on both methods. Performance comparison and system bias can be investigated by a standard statistical approach: linear regression’s and B&A plot data analysis ().

In the second protocol samples collected with specific sampling devices are going to be investigated with specific instruments, to obtain data on diagnostic accuracy.

Th79

Clinical validation of 'out of hours' critical laboratory results reduces admission rate to the emergency department

D Chandrajay, D Narayanan, JH Barth
Leeds General Infirmary, Chemical Pathology and Metabolic Medicine, Leeds, United Kingdom

Introduction: Communication of critical result is important to deliver urgent clinical care. Calling the requesting clinician or general practice over the telephone is feasible in working hours. However, the majority of blood test requests from primary care physicians arrive in the laboratory in the afternoon and analysed later in the day. This raises issues regarding communication of critical results to a responsible clinician.

Aim: Our study aimed to review the clinical validation of OOH critical results for out-patients and primary care patients.

Methods: A prospective study for 9 months involved data collection of patient demographics, clinical details, critical results, and the urgency of result communicated. The patient outcome was reviewed.

Results: A total of 311 OOH critical results were identified in the laboratory. After clinical validation 110 (35.4%) results were telephoned OOH urgently and 155 (49.8%) results were deferred to next day. 46 (14.8%) results were not telephoned. Following the urgent result communication 53/110 (48.18%) patients attended the hospital emergency department within 24 hours; and 17/110 (15.45%) had their repeat blood test by the GP within 48 hours. When the result was telephoned during working hours next day, only 15/155 (9.67%) attended the hospital acute services within 48 hours and 16/155 (10.32%) had repeat blood test at GP surgery.

Conclusion: The clinical validation of OOH critical results by a clinical biochemist helps to reduce the workload of out-of-hours community care services and may decrease admission rate to emergency department in the local hospital.

Th80

Patient percentile monitoring—a valuable quality indicator for the examination phase

K Van Uytfanghe1, D Stöckl2, LM Thienpont1
1Laboratory for Analytical Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Gent, Belgium, 2STT-Consulting, Horebeke, Belgium

Quality indicators (QI) are tools to evaluate the quality and effectiveness of laboratory testing. QI’s are necessary for monitoring the performance through the different examination phases. The latter consists of the testing of patient samples, hence, is an important part of the medical diagnosis, treatment and patient monitoring process. From this perspective, we propose the patient percentile monitoring project as QI for the examination phase.

The project consists of the daily monitoring of medians of twenty commonly measured analytes in outpatients. All type and sizes of laboratories can participate. They are expected to calculate and send their medians to us in an automated way. We collect all information in our database, but after exclusion of weekends. We then monitor the data by plotting of the moving median, but also the laboratories themselves can do it with help of a user-interface. We proposed preliminary limits for the assessment of the stability of performance. They are oriented on the biological variation, but, at the same time, respect the analytical reality. The laboratories are grouped by peer to allow instrument-specific comparison.

We have 85 participating laboratories with 153 different devices. We observe mid- to long term differences between different instruments, but also within-laboratory differences, sometimes accompanied by shifts or drifts. Another observation is that the moving median for certain analytes, e.g., C-reactive protein and gamma-GT, has higher variation in hospital laboratories. Focusing on outpatients seems promising for the assessment of laboratory bias.

The patient percentile monitoring tool gives laboratories a direct, real-time QI to monitor the examination phase in compliance with ISO 15189:2012 accreditation requirements. We believe it will allow laboratories to better evaluate the mid- to long term stability of performance. Observed stability issues may be an incentive for root cause analysis, so that finally the tool may contribute to improved performance.
Th81

Quality indicators to detect pre-analytical errors: a lighthouse in the night

A Aita, L Sciacovelli, A Padoan, M Plebani
University-Hospital of Padova, Laboratory Medicine, Padova, Italy

Scientific evidences demonstrate the negative impact of pre-analytical errors on patient safety and the use of quality indicators (QIs) as a strategic part of a quality improvement system in laboratory medicine. The coherent and standardized data collection of QIs is necessary to obtain a suitable data analysis and a prompt and effective implementation of corrective actions. This approach guarantees management focused on the patient. In this context our department, has implemented a QI system to monitor activities of the total testing process, performed by laboratory and non laboratory staff.

In this work we report QI data, collected in the blood collection sites inside and outside the laboratory (clinical wards inside our hospital, CW; internal blood collection sites, CL; blood collection sites outside the hospital, BCO), from 2010 to 2013, concerning the unsuitable samples: haemolysed (H), with inadequate sample-anticoagulant volume ratio (R), clotted (C), with incorrect container (I):
- H, 0.334–0.558:(CW, 0.325–0.548; BCO, 0.002–0.003; CL, 0.007–0.008);
- R, 0.149–0.129:(CW, 0.147–0.124; BCO, 0.001–0.003; CL, 0.001–0.002);
- C, 0.094–0.066:(CW, 0.084–0.056; BCO, 0.006–0.005; CL, 0.004–0.005);
- I, 0.076–0.047:(CW, 0.069–0.042; BCO, 0.006–0.003; CL, 0.001–0.001).

The data highlighted a general and significant (p < 0.001) improvement over time for all error typologies, except for H samples, demonstrating the effectiveness of corrective actions implemented (release of operative procedures, staff training, periodic meetings, etc.). The increasing of H samples, due to the introduction, in August 2012, of automated detection of haemolysis degree confirms the arbitrariness and unreliability of visual detection. Moreover the higher percentages concern samples sent from sites outside the laboratory where work non laboratory staff (CW) in respect to the laboratory staff (CL and BCO). Therefore the use of QIs is today necessary to monitor and improve all activities performed inside and outside the laboratory walls.

Th82

Improving the quality of ammonia results: tackling collection, handling and analytical issues at the Royal London Hospital

L Oliver, E Leach
Barts Health NHS Trust, Clinical Biochemistry Department, London, United Kingdom

Accurate plasma ammonia results are vital for the diagnosis of symptomatic hyperammonaemia, a medical emergency that requires prompt investigation and treatment. However, ammonia is an extremely unstable analyte and poor sample collection/handling is a common cause of elevated plasma ammonia. Audit of a 6 month period revealed that 10% of 402 samples received were unsuitable for analysis due to incorrect sample collection/handling—a significant underestimation of the scale of sample unsuitability due to uncertainty amongst laboratory staff about sample rejection criteria. At The Royal London Hospital ammonia analysis is further confounded by interference issues in the Roche Cobas NH3L assay. Two icteric indices are required: [conjugated bilirubin] about sample rejection criteria. At The Royal London Hospital ammonia analysis is further confounded by interference issues in the Roche Cobas NH3L assay. Two icteric indices are required: [conjugated bilirubin] > 171 μmol/L positively interfere, [unconjugated bilirubin] > 513 μmol/L negatively interfere. In addition, there have been several cases at The Royal London laboratory where results below the assay measuring range (< 10 μmol/L) were detectable on dilution.

To overcome these issues, a protocol was developed for sample collection, receipt and analysis. Before sample rejection criteria were implemented, the clinical and phlebotomy areas frequently requesting ammonia were identified by audit and targeted for education via presentations and written communications. To address laboratory issues, clear work flow algorithms were created for both sample reception and the automated laboratory detailing sample acceptance criteria, sample processing and analytical validation. Specifically, all samples are run immediately as neat and manual 1:2 dilution (to avoid problems with analyte stability) and a reminder in the laboratory interface prompts users to check the dilution results when the neat result is < 10 μmol/L. Reflex total and conjugated bilirubin testing was introduced on all ammonia requests and the icteric limit set at 171 μmol/L to trigger review of these results on analytical validation. The service will be re-audited in 6 months to assess the value of these changes and their effect on quality improvement.

Th83

Creatinine deltacheck to detect renal impairment and (pre-)analytical errors

IM Gillis', PW Schenk', CM Cobbaert', PJM van der Boog', N de Jonge'

'Leiden University Medical Center, Department of Clinical Chemistry and Laboratory Medicine, Leiden, Netherlands, 'Leiden University Medical Center, Department of Nephrology, Leiden, Netherlands, 'Bronovo Hospital, Department of Clinical Chemistry and Hematology, Den Haag, Netherlands

Introduction: Creatinine results may vary significantly depending on disease and treatment. Therefore, erroneous results due to (pre-)analytical problems can be missed easily. We investigated the utility of a deltacheck for early detection of (pre-)analytical errors. We also examined
whether the deltacheck is useful for detection of potentially clinically significant creatinine increases in transplanted kidney patients, given the importance of early detection of rejection. A deltacheck must distinguish between (patho)physiological changes and (pre-)analytical errors, must have a high efficiency (ratio of number of deltacheck alarms due to (pre-)analytical errors relative to total deltacheck alarms). Simultaneously it should not lead to unnecessary workload.

**Methods:** Serum creatinine assays are performed using an enzymatic assay on the Modular Analytics P800 (Roche). Sample volume required is 4 µl, CV = 2% at 80.5 µmol/L, CV = 1.4% at 362.5 µmol/L (source: GLIMS P5). For the setting of the deltacheck the functionality within the laboratory information system GLIMS (version 8.10.6; MIPS) is used.

**Results:** For detection of deterioration of renal function after kidney transplantation, we chose in consultation with nephrologists to automatically send an email to the attending nephrologist when a relative increase of 20% was observed in serial serum creatinine. After three months, an evaluation showed that all nephrologists considered the mailing valuable and no patients with a significant deterioration of renal function were missed. For the detection of (pre-)analytical disturbances we chose a relative deltacheck (positive or negative) of 34% above 100 µmol/L and an absolute deltacheck (increase or decrease) of 34 µmol/L below 100 µmol/L. This deltacheck gave an optimal ratio between detection of potential (pre-)analytical errors and confirmation of physiological 'normal' creatinine fluctuations. Creatinine results of dialysis and study patients are excluded.

**Conclusion:** For serum creatinine, the deltacheck is a useful tool for early detection of renal impairment and possible (pre-)analytical deviations.

---

### Th84

**Necessity of the critical values informing-clinicians needs**

_I Celap, I Vukasovic, N Vrkic_

Clinical Hospital Center Sestre Milosrdnice, Clinical Institute of Chemistry, Zagreb, Croatia

According to EN ISO 15189 critical values reporting is one of the most important requests. IFCC WG-LEPS (Working Group for Laboratory Errors and Patient Safety of International Federation for Clinical Chemistry and Laboratory Medicine) recommends critical values reporting as quality indicator 22 (QI-22). Recent investigation in our laboratory showed that only 35% critical results were communicated to physicians. However, the results are easily available through laboratory information system.

The aim of the study was to examine physicians’ requisites for immediate alert when critical results are obtained.

To obtain physicians’ needs for critical values alerts, we created a questionnaire which contains 9 questions with yes/no answers and one question with free text entry answer. Questions include physicians’ attitude with regard to immediate warning when laboratory obtains critical results, qualifications of the laboratory personnel who are reporting critical results and personal informing of critical results (specialist in laboratory medicine-clinician). Also, clinicians were asked to specify laboratory tests which require immediate notification if critical results are observed.

The questionnaire was answered by 49 clinicians: intensive care units (22/49; 45%), internal medicine department (12/49; 25%) and urology (14/49; 29%). The responders were mainly senior specialists with 5 years of experience (23/49; 47%) and residents (11/49; 22%). Results have shown that clinicians like to be warned about critical results (32/49; 65%); qualifications of the laboratory personnel who report critical results are not important (29/49; 59%); clinicians would ask for advice specialist in laboratory medicine if they consider it necessary (39/49; 80%); no need to inform clinician personally (32/39; 65%). Further, urgent informing of critical results is needed for electrolytes, glucose, haemoglobin, prothrombin time, platelets and troponin.

Results of the study showed that professionals in our laboratory have to raise awareness on necessity of critical results informing.

### Th85

**External quality assessment for trace elements: cobalt and chromium in blood**

_C Harrington, A Taylor_

Royal Surrey County Hospital, Clinical Biochemistry, Guildford, United Kingdom

**Aims:** The UKNEQAS for Trace Elements (TEQAS) includes assessment of performance for cobalt and chromium in blood, serum and urine. Following concerns about the increasing number of revisions in patients having metal-on-metal hip arthroplasty the MHRA issued a series of Medical Device Alerts concerning investigations of patients, including measuring concentrations of Co and Cr in blood. The advice stated that laboratories should participate in the TEQAS programme.

To show whether they can measure these elements in accordance with the MHRA guidance, the performance of laboratories was reviewed over 12 months and is continuously monitored by the scheme organisers.

**Methods:** Performance standards were determined in collaboration with colleagues in France and the USA. Using scheme results for 24 samples distributed in 2012, precision profiles were prepared over the concentration ranges 17-990 and 13-675 nmol/L, Co and Cr, respectively. The data were analysed as described by Thompson1 to give quality specifications2.
These specifications were used to calculate z-scores for performance assessment according to ISO 17043 and ISO 13528.

**Results:** The calculated quality specifications were ± 20% or ± 25 nmol/L and ± 20% or ± 40 nmol/L for Co and Cr, respectively.

**Conclusions:** The laboratories measuring cobalt and chromium provide results that are fit for purpose and should reassure surgeons and patients.

Additional work in progress involves the preparation and certification of a whole blood reference material in collaboration with LGC.

– Thompson M. Tr Anal Chem 2011;30:1168-75
– Harrington & Taylor 2012;344:e4017

**Th86**

**Software development for managing of genetic tests**

*F Rodriguez-Cantalejo1, FJ Chica-Alhambra2, I Caballero-Villarrasa2, A Benes-Diez2*

1IMIBIC/Reina Sofia Hospital/Cordoba University, Cordoba, Spain, 2Omnilab Iberia, Madrid, Spain, 3IMIBIC/Reina Sofia Hospital/Cordoba University, Clinical Analysis Service, Cordoba, Spain

**Introduction:** It is necessary to have general computer software in the clinical laboratories, due to the rise of genetic testing. This software tool has to meet the needs of technical staff and medical staff. In many laboratories, the hand records and paper records of these tests are still hindering traceability and patient safety.

**Objectives:** To develop a computer application from the clinical lab, that is capable of supporting all genetic tests and to ensure full traceability of the processing performed in each test.

**Material and methods:** The platform has been originated from system SMARTLIS. It has been implemented a specific module for this type of testing, taking advantage of the qualities this platform has. This computer application has web format and it is based on emerging technologies (django, jquery...). Therefore, it enables complete adjustment to the working environment (computers, tablets, mobile phones...) as well as the methodology used in the laboratory.

**Results:** Among the advantages achieved with the application are:

– It allows, for each test, all the necessary processes for obtaining results (DNA extraction, amplification...) so that you can know at any time what has happened in each test and maintain full traceability of performed procedure.
– It allows configuration of tailored plates with full traceability (quality control...).
– It contains a manual of technical assistance about the process to perform (incorporating video)
– It allows integration of images in any part of the process (karyotypes, gels...).
– Grouping of familial studies with printing of complete joint report.
– Integration with hospital information computer systems.
– Links to web support to the interpretation of results.

**Conclusions:**

– The development of SMARTLIS computing platform for Genetics allows full support for genetic tests of any laboratory, adapting itself to their needs
– It provides computerization of data and guarantees 100% traceability of the process, following the existing quality standards.
– It reduces significantly human error and increases patient safety.

**Molecular Genetics**

**Th87**

**Homocysteine metabolism and the associations of global DNA methylation with selected gene polymorphisms and nutritional factors in patients with dementia**


1Institute of Psychiatry and Neurology, Department of Genetics, Warsaw, Poland, 2Institute of Psychiatry and Neurology, First Department of Neurology, Warsaw, Poland, 3Viapath, Nutristasis Unit, Haemostasis & Thrombosis, London, United Kingdom, 4Viapath, Molecular Genetics, Haemostasis & Thrombosis, London, United Kingdom

Epigenetics (i.e. DNA methylation) together with the environmental and genetic factors are the key to understanding the pathogenesis of many diseases including dementia. Disturbances in DNA methylation have been already implicated in dementia. Homocysteine metabolism,
with folate and vitamin B₁₂ as essential cofactors, is integral to methylation processes. We evaluated the association of global DNA methylation, homocysteine, folate and B₁₂ status with dementia. Selected polymorphisms of genes previously associated with dementia development and the influence of various factors on DNA methylation were also investigated.

102 patients with dementia (53 with Alzheimer’s disease, 17 with vascular dementia and 32 with mixed dementia) were recruited. The control group consisted of 45 age-matched subjects without dementia and 46 individuals with mild cognitive impairment. Global DNA methylation was determined by Imprint Methylated DNA Quantification Kit (Sigma-Aldrich). Serum homocysteine, folate, B₁₂, creatinine, fasting glucose were determined by standard methods. Plasma and erythrocyte 5-methyltetrahydrofolate and plasma methylmalonic acid (markers of folate and B₁₂ status) were measured by HPLC. APOE, PON1 p.Q192R, MTHFR c.677C>T and IL1B-511C>T polymorphisms were identified using PCR-RFLP.

Subjects with dementia had significantly higher homocysteine (p = 0.012) and methylmalonic acid (p = 0.016) and lower folate (p = 0.002) and plasma 5-methyltetrahydrofolate (p = 0.005) than controls. There was no difference in DNA methylation between patients and controls. A tendency to higher DNA methylation in patients with vascular dementia (p = 0.061) was noted. Multivariate regression analysis of the whole group of investigated individuals demonstrated significant associations between DNA methylation and folate (p = 0.003), erythrocyte 5-methyltetrahydrofolate (p = 0.036), creatinine (p = 0.003) and glucose (p = 0.007) concentrations and IL1B-511C>T (p = 0.002) and PON1 p.Q192R (p = 0.044) genotypes. The association with MTHFR c.677C>T was significant only in controls (p = 0.017).

The biochemical results showed significantly lower folate and B₁₂ status in demented patients than controls. Global DNA methylation was associated with markers of folate status, creatinine, glucose and PON1 and IL1B polymorphisms.

Th88

Genetic variation in IL28B in chronic hepatitis C infected patients studied in Centro Hospitalar Lisboa Norte (CHLN) from October 2011-February 2014

H Brizido, LN Martins, GG Marques, J Melo-Cristino
Centro Hospitalar Lisboa Norte-Hospital Santa Maria, Lisboa, Portugal

Introduction: Nearly 3% of the world population is chronically infected with hepatitis C virus (HCV) a common cause of chronic liver disease and hepatocellular carcinoma. Currently only HCV genotype 1 patients are eligible for the new drugs available. The host polymorphism located upstream of IL 28B gene is a strong predictor of sustained virological response (SVR) for pegylated interferon alfa therapy. Patients with the IL 28B CC genotype polymorphism have higher rates of SVR than those with the CT/TT genotype. The high cost of triple therapy, adverse reactions and non-compliance are some of the factors justifying the selection of patients, setting out those who benefit with triple therapy and those who, without affecting the SVR, can be treated with dual therapy.

Objectives: The authors aimed to characterize the population of patients with HCV infection as to their genotype and evaluate the prevalence of IL 28B polymorphisms at CHLN.

Material and methods: The study included 391 patients with chronic hepatitis C. The genotype determination was performed with Siemens Technology (RNA extraction, amplification and LiPA). The IL 28B polymorphism (rs12979860) was determined by LightCycler® FastStart DNA Master System HybProbe using Magna Pure Compact (Roche) and LightMix® kit IL 28B (TIB Molbiol).

Results: Of the 391 patients studied 66.2% were genotype 1, 1.3% genotype 2, 18.7% genotype 3 and 13.8% genotype 4. In patients infected with HCV genotype 1 the IL 28B polymorphism distribution was 57% CT, 17% CC and 26% TT.

Conclusions: The most frequent HCV genotype is genotype 1 and in these patients the most prevalent IL 28B polymorphism genotype is CT. These are strong candidates for triple therapy since they do not have the favorable genotype for achieving SVR on standard therapy. The determination of HCV genotype and IL 28B polymorphism is crucial in deciding the therapeutic regimen.

Th89

Analysis of the C4 gene polymorphisms in patients with cryoglobulinemic vasculitis

E Menegatti1, S Baldovino2, M Messina1, V Oddone1, S Sciascia2, D Roccacello2
1University of Torino, Dept of Clinical and Biological Science, Torino, Italy, 2University Torino, CMID, Torino, Italy

Low C4 is one of the hallmarks of mixed cryoglobulinaemia (MC) and it is mostly due to early complement components consumption, but, several observations, suggest that other factors could be involved in C4 depletion.

C4 gene is located in a CNV region and can be present in multiple copies in a diploid genome. We investigated copy number of C4 genes and either C4A and C4B isotypes in patients with cryoglobulinemic vasculitis.

Genomic DNA isolated from 42 MC patients, 16 SLE patients and 78 healthy subjects was analyzed.
Gene copy number (GCN) of the C4 gene were evaluated by real time PCR. C4A6 allotype (p.Arg458Trp) and ins 2-bp mutation in exon 29 were screened by primer extension. Correlation with clinical signs of the disease (cutaneous ulcers, peripheral neuropathy, GN, purpura, hepatitis) have been performed by cluster analysis. (K-means algorithm).
MC patients have a non significant reduction in C4 GCN. We observed a significant increase of the frequency of the C4A6 allotype in cryoglobulinemic patients (6 /42 MC vs 0/78). C4A6 shows reduced ability to bind C5 within the C5 convertase complex. It is intriguing the unexpected high frequency of p.Arg458Trp variant in MC patients. Few data are available on the biological consequences of the presence of C4A6 allotype in immunocomplexes removal and antiviral defence mechanisms.
Cluster analysis allow us to identify cluster1 (> three copies of C4 gene) that showed a greater prevalence of glomerulonephritis, severe cutaneous ulcers and hepatitis. Cluster2 had the highest prevalence of patients suffering for neuropathy and purpura. These findings may suggest that cryoglobulinemic vasculitis may be viewed as a disease with a bipolar spectrum of manifestation and distinguishing between high Vs low level of C4 copies may be helpful and it could in, the future, represent a valid tool in predicting the progression of the disease.

Th90

SNPs of the corticotropin releasing hormone receptor 1 and 2 and their association with genetic risk for postnatal depression

AR Frank1, SCH Smith1, N Engineer1, DK Grammatopoulos1,2
1University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, 2Warwick Medical School, Coventry, United Kingdom

Background: Postnatal depression (PND) affects approximately 15% of women and if left untreated, it has profound consequences on family life and offspring development. Early identification and intervention can reduce the severity of the condition. Functional anomalies of the hypothalamic pituitary adrenal driven hormonal responses appear to be involved in pathogenesis of PND. Single nucleotide polymorphisms (SNPs) associated with abnormal HPA responses may be suitable genetic biomarkers for PND. Previously we reported over-expression of the rs242939 SNP of CRHR1 in women at high risk of PND. In this study additional SNPs of the CRHR1 and CRHR2 receptors previously associated with depression were investigated for association with PND.

Methods: Patients (n=250) were recruited to the Coventry and Warwickshire genetic association of PND cohort at 24-28 weeks gestation. A blood specimen was obtained and an Edinburgh Postnatal Depression Scale (EPDS) questionnaire completed both at recruitment and 2-8 weeks postnatally. An EPDS score ≥10 was used to identify women at high risk of PND. Genotyping was performed using mutation specific primers and qPCR. SNPs of the CRHR1 gene (rs7209436/rs242924/rs242940/rs173365/rs110402) and CRHR2 (rs3779250) were investigated. Allele and haplotype frequencies were calculated using the SNPstats software.

Results: Sixty percent of women (n=145) completed the full protocol; 30 had an EPDS≥10 at recruitment, 36 women had an EPDS≥10 postnatally, 16 had EPDS≥10 pre and postnatally. The TATGG haplotype present in 32.5% patients tested, had a protective effect for PND with an OR 0.44 (95%CI 0.22-0.86, p=0.017). Two SNPs individually (rs242924, rs173365) had a statistically significant protective effect with OR 0.41 (0.19-0.90) p=0.024 and OR 0.37 (0.19-0.90) p=0.024 respectively.

Conclusion: This study identified a significant protective association of individual SNPs of the CRHR1 gene and PND. The TATGG haplotype previously linked with major depressive disorders in adults was shown to be protective for PND in this cohort.

Th91

Evaluation of HLA-B27 assay using real time PCR

G Sukumar, S Sankaralingam, V Sundaresan
Princess Alexandra Hospital NHS Trust, Harlow, United Kingdom

Background: Human leukocyte antigen B27 is strongly associated with Ankylosing Spondylitis (AS). HLA-B27 typing is of diagnostic value because 90% of patients with AS carry B27 surface antigen compared to 8-10% of healthy individuals. Conventionally HLA-B27 is typed by detecting the HLA antigens at the cellular surface, either by cell cytotoxicity tests or by fluorescence serology with specific antibodies, but known to give false positive or negative results due to cross reactivity with antibody. DNA based allele-specific polymerase chain reaction (PCR) is a preferred alternative but time consuming and prone to contamination. In real time PCR, samples are amplified with allele specific primers and products identified by melting curve analysis in the same reaction tube thereby reducing labour and removing the possibility of contamination.

Aim: To evaluate a commercial kit and an in-house primer mix for the identification of HLA-B27 using real time PCR.

Methods: The first part of this study evaluated the LightMix® Kit HLA-B27 by TIB MOLBIOL Germany, which was designed for use on any LightCycler instrument. In the second part of the study, an in-house PCR mix developed using same specific primers as in the LightMix® Kit,
Carrier frequency of 5-alpha reductase 2 deficiency in Hong Kong Han Chinese

AOK Chan¹, PM Tong², CK Lee²
¹Queen Mary Hospital, Division of Clinical Biochemistry, Hong Kong, Hong Kong, ²Hong Kong Red Cross Blood Transfusion Service, Hong Kong, Hong Kong

5-alpha reductase 2 is an enzyme essential in the conversion of testosterone to dihydrotestosterone, which is a more potent androgen than testosterone. Deficiency in this enzyme causes under-virilization in males and is the most common cause of 46,XY disorders of sex development in Hong Kong. It is an autosomal recessive disease due to mutation in the SRD5A2 gene. The worldwide carrier frequency and incidence rate of this disease is unknown. The objective of this study is to estimate these two figures in our population, where over 90% of it is made up of Southern Han Chinese. 300 blood donors of ethnic Chinese were recruited. DNA was extracted from their peripheral blood. All the coding exons and the exon-flanking introns (at least 30 bp) of the SRD5A2 gene were amplified by polymerase chain reaction followed by direct DNA sequencing. It was found that two subjects were heterozygous for a known disease-causing mutation NM_000348.3:c.680G>A, NP_000339.2:p.Arg227Gln. One novel missense variant NM_000348.3:c.196G>A, NP_000339.2:p.Gly66Arg was detected in heterozygous state in one subject. In silico analyses using PolyPhen2 and Align GVGD predicted this variant to be probably pathogenic, while multiple sequence alignment showed that glycine at position 66 is highly conserved across mammal species. Based on these findings, the overall carrier frequency is 1 in 100. By Hardy-Weinberg equilibrium, the estimated incidence of having an affected male is 1 in 80,000 in the Hong Kong Chinese population. In conclusion, the estimated carrier frequency and incidence rate of 5-alpha reductase 2 deficiency are probably more common than many other inherited metabolic diseases in the Hong Kong Chinese population. Implementation of routine premarital genetic counseling and genetic testing should be considered such that affected couples could have an informed option on reproduction.

An evaluation of next generation sequencing for the genetic diagnosis of the Primary Hyperoxalurias

EAL Bagg¹, M Mueller¹, TJ Aitman¹, G Rumsby¹, EL Walker¹
¹Imperial College Healthcare NHS Trust, Clinical Biochemistry, London, United Kingdom, ²Imperial College London, MRC Clinical Sciences Centre, London, United Kingdom, ³University College London Hospitals NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom

The Primary Hyperoxalurias (PH) are autosomal recessive diseases of glyoxylate metabolism characterised by the excessive production and excretion of oxalate. Hyperoxaluria may lead to nephrocalcinosis, urolithiasis and consequent renal failure if treatment is not initiated. The three known types of PH are PH1, due to mutations in the AGXT gene, PH2, caused by mutations in the GRHPR gene and PH3, arising from defects in HOGA1.

The genetic diagnosis of Primary Hyperoxaluria currently relies on a step wise analysis of the AGXT, GRHPR and HOGA1 genes using Sanger sequencing. Sequential analysis can be time consuming, relatively expensive and may contribute to delays in the diagnosis and effective treatment of the disease. We have evaluated a next generation sequencing (NGS) assay for the simultaneous analysis of these genes using the Illumina TruSeq Custom Amplicon system.

Targeted sequencing of samples from 90 patients previously diagnosed with PH was performed in parallel on an Illumina MiSeq sequencer and the variants identified were compared to the results of Sanger sequencing. NGS showed 98% agreement with Sanger sequencing and correctly established the disease status in 97% of patients. There were no false positive diagnoses of PH and thus the NGS assay showed 100% diagnostic specificity. For PH1, diagnostic sensitivity was 97% for Sanger sequencing and 95% for NGS and for PH2 and PH3 both approaches had 100% diagnostic sensitivity. Three previously diagnosed PH1 patients were found to be heterozygous for disease-causing variants in the HOGA1 gene, which may contribute to the clinical heterogeneity observed in PH1. NGS analysis showed comparable diagnostic performance to Sanger sequencing for the diagnosis of PH and, if implemented, would enable the simultaneous testing for all forms of PH ensuring a more rapid diagnosis at potentially decreased cost.
The Bristol Genetics Laboratory (BGL) has provided a comprehensive Familial Hypercholesterolaemia genetic testing service for dFH/pFH cases (Simon Broome criteria) referred through UK lipid clinics using Sanger sequencing/MLPA. Since Sept 2013 testing has moved to a comprehensive 4 gene next generation sequencing assay.

Over a five year period a variant was found in 329/1014 diagnostic cases (32%). 184 distinct variants were identified, with 18 copy number variants (4 deletions and 4 duplications) detected. The most common mutation identified APOB: c.10580G>A p.(Arg3257Gln) accounts for 13% of positive cases, with LDLR c.1049G>C p.(Arg350Pro), c.2054C>T p.(Pro685Leu), c.301G>A p.(Glu101Lys) and c.313+1G>A frequently represented. LDLR c.1436T>C p.(Leu479Pro), accounting for 3% of cases, is found almost exclusively in the SW of England and is associated with a more severe presentation.

54 are novel variants: 31 missense, 4 nonsense and 5 splice-site (not reported to the LDLR locus specific database http://www.ucl.ac.uk/ldlr/Current/ or the literature). 10 novel variants are large deletions/duplications highly likely to be pathogenic.

6 cases were identified as homozygous or compound heterozygous FH, these results were shown to correlate with the severe phenotype presenting in these patients.

Only 222 cascade tests have been referred from 329 positive index cases giving a cascade test index of 0.67. 57% of cascade tests are positive. The low referral rate for cascade testing reflects in part the lack of commissioning investment for a cascade testing programme in England. Recent (2014) investment in FH cascade nurses by the British Heart Foundation is likely to considerably improve this position.

We present a 5 year audit of our genetic testing service.

NICE recommends comprehensive genetic testing in all patients clinically diagnosed with FH and genetic cascade testing of at-risk relatives, however, the cost of FH genetic testing still remains a barrier to commissioning.

Bristol Genetics Laboratory (BGL) has developed a comprehensive, high throughput diagnostic genetic test for FH using next generation sequencing. The custom-designed targeted capture assay (HaloPlex, Agilent) sequences 4 FH genes; LDLR, APOB, PCSK9 and SLCO1B1 and the SLCO1B1 variants (rs2306283 and rs4149056) associated with statin-induced myopathy. Data analysis uses an open-source pipeline; alignment (bwa), variant calling (GATK), variant annotation (Geneticist Assistant, SoftGenetics), and copy number analysis (CONTRA/brspeak CNV tool). Validation on 76 samples showed 100% concordance for 228 variants including 23 CNV (deletions and duplications).

BGL has tested >1000 FH index cases since 2008 and launched the first UK NGS service in October 2013. To date, 214 patients have been reported with 48 distinct mutations and a 29% (62/213) positive detection rate, the most common mutations being APOB c.10580G>A and LDLR c.313+1G>A. A further 9% (19/213) of patients have variants of uncertain significance (VUS) with 16 of these found in APOB and 2 in PCSK9. NGS CNV analysis detected 7 LDLR deletions.

The NGS assay has detected 15 additional variants compared with historic methods. One patient has a PCSK9 FH associated mutation c.1486C>T, and another a likely FH causing novel PCSK9 variant c.1555G>A. 3 patients have PCSK9 lipid lowering/raising variants. We have detected a previously reported APOB mutation, 4 candidate novel APOB variants and 6 rare APOB variants of uncertain significance.

NGS offers a single high throughput assay for point mutations and copy number at 23% reduced cost and detects additional clinically significant variants in APOB and PCSK9. We report on our service development, and future prospects illustrated by interesting case studies.
Th96

OTX2 gene involvement in eye malformations

E Tejedor Hernández, T Vendrell Bayona, A Fernández Rodríguez, N Castells Sarret, M Mosquera Parrado, A Plaja
HU Vall d’Hebron, Department of Clinical Biochemistry, Barcelona, Spain, HU Vall d’Hebron, Department of Genetics, Barcelona, Spain

Background: Severe eye malformations are a rare group of developmental disorders with a live birth prevalence of 1/10,000. At the severe end of the spectrum are anophthalmia and extreme microphthalmia. Our patient, a 2-year-old male presented right anophthalmia and left microphthalmia with absence of both optic nerves, ventriculomegaly, Blake cyst and cerebellar vermis abnormality. Percentile length 3 and occipito-frontal head circumference (OFC) at -2SD. He was the fourth child of healthy non-consanguineous parents with no known family history of malformations. Pregnancy follow-up was almost nonexistent, but at the third trimester a bilateral ventriculomegaly was observed. At the 41th weeks of pregnancy the child was born with a birth weight of 3180g, length of 50cm and OFC of 35 cm.

Methods: High resolution array comparative genomic hybridization (Agilent G4827A CGH ISCA v2, 8x60K), was performed according to the manufacture’s protocols. Subsequently to the identification of a deletion, we confirmed results and performed familiar study with CNV-specific BAC-FISH applying the probe RP11-10p7 (14q22.3,SG, [hg19]55968549-56129687) and marker RP11-123m6 (14q.32.2,SG).

Results: a-CGH analysis identified a heterozygous deletion of approximately 1.67Mb in 14q22.3, involving 5genes described on the OMIM database. FISH analysis showed the same deletion in the mother.

Conclusions: Heterozygous mutation in the OTX2 gene is related to syndromic microphthalmia type 5 (OMIM 610125). Heterozygous loss of function of OTX2 gene accounts for about 2-8% in patients with anophthalmia and/or severe microphthalmia and commonly occurs de novo in affected children. Our patient inherited the mutation from her normal mother. Since almost 35% of the cases OTX2 mutations are inherited from a normal parent, it has been suggested that OTX2 mutations alone may not lead to full expression of the phenotype. Additional genetic factors, environmental and stochastic variation on development have been invoked as coadjutant factors to full expression of OTX2 malfunction phenotypic effects.

Immunology

Th97

There are no differences in IL-6, CRP and homocysteine concentrations between female descendants with or without a family history of AD

S Devcic, N Ruljancic, L Glamuzina, M Mihanovic
Psychiatric Hospital Sveti Ivan, Department of Psychogeriatry, Zagreb, Croatia, Psychiatric Hospital Sveti Ivan, Department of Laboratory Diagnostics, Zagreb, Croatia, Psychiatric Hospital Sveti Ivan, Head of Psychiatric Hospital Sveti Ivan, Zagreb, Croatia

Background: Alzheimer disease (AD) is most present among dementias. A wide range of recent studies have detected inflammation as one of the most influent factors in the appearance and spreading of neurodegenerative brain diseases.

Methods: We aimed to understand the influence of IL-6, CRP and homocysteine on 55 female patients suffering from AD, 51 middle-aged daughters of the diseased patients, and 53 middle-aged subjects without positive family history of AD.

Results: The results of the conducted research are in accordance with the present scientific knowledge, thus a statistically significant difference for examined parameters has been determined between women suffering from AD and their middle-aged daughters and middle-aged subjects without positive family history of AD. There were no significant difference in hsCRP (p=0.601), IL-6 (p=0.582) and Hcy (p=0.188) concentrations between female descendants with or without a family history of AD. There were obtained positive correlation between Hcy and IL-6 and between IL-6 and hsCrp in AD patients while there is no such correlation between female subjects with or without a family history of AD.

Conclusion: Obtained results indicate that the increased concentration of inflammatory factors and Hcy in AD patients is a symptomatic rather that causal factor. These findings of an association between Hcy and IL-6 levels and between IL-6 and hsCrp, support the hypothesis that Hcy stimulates low-grade inflammation. However, our finding that there is a lack of correlation between Hcy and CRP in AD patients suggests that Hcy levels may not be a simple reflection of inflammation.

Th98

Lymphocyte subsets and cytokine production in patients with neuromyelitis optica

M Hindos, L Skacikova, P Nytrova, T Zima
General Faculty Hospital in Prague and 1st Faculty of Medicine of Charles University, Prague, Czech Republic

Background: Neuromyelitis optica (NMO) is an inflammatory disease of central nervous system (CNS) characterized by production of antibodies against aquaporin-4 (NMO-IgG/AQP4-IgG) that play a crucial role in the pathogenesis. However, the role of cellular mechanisms and cytokine crosstalk in NMO remains elusive.
Methods: Tested groups were 20 patients with NMO and 23 controls with other neurological diseases. NMO patients were treated with usual immunosuppressive therapy or with rituximab. Observation window was approximately 41 months (from 17 to 60). All individuals were tested from peripheral blood for lymphocyte subpopulations CD3+, CD3+CD4+, CD3+CD8+, NK cells, B-cells and intracellular cytokine production (IFNγ, IL-12, IL-2, IL-4, IL-6, IL-10) using flow cytometry. Results were compared between NMO and control group, NMO patients treated with rituximab and NMO with other therapy and relapsing NMO vs. disease in remission.

Results and conclusions: Patients with NMO had significantly lower absolute lymphocyte count as well as relative and absolute count of B-lymphocytes compared to controls. On the contrary, relative CD8+ count was significantly higher in patients with NMO and even higher in relapsing patients. Patients treated with rituximab showed significantly higher TNFα production and also increased levels of CD8+ T lymphocytes. This is probably due to depletion of B-lymphocytes and regulatory Treg subpopulation responsible for controlling monocytic TNFα production resulting in Th1 response support. Higher numbers of CD8+ lymphocytes in patients with NMO compared to control group could support the theory of cellular contribution to pathogenesis of NMO. However, increased CD8+ levels after treatment with rituximab confirm B-lymphocytes and production of anti-AQP4 autoantibodies as the major pathogenetic mechanism in NMO. The possible pathogenetic role of CD8+ lymphocytes is probably minor, but our results indicate that increasing levels of CD8+ lymphocytes in circulation could be a marker for predicting disease relapse.

Supported by research project PRVOUK P25/LF1/2.

Th99

Cryoglobulins-our experience

E Modric, M Gorenjak
University Clinical Center Maribor, Department of Laboratory Diagnostics, Maribor, Slovenia

Cryoglobulinaemia is associated with many illnesses: infections (especially hepatitis C), autoimmune disorders and malignancies and can be life-threatening. Cryoglobulins testing is important for diagnosis, treatment and follow up of patients (1). Our laboratory started with improved cryoglobulin testing in November 2004 and received more than 2000 requests until December 2013. Specimens were transported at 37°C and after seven days of cryoprecipitation at 4°C, washing and dissolving of the precipitate with re-warming, cryoglobulin concentration was measured. Lowry’s method was used for quantification of proteins, but became time consuming, because number of samples raised considerably in 2011. We introduced automatized nephelometric method with benzethonium chloride and established an algorithm with automatic further testing for positive results to determine the type of cryoglobulinaemia in 2012. From 2015 samples tested, 33 percent of the samples were found positive (> 100 mg/L cryoglobulin), among 110 positive results in 2012 and 2013, 56 of them were tested with immunofixation. Results showed 29 Type III, 12 Type II and 5 Type I cases of cryoglobulinaemia respectively. Most of the samples in our laboratory were sent from hematology and rheumatology departments and considerable number of results was just below the reference value. After evaluating albumin contamination of samples, we plan to reconsider our reference values, because there is no relationship between the cryoglobulin concentration and severity of the symptoms. Detection, analysis and reporting of cry globulins needs standardization, because variations in the suggested reference values alone are confusing (2).


Th100

Low-grade systemic inflammation revealed by plasma biomarkers in acute intermittent porphyria

E Storjord1, JA Dahl1, A Landsem1, H Fure1, J Krey Ludviksen1, E Waage Nielsen2, TE Mollnes3, O-L Brekke4
1Nordland Hospital, Department of Laboratory Medicine, Bodø, Norway, 2Institute of Clinical Medicine, University of Tromsø, Department of Anesthesiology, Nordland Hospital, Bodø, Norway, 3Institute of Immunology, University of Oslo, Rikshospitalet University Hospital, Oslo, Institute of Clinical Medicine, University of Tromsø, Department of Laboratory Medicine, Bodø, Norway, 4Institute of Clinical Medicine, University of Tromsø, Department of Laboratory Medicine, Nordland Hospital, Bodø, Norway

Aim: Acute intermittent porphyria (AIP) is a rare autosomal dominant metabolic disorder of haem synthesis caused by a mutation in the porphobilinogen deaminase enzyme. AIP patients have enhanced risk of liver fibrosis and primary hepatoma. AIP can be worsened by infections. The aim of this study was to examine whether AIP patients have increased systemic inflammation and in case if such inflammation is correlated with AIP disease activity and liver fibrosis.

Methods: The study design was case-control with inclusion of 50 persons with the AIP mutation W198X and 50 healthy controls matched for gender, age, and place of residence. Plasma cytokines, chemokines and growth factors were analysed with multiplex technology. Long
pentraxin-3 (PTX3), prothrombin F1+2, the complement activation products C3bc and TCC were analyzed using ELISA. Urine porphobilinogen (PBG), hematological and biochemical tests were analysed using routine methods. The FIB-4 liver fibrosis score, was calculated from age, AST, ALT and platelets. Symptoms of AIP, medication and other diseases were obtained using a questionnaire. Wilcoxon matched-pairs signed-rank test was used on the matched case-control data. Non-parametric Spearman correlations giving r and two tailed p were computed in the AIP group.

**Results:** The levels of 27 inflammatory cytokines, chemokines and growth factors were significantly (p < 0.0001-0.0004) higher in persons with AIP compared to the matched controls. Blood monocyte numbers (p = 0.0037), C3bc (p = 0.0015) and serum IgG levels (p = 0.0326) were significantly increased in the AIP patients. The FIB-4 marker of liver fibrosis was correlated with TCC (r = 0.41, p = 0.0031), prothrombin F1+2 (r = 0.33, p = 0.0187) and PTX3 levels (r = 0.31, p = 0.0263). The AIP disease activity (PBG μmol/mmol creatinine), was correlated with prothrombin F1+2 (r = 0.54, p < 0.0001), PTX3 (r = 0.38, p = 0.0061), TCC levels (r = 0.33, p = 0.0208) and FIB-4 (r = 0.32, p = 0.0237).

**Conclusions:** AIP is associated with systemic low-grade inflammation. AIP disease activity is correlated with inflammatory markers and with liver fibrosis.

---

**Th101**

**Haploidentical T-alpha/beta and CD19-depleted hematopoietic stem cell transplantation in SCID**

_F Tosato1, G Bucciol2, G Pantano1, MC Putti2, MC Sanzari1, G Basso2, M Plebani1_

1University Hospital of Padova, Laboratory Medicine Department, Padova, Italy, 2University Hospital of Padova, Pediatric Hematology Oncology Department, Padova, Italy

**Background:** Severe combined immunodeficiency (SCID) is a primary immunodeficiency (PID) with incidence < 1:100,000 newborns. Neontal screenings are not implemented in our country. It is characterized by early onset, failure to thrive, diarrhea, infections. Treatment of choice is urgent hematopoietic stem cell transplantation (HSCT).

We report the case of a baby referred for Pjioveci bronchopneumonia.

**Methods:** 4-month-old female baby, weight 6.3kg, height 60cm (both 50th percentile), past medical history: two perforated otitis, oral thrush, bronchopneumonia. Admitted in ICU for severe respiratory failure, Pjioveci pneumonia, Rotavirus gastroenteritis, requiring assisted ventilation and specific antimicrobial treatment. CBC showed leukopenia with lymphocytopenia (400 lymphocytes/mm3). Immunophenotype was assessed in the suspicion of PID.

**Results:** Immunophenotype: 8% CD3+CD4+CD8-CD45RA+CD45RO-cells (absence of memory T-cells rules out maternal chimerism), 91% CD16/56+, 0% CD19 and CD8; negative CD25, CD38, HLA-DR.

Molecular analysis identified RAG1 mutation, supporting SCID T-B-NK+ diagnosis.

We performed haploidentical Ta/β-CD19-depleted HSCT from father, after conditioning with Treosulfan, Fluoradabine, rabbit antilymphocyte serum, with no other immunosuppressant. PMN and platelets engraftment at day +17 and +15 respectively led to progressive clinical and radiologic improvement, extubation on day +14. 100 days after HSCT CBC is stable at 700 lymphocytes/mm3; immunophenotype shows 2% CD3 (1% CD4, 1% CD8), 82% CD19, 13% CD16/56. Engraftment is confirmed by 100% donor chimerism, XY chromosome detected by FISH. The baby is well at home, without infections. 9 months after HSCT CBC is stable at 1840 lymphocytes/mm3; immunophenotype shows 79% CD3 (57% CD4, 15% CD8), 13% CD19, 7% CD16/56. Also the relative distribution of naïve and memory T lymphocytes, the activation pattern, and the B lymphocytes subpopulations are normal. Engraftment is confirmed by the presence of 54% donor cells in peripheral blood. The baby is well at home, without infections.

**Conclusions:** Haploidentical Ta/β-CD19-depleted HSCT can be useful in SCID cases with severe active infections.

---

**Th102**

**“Real world” performance of ELISA tests for anti DSG1/DSG3 and anti BP180/230 antibodies**

_S Baldovino1,2, S Sciaccia1,2, D Roccatello1,2, A Boeri1, L Solfitti1, D Di Simone1, K Giancaspero1,2, E Menegatti1_

1Turin University, Center of Research of Immunopathology and Rare Diseases, Torino, Italy, 2Turin University, Clinical and Biological Science Department, Orbassano, Italy

**Introduction:** Pemphigus and pemphigoid are rare autoimmune bullous dermatosis that can affect skin or mucous membranes. Pathogenesis is due to the presence of autoantibodies against desmosomes in pemphigus or hemidesmosomes in pemphigoid. The main antigenic determinants are desmoglein 1 and 3 (DSG1 and 3) or BP 180 and 230. Diagnostic gold standard is cutaneous or mucous biopsy with direct immunofluorescence. ELISA testing is useful when biopsy is contraindicated and for patients follow-up.

**Methods:** We evaluated clinical performances of four kits for anti DSG, DSG3, BP180, and BP230 antibodies in 47 Patients affected by pemphigus vulgaris (27) or by pemphigoid (20). As a negative control we used 11 normal subjects and 7 subjects affected by cutaneous SLE. ELISA testing was performed using kits MESACUP for Dsg1, Dsg 3, BP180, and BP230 (Medical& Biological Lab. Co., LTD, Japan).
**Results**: Sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were evaluated for DSG1 (SE: 0.3; SP: 1; PPV: 1; NPV: 0.49), DSG3 (SE: 0.81; SP: 0.94; PPV: 0.96; NPV: 0.77), BP180 (SE: 0.3; SP: 1; PPV: 1; NPV: 0.56), BP230 (SE: 0.15; SP: 1; PPV: 1; NPV: 0.51). Diagnostic performance were better using combined analysis such as DSG1+DSG3 (SE: 0.89; SP: 0.94; PPV: 0.96; NPV: 0.85) and BP180+BP230 (SE: 0.3; SP: 1; PPV: 1; NPV: 0.56). We evaluated also the correlation between clinical manifestations and antibodies titer during follow-up.

**Discussion**: We observed a lower sensitivity than expected for antiDSG1, antiBP180, and antiBP230. A possible explanation is the substantial lack of patients affected by cutaneous pemphigus in our sample and the possible involvement of other proteins, such as laminin V in the pathogenesis of pemphigoid. Moreover we observed different trends in antibody titer during therapy indicating the need for a close customization of the laboratory follow-up.

**Th103**

**Diagnostic value of serum MRP8/14 in preterm deliveries**

*K Malíčková1, M Kouckỳ2, I Francová1, A Parízek2, Z Hájek2, T Zima1*

1General University Hospital & 1st Faculty of Medicine of Charles University, Institute of Medical Biochemistry and Lab Diagnostics, Prague, Czech Republic, 2General University Hospital & 1st Faculty of Medicine of Charles University, Dept of Obstetrics and Gynecology, Prague, Czech Republic

**Objective**: To evaluate the association between serum MRP8/14 (S100A8; calprotectin) levels soon after the appearance of signs of preterm delivery and preterm delivery within 48 hours, before the 34th and 37th gestational weeks, and the possible additional value of concurrently evaluated ultrasound vaginal cervicometry with serum MRP8/14 measurement.

**Methods**: A total of 60 females were included. Serum MRP8/14 was measured by an enzyme immunoassay. Sonographic evaluation of cervical length in all females was conducted by transvaginal ultrasound.

**Results**: Patients who delivered within 48 hours after analysis showed significantly higher MRP8/14 concentrations compared to females with term deliveries (14.50 {6.75;29.20} and 2.52 {1.25;4.52} respectively), p = 0.001. Higher MRP8/14 was proven also for deliveries before/after weeks 34 and 37 (p = 0.001 and p = 0.014, respectively). A combined finding of cervical length shortening below 18 mm and MRP8/14 level increasing above 10.50 μg/mL could point to the significantly higher risk of preterm delivery (OR 22.0 {2.81;104}).

**Conclusions**: MRP8/14 proteins are regarded as markers for a number of inflammatory diseases in humans. Elevated maternal serum concentration of MRP8/14 could be an independent and relevant risk factor for preterm delivery.

**Acknowledgements**: Supported by the research projects PRVOUK P25/LF1/2 and RVO VFN 64165/2012 of General University Hospital in Prague.

**Th104**

**Re-audit of primary care allergen requesting practice**

SK Moore, AS Everitt
Basildon Hospital, Clinical Biochemistry, Essex, United Kingdom

**Aims**: To re-audit the quality of Primary Care allergen request forms and compare requesting patterns with a previous audit in 2011.

**Method**: 441 requests for IgE/IgE specific allergens received from Primary Care from November 2011 to October 2012 were reviewed in terms of requesting patterns by practice/GP, age of patients and IgE specific allergen tests/results. Fifty request forms were selected at random and audited against the following four standards: 100% of request forms should have legible/relevant clinical data,

1. should be accompanied by an IgE allergen pro-forma,
2. should indicate which IgE specific tests are required and
3. should indicate whether the suspected allergy is seasonal or perennial.

**Results**: House dust mite (HDM) and IgE specific to cow’s milk were the most commonly requested allergens. 13% of all the allergy requests were requested by one GP practice; 36% were on children ≤16 and 2% were requested on patients >75 years of age. Of the 50 request forms audited 92% (86%), 26% (26%), 86% (49%) and 18% (25%) met standards one to four respectively (results of previous audit in brackets). The most common terms used were “RAST” (38%) and “IgE specific allergen” (26%).

**Conclusions**: Request forms showed improvement from previous audit in quality of clinical data and indication of which IgE specific tests were required. However, still only 26% of requests were accompanied by an IgE allergen pro-forma.
Recommendations: All requests should have clearly legible clinical details and be accompanied by an IgE allergen pro-forma; available in Pathology Handbook (www.baspath.co.uk). All requests should clearly indicate which IgE specific test / allergen is required based on clinical history & examination, and not rely on the laboratory to determine which allergens to request. To improve quality and appropriateness of requests, provide more education / guidance to users via Pathology handbook and reports.

Management

Th105

From monovalency to polyvalency: the Slovak experience

O Kovac, A Porubanova
Slovak Medical School, Institute of Laboratory Medicine, Bratislava, Slovakia

Method: Listing and commenting outcomes reached in the process of change.
Results: In 1994 Slovak Society for Laboratory Medicine (SSLM) started studying financing systems and organisational structure of laboratory diagnostic in EU and USA. In 1997 costing based system for financing laboratory diagnostics was implemented. Since 1997 SSLM organizes annual conferences with invited speakers from EU. In 1999 the process of accreditation was introduced. In 2000 SSLM and Slovak Medical School (SMS) established new polyvalent discipline: laboratory medicine based on UEMS Blue Book (BB), EC4 syllabus (EC4S) and McClatchey's Clinical Laboratory Medicine textbook. Since 2004 Institute of Laboratory Medicine (ILM) at the Slovak Medical School is accountable for postgradual education in polyvalent laboratory medicine. In 2005 SSLM in cooperation with University hospital established the first integrated and consolidated polyvalent department of laboratory medicine in Slovakia. Since 2008 SSLM in cooperation with SMS, Slovak Health Insurance Institution (SHII) and Alphamedical Ltd run case studies focused on quality indicators (2010), personal benchmarks (2012), quality systems (2013) and quality monitoring (2014).
Conclusions: SSLM in cooperation with SMS, SHII, Alphamedical Ltd, UEMS and EC4 changed the shape of laboratory diagnostics in Slovakia: system of financing, integration and consolidation, accreditation and laboratory medicine (as a polyvalent discipline with accredited curricula harmonized with BB and EC4S) represent the main tools. 30 accredited clinical laboratories, more than 10 polyvalent departments and about 40 polyvalent specialists in laboratory medicine with postgradual state exam document that change.
Acknowledgment: To Huib Storm, Gilbert Wieringa and all SSLM members and stakeholders for their support and cooperation.

Th106

Faecal calprotectin audit

LD Ferguson, M Panarelli
Glasgow Royal Infirmary, Clinical Biochemistry, Glasgow, United Kingdom

Background and aim: Faecal calprotectin is a sensitive gastrointestinal inflammatory marker used to discriminate inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS). Demand is increasing but funding remains limited. This audit aimed to quantify the scale of faecal calprotectin requests, requestor source, potential reasons for increased demand, and the impact of demand management to improve service delivery.
Methods: A Telepath search was performed for all faecal calprotectin requests sent to Glasgow Royal Infirmary Biochemistry from January 2011 to January 2014. The number, source, and duplication rate of faecal calprotectin requests were recorded. A minimum 3 month interval between requests began in August 2013 and following a 2 month run in period, requests from October 2013 to January 2014 were logged, categorised by requestor source, and the number of duplicate requests recorded.
Results: In 2011, the average number of faecal calprotectin requests was 706 samples/month. 32% were repeat requests. By 2013, average total requests increased by 60% to 1131 requests/month prior to service change. After introducing demand management, re-audit showed repeat requests decreased to 10.5%. Average total monthly requests remained similar. 24% of requests were from gastroenterologists, 24% from GPs, 10% from non-GI hospital clinicians, 22% from other biochemistry departments, and 20% were external chargeables.
Conclusion: Demand management reduced the percentage of repeat faecal calprotectin requests. However, total monthly requests remained similar reflecting increased demand overall. Collaboration with clinicians is required to ensure appropriate requesting. A significant percentage of requests were from primary care as well as gastroenterology. This may reflect recent NICE guidance that faecal calprotectin is a cost-effective way to distinguish potential IBD from IBS in the community, reducing colonoscopy referrals. Increased demand from secondary care reflects the role of faecal calprotectin in disease monitoring, predicting relapse, and assessing treatment response in known IBD.
Th107

Homozygous Tangier disease (analphalipoproteinaemia) with undetectable serum high-density lipoprotein cholesterol and no clinical manifestations

C Van Heyningen1, JC Gell2

1NHS, Southport, United Kingdom, 2University of Liverpool, Liverpool, United Kingdom

Tangier disease, or analphalipoproteinaemia, is a rare form of familial high-density lipoprotein (HDL) deficiency with only about 100 cases reported. The biochemical findings include serum HDL cholesterol concentration below 0.12 mmol/L, apolipoprotein A-I levels below 0.05 g/L, low total cholesterol and normal or high triglycerides. The clinical signs of Tangier disease result from the deposition of cholesteryl esters in nonadipose tissues causing hyper-plastic orange-yellow tonsils, hepatosplenomegaly, neuropathy and corneal opacities. These clinical signs combine differently in each patient. An association with early cardiovascular disease is variable. We report a patient without physical signs identified by finding undetectable serum levels of HDL cholesterol.

A 40-year-old female was referred to the clinic because of persistent undetectable serum HDL cholesterol (HDL-C) levels of < 0.1 mmol/L measured by homogenous direct enzymatic colorimetric assay (Roche autoanalyser). Serum total cholesterol varied from 2.9 to 2.3 mmol/L and triglycerides from 2.5 to 1.7 mmol/L. Her family members have reduced HDL-C levels. Her father presented at age 40 years with a stroke and ischaemic heart disease. Computed tomography studies showed mild calcification of her right coronary artery but no arterial stenosis was shown on angiography. Artefactual and secondary causes of low HDL-C were excluded.

Genetic analysis, costing 1576 Euros/£1300, was undertaken in Oslo, Norway. Sequencing of apoA1 and LCAT genes did not identify any mutation. Sequencing of the ATP-binding cassette transporter A1 (ABCA1) gene revealed a homozygous mutation resulting in a non-functional protein and indicates a diagnosis of Tangier disease.

This case study illustrates how Tangier disease without clinical features may be identified by finding an undetectable serum HDL-C level. The diagnosis must be confirmed by genetic analysis of genes associated with low HDL-C. The degree of risk for cardiovascular disease may be estimated from the family history of lipid abnormalities and cardiovascular events.

Th108

Hyponatraemia: how low could it go...!? 

M Khedr, AS Davison

Royal Liverpool University Hospital, Department of Clinical Chemistry and Metabolic Medicine, Liverpool, United Kingdom

65-year old man was taken to the Emergency Department after he was found on the floor following a fall. Past medical history included chronic pancreatitis due to alcoholism, hypertension and previous perforated peptic ulcer. Drug history included creons, amlodipine and multivitamins. Glasgow Coma Scale was 15/15. He had no focal neurological signs. Biochemically: Sodium, potassium, chloride, urea, and creatinine were 98mmol/L, 4.7mmol/L, 55mmol/L, 2.7mmol/L and 42umol/L, respectively. Sodium result was confirmed on a repeat sample and on direct ion selective electrode measurement. Bilirubin, ALT, GGT, ethanol and CK levels were 32umol/L, 102U/L, 74U/L, 2.2mmol/L and 18713U/L, respectively. Cortisol level and thyroid functions were normal. Arterial blood gases revealed a fully compensated metabolic acidosis (anion gap 10). Whole blood lactate, ketones and random glucose were 5.9, 2.6 and 11mmol/L, respectively.

A clinical impression of hypovolaemic hyponatraemia was made. This was supported by the findings of urinary sodium of 12mmol/L and urine osmolality of 513mosmol/kg H2O. He was commenced on intravenous 0.9% saline aiming at raising sodium by 10mmol/ 24 hours. Over the following 6 days, the sodium rose gradually to 131mmol/L and CK dropped to 197 U/L. Renal function remained stable. He recovered well after 11 day stay in hospital and was discharged home with support.

Management of profound hyponatraemia can be challenging. Hypertonic saline should be infused if the severe hyponatraemia was thought to be acute and symptomatic. Otherwise, establishing the patient volume status is fundamental before embarking on any therapeutic intervention. When 0.9% saline is to be given to treat hypovolaemic hyponatraemia, this should be done slowly and very carefully with the aim of raising serum sodium no more than 12mmol/24 hour. This would require regular assessment of U&E’s after each 0.9% saline infusion. Failure to do so may put the patient at risk of neurological complications from central pontine myelinolysis.
**Th109**

**Bicarbonate: to measure or not to measure...?**

K Csenki¹, M Khedr², AS Davison²

¹Royal Liverpool and Broadgreen University Hospitals Trust, Department of Surgery, Liverpool, United Kingdom, ²Royal Liverpool University Hospital, Department of Clinical Chemistry and Metabolic Medicine, Liverpool, United Kingdom

57-year-old man presented with vomiting and high stoma output. Past medical history included hypertension, chronic obstructive pulmonary disease, right hemicolectomy, end colostomy and ileostomy for a perforated appendix. He had a white cell count of 19.5 x10⁹/L (neutrophil count 17.1 x10⁹/L). Biochemically: Sodium, potassium, chloride, urea and creatinine were 107mmol/L, 3mmol/L, 75mmol/L, 35.5mmol/L and 257umol/L, respectively. This was consistent with profound hyponatraemia and acute kidney injury stage 2. Since he was dehydrated and hypotensive, a diagnosis of hypovolaemic hyponatraemia was made.

He was carefully treated with intravenous 0.9% saline following which his sodium, chloride, urea and creatinine improved to 129mmol/L, 104mmol/L, 8.2mmol/L and 115umol/L, respectively. Bicarbonate remained low, between 10-15 mmol/L. Following discussion with the Duty Biochemist, the medical team arranged the measurement of arterial blood gases, which revealed a partially compensated metabolic acidosis (anion gap 18) and severe hypoxia. Whole blood lactate and glucose were 1.2 and 6.5mmol/L, respectively. Clinically, he had signs suggestive of bilateral pneumonia, which was associated with rapidly developing septic shock and type 1 respiratory failure requiring intubation, ventilation and inotropic support. He was transferred to the intensive therapy unit where he spent 6-weeks requiring intermittent ventilatory support. Subsequently, he was discharged to a general ward for general rehabilitation and convalescence.

This is an example of where bicarbonate had a key role to pointing toward a persisting normal anion gap metabolic acidosis resulting from AKI and sepsis. This case demonstrates the clinical utility for bicarbonate measurement and adds weight to the argument for routinely measuring bicarbonate. A close liaison between biochemistry team and the medical team was paramount in uncovering the underlying pathology and in ensuring a timely communication and transfer to intensive care unit.

**Th110**

**A rare cause of neonatal hyperbilirubinaemia**

VE Clough, F Akinlade

Barking, Havering and Redbridge University Hospitals NHS Trust, Clinical Biochemistry, Essex, United Kingdom

A 12 day old male was admitted to NICU with sepsis and severe jaundice. He was found to have unconjugated hyperbilirubinaemia, total bilirubin 634 μmol/L and conjugated bilirubin 16 μmol/L. Total bilirubin decreased in response to phototherapy but increased when treatment was stopped. There were concerns over the risk of kernicterus. All other biochemistry results were within the reference range with no evidence of liver disease. Hb was 162 g/L with a low reticulocyte count of 0.7% (2-6).

Over the next week the patient was investigated at length for haemolytic disease. The blood film showed no evidence of haemolysis. Occasional spherocytes were seen but not suggestive of hereditary spherocytosis. No haemoglobin variants were detected by haemoglobinopathy screening. Galactose-1-phosphate uridyltransferase and Glucose-6-phosphate dehydrogenase (G6PD) screens were both normal, excluding classical galactosaemia and G6PD deficiency. There was no evidence of pyruvate kinase deficiency. Hypothyroidism was also excluded.

On day 22, following discussion with the laboratory, a sample was sent for genetic testing for Crigler-Najjar (CN) syndrome which was later confirmed as the diagnosis. CN syndrome results in unconjugated hyperbilirubinaemia due to reduced bilirubin-uridine diphosphate glucuronosyltransferase activity. The patient was found to be homozygous for a c.1043delA mutation in the UGT1A1 gene complex.

The patient was discharged and is continuing 14-16 hours of daily phototherapy at home. Total bilirubin remains between 200 and 300 μmol/L. Treatment with phenobarbital was ineffective. The initial bilirubin concentration and the amount of phototherapy required suggest this is Type 1 CN syndrome, with a complete absence of enzyme activity. The parents were not found to be carriers of the mutation suggesting a de novo mutation. An auxiliary liver transplant is being considered.

Crigler-Najjar is a rare cause of unconjugated hyperbilirubinaemia; diagnosis is vital to ensure appropriate on-going treatment with phototherapy and/or phenobarbital to avoid kernicterus.
Th111

Anaemia, neutropenia and myelopathy due to acquired copper deficiency presenting as myelodysplastic syndrome

Il Scargill, M Saif, D Gosal

Salford Royal NHS Foundation Trust, Department of Clinical Biochemistry, Manchester, United Kingdom, Salford Royal NHS Foundation Trust, Department of Haematology, Manchester, United Kingdom, Salford Royal NHS Foundation Trust, Department of Neurology, Manchester, United Kingdom

A 46 year old male patient presented to his GP with a 6 week history of extreme lethargy. Initial investigations showed severe anaemia and neutropenia (Hb 46g/L, neutrophils 0.2x10^9/L), with slightly reduced Vitamin B12 (175ng/L) and macrocytosis (MCV 103fl). Additional pathology and imaging tests were unremarkable.

A blood film showed dysplastic changes in neutrophils but no overt features of B12 deficiency. A bone marrow examination was undertaken to evaluate his cytopenias which confirmed changes consistent with Myelodysplastic Syndrome (Refractory Cytopenia with Multilineage Dysplasia). Intramuscular B12 replacement therapy was commenced to ensure that the underlying changes were not due to B12 deficiency. No improvement in counts was observed despite adequate B12 therapy. A repeat marrow biopsy six months later showed persistent dysplastic changes.

Following his initial diagnosis, the patient subsequently developed sensory features consistent with a peripheral neuropathy with sensory ataxic features and neurophysiology investigations demonstrated a sensory predominant neuropathy with axonal length dependent features. An MRI of the cervical spine demonstrated Subacute Combined Cord Degeneration. Serum copper and caeruloplasmin, and urine copper were undetectable (<1 μmol/L, <0.03g/L, and <0.1 μmol/L respectively). Serum zinc was markedly raised at 35 μmol/L.

Acquired copper deficiency is a rare cause of refractory anaemia and neutropenia, and a recently recognised cause of Myelodysplasia. It is also an increasingly recognised cause of neurological manifestations, clinically and radiologically similar to those seen in Vitamin B12 deficiency. Common causes of hypocupraemia include upper GI surgery or malabsorption and zinc excess, due to competing absorption mechanisms.

The patient was treated with oral copper supplementation. Normalisation of serum copper concentration resulted in correction of cytopenias, and stabilisation of neurological symptoms. Interestingly, the patient reported liberal usage of over the counter dental filling pastes, which contain high levels of zinc oxide. Zinc concentration normalised upon cessation of use of these products.

Th112

A case of hypocalcaemia in osteoblastic bone metastasis which demonstrates the importance of vitamin D analysis prior to bisphosphonate treatment

SL Davies, M Khedr, AS Davison

Royal Liverpool & Broadgreen University Hospitals NHS Trust, Department of Clinical Biochemistry & Metabolic Medicine, Liverpool, United Kingdom

A 65 year old male presented with a swollen arm following a fall. Admission biochemistry was in keeping with rhabdomyolysis and acute kidney injury (creatinine=3391μmol/L, urea=21.9mmol/L, creatinine=318 μmol/L; reference urea=3.9mmol/L, reference creatinine=68 μmol/L). X-ray of the right arm revealed a pathological fracture of the humerus. Computerised Tomography scanning showed a possible primary tumour in the bladder or prostate with diffuse sclerotic skeletal metastases which were evident on an isotope bone scan. This was corroborated by a high prostate specific antigen (PSA=150 μg/L). Admission bloods revealed: adjusted calcium (ACa)=2.16mmol/L, albumin (Alb)=28g/L, phosphate (Pi)=1.66mmol/L, alkaline phosphatase (ALP)=911U/L and magnesium (Mg)=0.61mmol/L.

Parathyroid hormone measurement suggested secondary hyperparathyroidism (PTH=14.5pmol/L). During a similar episode 3-months earlier, U&Es, calcium profile and Mg were normal and PSA was 53 μmol/L. Retrospective vitamin D measurement corroborated by a high prostate specific antigen (PSA=150 μg/L). Despite intensive calcium and active vitamin D supplementation, the hypocalcaemia remained refractory and necessitated 6 weeks of hospitalisation.

Following oncology review, 4mg of intravenous bisphosphonate (zoledronic acid) was administered. ACa dropped precipitously post-treatment to a trough of ACa=1.41mmol/L, Alb=22g/L, Pi=1.00mmol/L, ALP=734U/L and Mg=0.57mmol/L. Retrospective vitamin D measurement showed vitamin D deficiency (25nmol/L). This case demonstrates that hypocalcaemia can be associated with malignancy, particularly in breast and prostate carcinomas with osteoblastic bone metastases. Vitamin D deficiency and hypomagnesaemia are other contributory factors in this case. The adverse patient outcomes associated with bisphosphonate treatment in a vitamin D deficient patient exemplifies that calcium and vitamin D measurement and replacement (if required) are mandatory in patients being considered for bisphosphonates or denosumab. This prevents secondary hyperparathyroidism and hypocalcaemia if there is insufficient calcium for bone healing/repair.
Th113

An inherited metabolic disorder causing respiratory difficulties with a fatal outcome

F Meakin, M Yanney

‘King’s Mill Hospital, Clinical Chemistry, Nottinghamshire, United Kingdom, ‘King’s Mill Hospital, Paediatrics, Nottinghamshire, United Kingdom

A female born at 38 weeks following a normal vaginal delivery was identified as having mild conductive hearing loss from the newborn hearing screen. At 10 months old she was admitted electively for a sedated audiogram but needed review by the paediatric team after developing breathing difficulties and low saturations following sedation. On examination hepatosplenomegaly was noted. She also had developmental delay and a chest x-ray showed some perihilar patchiness consistent with a lower respiratory chest infection. Past medical history revealed a background of recurrent chest infections from the age of 5-6 months.

Blood investigations showed pancytopenia, with haemoglobin 87g/L (111-141), white cell count 4.0 x10^9/L (6-17.5) and platelets 86 x10^9/L (150-450). A bone marrow biopsy was performed to rule out leukaemia, but this was essentially normal. A metabolic screen including plasma and urine amino acids, urine organic acids and GAG electrophoresis did not identify a specific metabolic abnormality but showed some metabolites associated with hepatic dysfunction. White cell enzyme analysis demonstrated reduced beta-glucosidase activity 0.34 μmol/g/h (1.0-5.0) and significantly elevated chitotriosidase 21767 μmol/l/h (4.0-120) consistent with a diagnosis of Gaucher disease.

She went on to develop neurological signs including ophthalmoplegia, bilateral squint, poor swallowing, stridor, hypotonia and brisk tendon reflexes. The early onset of these signs and rapid progression pointed to the severe form of neuronopathic Gaucher disease (type II). Genetic analysis confirmed compound heterozygosity for c.1448T>C, p. (Leu483Pro) [L443P] and c.508C>T, p. (Arg170Cys) [R131C] mutations in the GBA gene.

She continued to deteriorate in terms of her health and development and died aged 17 months following haemorrhage and a respiratory arrest. Confirmation of the genotyping has enabled carrier testing of the family and prenatal testing for future pregnancies.

This case highlights the progressive nature of classic type II acute neuronopathic Gaucher disease.

Th114

Psuedohyponatraemia with normal serum total protein and triglycerides: the X factor!

GN De Costa, S Ugrinovic, DJ Halsall

‘Clinical Biochemistry and Immunology, Addenbrookes Hospitals NHS Foundation Trust, Cambridge, United Kingdom, ‘The Pathology Partnership, Biochemistry and Immunology Department, Cambridge, United Kingdom

A patient previously diagnosed to have primary biliary cirrhosis (PBC) and familial hypercholesterolemia was admitted with worsening jaundice and lethargy. Serum sodium, when analyzed using indirect ion-selective electrodes (ISE) method, was markedly reduced at 115mmol/L (135-145). Serum osmolality was 289 mOsm/Kg (280-300) suggesting a diagnosis of pseudohyponatraemia. This was confirmed by a direct ISE result of 143mmol/L (135-148). Neither hypertriglyceridaemia nor hyperproteinemia were present (Triglyceride 1.7 mmol/L, Total Protein 64g/L (63-83)). However, the globulin fraction was high (55.5g/L) predominantly due to a polyclonal increase in IgM. Cholesterol was elevated at 13.8mmol/L, but this was thought unlikely to be associated with pseudohyponatraemia of this magnitude. Serum electrophoresis performed by Capillary Zone electrophoresis (Sebia, UK) on a subsequent sample showed an atypical anodally migrating band typical of the presence of lipoprotein X (LpX). Consistent with the presence of LpX, this band was not visible using agarose gel electrophoresis.

LpX is an abnormal phospholipid thought to be formed by cholestatic reflux of lipoprotein-rich bile into the serum. This binds albumin to form a cholesterol rich lipoprotein with apoplipoprotein C at its surface. It is thought to be responsible for hypercholesterolaemia seen in PBC. LpX has been shown to be a rare cause of pseudohyponatraemia. However, in this patient, the degree of contribution to the pseudohyponatraemia by LpX and the raised globulins remains unknown.

Whilst the use of direct ISE and osmolality clearly shows the presence of pseudohyponatraemia in this patient, reliance on total protein and triglyceride measurement would not. LpX should be considered as a possible cause of pseudohyponatraemia in samples from patients with cholestatic jaundice and high serum cholesterol. As well as pseudohyponatraemia, LpX and the high gamma globulins present in this patient may also cause assay interference with other analytical methods resulting further diagnostic confusion.

Th115

Unusual triad of problematic antiphospholipid syndrome, haemolytic anaemia and thyroid dysfunction

A Hassan

York Teaching Hospitals, Biochemistry, York, United Kingdom

A 15 y boy presented with redness, swelling and pain of left leg, with no trauma. Ultrasound confirmed femoroilacic deep vein thrombosis. He was thrombocytopenic, platelet count 102, APTT 80.7, PT 16.9, fibrinogen >4.5. Anticardiolipin and Lupus coagulant antibodies were positive,
consistent with antiphospholipid syndrome. He continued anticoagulation treatment for 6 months. Anticardiolipin antibody remained strongly positive, titre >120 GPL u/ml, anticardiolipin IgM 2.7 MPL u/ml. He then presented with tiredness and jaundice, haemoglobin 9.9 g/dl, with positive direct antiglobulin test, characteristic changes in blood film, elevated bilirubin, reticulocytosis, consistent with autoimmune haemolytic anaemia. He started on Rituximab to allow cessation of steroids. Rituximab improved autoimmune haemolysis but he developed acrnome rash, remained lupus anticoagulant and anticardiolipin antibody positive, had recurrent infections reflecting immunosuppression, unusual, following Rituximab monotherapy. He developed constellation of symptoms: persistent migrainous headaches with normal CT, gastric intolerance, 2 stones weight loss over 2 months, musculoskeletal pain, neutropenia, petechial rash, low platelets, and intermittent haemolysis treated by splenectomy. He then presented with left sided chest pain with no cardiac symptoms, which was not further investigated. It was stated that there was “no cardiac risk factors”. He complained of thirst and tiredness and was prescribed Propranolol to cope with palpitations. Tiredness was attributed to increased dose of Propranolol. Eventually, he was diagnosed with hyperthyroidism. During admission for pneumonia, raised calcium & alkaline phosphatise, deranged LFTs were thought to be caused by hyperthyroidism.

Highlights of the case:
1. Antiphospholipid syndrome resistant to treatment posing high risk for developing organ failure, so management should be multidisciplinary,
2. Hyperthyroidism was initially overlooked in context of less common APLS,
3. Doctor should measure blood pressure, glucose and cholesterol to rule out hypertension, diabetes, hyperlipidaemia, and patients stop smoking, maintain healthy weight, and take exercise to reduce other thrombosis risk factors.

Th116

Discordant concentrations of cardiac troponin T and I in a case of inflammatory polymyositis

SC Benton1, J Bailey2, A Salek-Hadadi3, DC Gaze4
1Surrey Pathology Services, Royal Surrey County Hospital NHS Foundation Trust, Clinical Biochemistry, Guildford, United Kingdom, 2Barts Health NHS Trust, Clinical Biochemistry, London, United Kingdom, 3Barts Health NHS Trust, Department of Neurology, London, United Kingdom, 4St George’s Healthcare NHS Trust, Department of Chemical Pathology, London, United Kingdom

A 26-yr old West African male, previously well, presented to the Eye-Casualty Department with a dropping upper eye-lid and uveal inflammation. A diagnosis of ocular myasthenia was made. He also had distal weakness and increased fatigue.

1-month later he re-presented with significantly increased eye and proximal weakness and severe fatigue. Physiological and electromyography studies diagnosed active myositis. Immunology confirmed an antibody-mediated inflammatory polymyositis (Anti-PM-scl).

Blood tests identified markedly elevated CK(22,300U/L) and Roche cardiac troponin (cTn) T(5,279ng/L). Elevated CK was explained by the polymyositis. Persistently elevated cTnT raised concern about his cardiac function leading to extensive cardiology investigations; all were normal. Cardiac involvement is not typical of Anti-PM-scl-polymyositis. cTnT analysis on a Radiometer assay agreed with Roche. cTnT and CK remained elevated, decreasing simultaneously with steroid treatment.

Lack of concordance with cardiac investigations questioned the specificity of the assay for cTnT. No assay interference was confirmed; samples diluted linearly and had good recovery post PEG precipitation. cTnI measured by Siemens, Abbott, Abbott-iSTAT and Radiometer were elevated were but all were less than 2x-99th percentile for the respective assays; demonstrating a lesser degree of elevation than cTnT. Serum Western blot analysis demonstrated the presence of cTnT and cTnI.

The values above 99th percentile for cTnI and serum Western blotting suggests the presence of cardiac cell necrosis. We cannot currently confirm if the excessive cTnT is cardiac or possibly skeletal in origin.

A previous case-report describes similar findings; biopsy studies confirmed presence of cTnT in skeletal muscle, due to re-expression of cTnT mRNA in regenerating tissue. We hypothesise a similar mechanism caused elevated cTnT in this patient and are undertaking further work to assess cTn content from a skeletal biopsy. We consider it important that biochemists and clinicians using the cTnT assay are aware of this phenomenon in conditions leading to large amounts of skeletal muscle regeneration.

Th117

Giant ectopic parathyroid adenoma with multiple brown tumours-a case report

F Ahmed1, P Prinsloo1, D Hosking2
1Nottingham University Hospitals, Clinical Pathology, Nottingham, United Kingdom, 2Nottingham University Hospitals, Medicine, Nottingham, United Kingdom

Primary hyperparathyroidism is a relatively common disorder, particularly in postmenopausal females. Indeed, it is the third most common endocrine problem after diabetes and thyroid disorders. We present a case of primary hyperparathyroidism in a 70 year old female due to a
giant ectopic mediastinal parathyroid adenoma with multiple “brown” tumours throughout the skeleton. The parathyroid adenoma measured 8.5cm × 4cm × 3cm, weighed 43g and appears to be one of the biggest PTH secreting functioning adenomas operated on in the UK. Our patient presented in an unusual way which posed diagnostic challenges among physicians and radiologists. PTH concentration fell from 2680 to 690pg/mL post operatively and the adjusted calcium decreased to a nadir value of 1.9 mmol/L consistent with the “hungry bone syndrome”. PTH fell to a nadir value of 22pg/mL 2 days after the surgery and then increased to between 100 to 200pg/mL in response to hypocalcaemia. Despite very high levels of PTH pre-operatively there was relatively immediate resumption of PTH secretion post-operatively, without evidence of prolonged suppression of the remaining parathyroid glands. 5 months post-surgery, the patient remained normocalcaemic without the requirement for calcium supplements.

Th118

Analytical interference of IgM paraprotein with urea measurement

SJ Pattman, J Lisle
South Tees Hospitals NHS Foundation Trust, Department of Biochemistry and Immunology, Middlesbrough, United Kingdom

Aim: We report the case of a 65 year old male with an elevated urea measurement which along with a raised globulin fraction led to the diagnosis of Waldenström’s Macroglobulinaemia. We describe simple clinical laboratory methods to demonstrate the presence of a paraprotein and the interference with the urea assay.

History: Clinical authorisation of a sample from primary care revealed a urea (Siemens Advia) result of 15.9mmol/L which was out of keeping with other parameters (Creatinine 85mmol/L, Sodium 134mmol/L) and without any evidence of a gastrointestinal bleed. An elevated globulin fraction of 56 g/L was found on the same sample. Immunoglobulin analysis and serum electrophoresis revealed an IgM paraprotein of 47.66 g/L. Previous blood results in 2012 showed a normal urea of 7.5mmol/L along with a globulin fraction of 43g/L. Further bone marrow analysis showed excess mature lymphocytes in keeping with a diagnosis of Waldenström’s Macroglobulinaemia.

Results of further investigations: Demonstration of the presence of a paraprotein was shown by the Sia Euglobulin Precipitation test. In our test, addition of a hypotonic solution (distilled water) to the sample caused precipitation of the abnormal protein. We also showed, in vitro, the production of a turbid solution following manual addition of Reagent 1 and 2 from the urea method kit. Analysis of the reaction curves revealed abnormal plots with the effect on absorbance at both the primary (340nm) and sub wavelengths (410nm).

Conclusion: Paraproteins are recognised to interfere variably with a number of analytes. In our case the progression of disease appears to relate to the disturbance in the urea measurement. Careful review of unusual results, previous trends and automated analyser data from patients with monoclonal proteins should be made to identify this problem. Simple addition of distilled water to a sample can reveal the possibility of paraprotein interference.

Th119

Autoimmune disease, an important consideration for complex neurological presentations

KME Gallagher¹, M Woodall¹, C Darby¹, A Vincent²
¹University Hospitals Coventry and Warwickshire, Biochemistry, Coventry, United Kingdom, ²John Radcliffe Hospital, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom

A 3 year old male of British-Caribbean origin presented with sudden bilateral loss of vision and poor balance. He was said to be “clumsy” but previous testing had excluded muscular dystrophy and Di George syndrome. Fundoscopy revealed swelling of the optic discs. An MRI of the brain and orbits ruled out a space occupying lesion, but identified swelling of the left optic nerve. Several possible causes of optic neuritis were investigated. Results for very long chain fatty acids were normal, excluding a defect of peroxisomal fatty acid beta-oxidation. CSF was negative for HSV, VZV, CMV and EBV by PCR. Oligoclonal bands were not detected in CSF making a diagnosis of MS unlikely. However, Aquaporin-4 antibodies were high (>1:6000) consistent with neuromyelitis optica (NMO). NMO is a rare autoimmune disease that affects mainly the optic nerves and spinal cord. The disease can occur in children and early age at onset carries a risk of permanent blindness.

The patient was given methyl prednisolone followed by oral prednisone; vision in the left eye improved but not in the right eye. He suffered a series of relapses of optic neuritis and transverse myelitis associated with spinal cord and brain demyelination on MRI. The patient has received extensive immunotherapies including steroids, plasma exchange, azathioprine and ofatumumab which eventually stabilised his condition (AQP4-antibody titre 1:800), but with little further improvement in vision.

This case illustrates the wide differential diagnoses in young children with neurological symptoms, and the importance of considering autoimmune diseases that can be stabilised and often improved by immunotherapies.
Th120

Hot glands-three new cases of phaeochromocytoma presenting in a district general hospital

PS West¹, A Theodoraki¹, L Farouk², P Hyatt¹, C Nethaji²
¹North Middlesex University Hospital NHS Trust, Biochemistry, London, United Kingdom, ²North Middlesex University Hospital NHS Trust, Endocrinology, London, United Kingdom

Catecholamine secreting tumours are neuroendocrine tumours arising from the chromaffin cells of the adrenal medulla (phaeochromocytomas 90%) or the sympathetic ganglia (paragangliomas 10%). They are rare and are causes of secondary hypertension in less than 1 in 1000 hypertensive patients. Presentation can be variable with orthostatic hypertension, anxiety, dyspnoea, weight loss, hyperglycaemia, cardiomyopathy and abdominal pain.

Around 10% are discovered incidentally in scans performed for other reasons.

Three such patients presented to our endocrine clinic within a year of each other.

The first was a 58 year old female presenting with a one day history of diarrhoea and bleeding per rectum and a previous history of hypertension and hypercholesterolaemia. A metaiodobenzylguanidine (MIBG) scan showed necrotic changes arising from the left adrenal gland suggestive of a phaeochromocytoma.

The second was a 38 year old male presenting with a one week history of right upper quadrant colicky abdominal pain and vomiting with a previous history of hypertension. MIBG scanning showed findings consistent with a right adrenal phaeochromocytoma and the third was a 68 year old female presenting three days after an elective right inguinal hernia repair with inspiratory chest pain and background history of diet controlled type 2 diabetes, hypertension and hypercholesterolaemia. MIBG scanning showed an appearance likely to represent an adrenal carcinoma or phaeochromocytoma.

All three subsequently had 24 hour urine collections for catecholamines with all showing grossly abnormal normetadrenaline and metadrenaline excretions.

In a patient with an adrenal nodule and hypertension, investigations to exclude a phaeochromocytoma need to be undertaken, especially if the lesion is not lipid rich on imaging.

Th121

An atypical case of liver injury caused by drug induced cholestasis

JM Wadsworth, M Khedr, AS Davison
Royal Liverpool Hospital, Clinical and Metabolic Medicine, Liverpool, United Kingdom

A 49-year-old woman developed jaundice soon after completing a 5-day course of amoxicillin. Past medical history included type 1 diabetes and hypertension. Medications included atorvastatin, ramipril and subcutaneous continuous Humalog insulin infusion. She had no previous history of drug allergy. She denied drinking alcohol or using herbal or complementary medicines. Clinically, she was well and jaundiced but had no peripheral signs of hyperlipidaemia. Her bilirubin, ALT, ALP and GGT were 109 umol/L, 204 U/L, 2631 U/L and 2759 U/L, respectively. Her total cholesterol, triglycerides, LDL and HDL cholesterol were 34.7 mmol/L, 2.4 mmol/L, 0.4 mmol/L and 33.2 mmol/L, respectively. She had normal albumin and clotting screen.

She was admitted for further investigations and was commenced on ursodeoxycholic acid. Atorvastatin was stopped. Autoimmunity screen was negative for Anti-Nuclear Antibodies, Anti-Liver Kidney Microsomal Antibodies, Anti-Mitochondria Antibodies and Anti-Smooth Muscle Antibodies. Hepatitis B screen was negative while EBV and CMV IgGs were positive, indicating previous exposure. CT scan of the abdomen did not show any evidence of intra or extra-hepatic obstruction. In view of the diagnostic uncertainty, a liver biopsy was arranged. The biopsy showed acute features in keeping with drug related liver injury.

She was discharged from hospital after a 2-week stay with improvement in her lipid and liver profile. A month later, her bilirubin, ALT, ALP and GGT were 14 umol/L, 35 U/L, 579 U/L and 651 U/L, respectively. Her total cholesterol, triglycerides, LDL and HDL cholesterol were 10.5 mmol/L, 1.1 mmol/L, 2.3 mmol/L and 7.7 mmol/L, respectively. Cholestasis is a well-recognised cause of secondary hyperlipidaemia. In our case, it was likely caused by amoxicillin. Of interest, was the improvement in lipid profile following the resolution of cholestasis. It is thought that lipoprotein X has a role in developing hypercholesterolaemia of cholestasis as it fails to inhibit the synthesis of cholesterol.
Th122

An unusual presentation of hyperparathyroidism and hyperthyroidism in a pregnant woman

CL Thomas, N Engineer, N Murthy, C Darby
University Hospitals Coventry and Warwickshire NHS Trust, Coventry, United Kingdom

A 32 year old female presented to her GP with hypertension in early pregnancy (13/40). Initial investigations showed evidence of acute kidney injury. The patient was later admitted to the emergency department with hypertension, tachycardia and hyperemesis. She was stabilised on IV fluids and discharged.

At an antenatal clinic appointment over two weeks later her serum calcium was checked. She was found to be hypercalcaemic with an adjusted calcium of 3.74 mmol/L. Further tests showed a PTH of 28.6 pmol/L, consistent with a diagnosis of primary hyperparathyroidism. USS of the thyroid showed diffusely large lobes and a well defined parathyroid adenoma on the lower right lobe. Further tests revealed hyperthyroid TFTs: TSH < 0.02 mU/L, free T3 13.0 pmol/L and free T4 44.3 pmol/L.

The patient underwent a parathyroidectomy at 18/40 weeks. PTH and calcium concentrations returned to within reference range two days post-op (PTH 1.1 pmol/L, Adj Calcium 2.8mmol/L). Histology confirmed a parathyroid adenoma with no signs of malignancy. The patient was treated with propylthiouracil (PTU) until her thyroid function tests returned to within reference ranges after approximately 2 months. She was also tested for TPO and TSH receptor antibodies and was negative for both. When PTU was withdrawn at 30/40 the patient remained stable and her hyperthyroidism is now thought to be due to hyperemesis related thyrotoxicosis.

The patient delivered a healthy baby boy after induction of labour at 37+6 for pre-eclampsia. The TFTs of the patient post delivery were normal: TSH 0.9 mU/L, free T3 2.9 pmol/L and free T4 10.3 pmol/L, as were those of the neonate which were checked on day 5 of life: TSH 1.84 mU/L, free T3 6.7 pmol/L and free T4 27.2 pmol/L.

This case emphasises the importance of diagnostic testing in the differential diagnosis of hyperemesis in this patient cohort.

Th123

Butyrylcholinesterase deficiency: the case of the missing genotype

A Corrigan, N Unsworth, A Marinaki, Z Arkir
Viapath, Biochemical Sciences, London, United Kingdom

Aim: Butyrylcholinesterase (BChE, EC3.1.1.8) hydrolysates short-acting muscle relaxants. BCHE variants in some patients results in decreased catalytic activity (qualitative variants) and/or decreased concentration of enzyme in plasma (quantitative variants). This results in prolonged paralysis following drug administration. We present case studies of two female adult patients with low total BChE activity not fully explained by their phenotypic inhibition profile.

Methods: Total BChE activity was determined using dithiobis-2-nitrobenzoic acid as a substrate and reaction monitoring at 30°C, 408nm. Phenotyping was assigned using BChE activity in the presence and absence of inhibitors (Pancuronium bromide, Sodium fluoride, Dibucaine, Propanol and R02-0683). Realtime TaqMan® allelic discrimination assays were used to genotype A, K and F variants. The coding region of the BCHE gene was sequenced.

Results: Pregnancy, severe illness and secondary causes of low total BChE activity were excluded in both patients. Patient 1 had inconclusive inhibition studies with low total BChE activity (BChE = 1.8 IU/ml RR: 3.0-9.0). She was heterozygous for A and K variants but BChE activity was low for this genotype. Sequencing revealed a heterozygous variant c.1142G>T, p.Gly381Val (rs373114728). The amino acid substitution is predicted to be deleterious to protein structure. This rare variant has not been previously reported in association with BChE deficiency.

Patient 2 had USUAL phenotype with unexpectedly low total BChE activity (BChE=2.6 IU/ml), and was wildtype for A, K and F variants. A heterozygous insertion-deletion event within exon 2, c.A36delTinsAG caused a premature stop codon. This variant has been previously reported in individuals with low BChE activity.

Conclusions: Total BChE activity results from a complex interplay between genetic and epigenetic factors. Screening for common variants is not sufficient where total BChE activity is unexpectedly low. A combination of biochemistry with genotyping improves risk assessment. Cascade testing of first degree relatives in these families is advised.
Th124

Blue book versus EC4 syllabus: improving visibility and recognition of the laboratory medicine profession

G Kovac, A Porubenova
Slovak Medical School, Institute of Laboratory Medicine, Bratislava, Slovakia

Aim: Contribution to the improvement of adequate visibility and recognition of the laboratory medicine profession in extensive discussions on finding way for moving ahead.

Method: Analysis and comparison of the structure and content of the Blue Book (BB) and the EC4 Syllabus (EC4S).

Results: BB deals with the overlap between specialties, polyvalence versus monovalence, frame and arrangement of training programs, logbook, fellowship, continual medical education, accreditation and visitation of training centres. These points are not directly mentioned in EC4S. In comparison to EC4S, BB is more ramified and (may be) less transparent. Main problem of BB are lacking chapters: “Monovalent Microbiology”, „Genetics” and not sufficiently extensive chapter „Monovalent Clinical Chemistry”. EC4S In comparison to BB is focussed prevailingly on the direct content of postgradual study topics. The basis was clinical chemistry and immunology with subsequent extension by microbiology, hematology and genetics: some of them are in comparison with BB rather restricted. Text and formal structure of EC4S may seem to be more concise and transparent.

Conclusions: BB was developed from the beginning on clinical, contextual and polyvalent approach. It takes into account the external environment of the educational process. EC4S approaches the problem more analytically and contentually: chemistry and immunology as the starting point with extension by other monovalent disciplines streamlining into final polyvalent shape. Both documents complement each other and represent good frame for shaping modern laboratory diagnostics.

Acknowledgment: to Gerhard Zlabinger, Augusto Machado, Wytze Oosterhuis, Ludo Marcelis and Vanna Kokkinu for motivation and valuable comments.

Th125

Do doctors cause more hemolysis during venepuncture? Review of hemolysis rate during blood taking in 4 acute medical wards

L Ong, A Pajarillaga, S Saw, S Sethi
NUHS, Singapore, Singapore

Background: Hemolysis during blood taking is one of the common pre-analytical errors and is operator and technique dependent. Therefore we looked into differences in rate of hemolysis during venepuncture and its impact.

Methods: We reviewed biochemistry laboratory results for hemolysis and other tests from 6 to 19 March 2013, in 4 acute medical wards, and information about the phlebotomist, type of tests, time of testing and severity of hemolysis were collected. Hemolysis was defined as free hemoglobin ≥100mg/dl, and gross hemolysis ≥500mg/dl. Hemolysis was measured as part of indices measurement on Advia Centaur and Vitros 5600.

Results: 8375 laboratory requests (individual tests or panels) were performed by 1533 phlebotomists. There were 171 doctors, 665 nurses and 697 professional phlebotomists. 87(5.7%) were hemolyzed and 13 were grossly hemolyzed, of which 6.2%, 10.5% and 1.0% were performed by doctors, nurses and phlebotomists respectively.

More samples performed after office hours were hemolyzed (7.4% compared to 4.9% during office hours). During office hours, 0.9%, 7.5% and 16.8% of phlebotomist, doctor and nurse drawn samples were hemolyzed. After office hours, 1.7%, 5.1% and 9.7% of phlebotomist, doctor and nurse drawn samples were hemolyzed.

50% of the phlebotomies required potassium measurement, with similar numbers in both hemolyzed and non-hemolyzed groups. In gross hemolysis, the results were invalidated and required repeated testing in 13 requests.

Discussion: 5.7% of phlebotomies had hemolysis and 0.84% was grossly hemolyzed. Professional phlebotomists had lowest rates of hemolysis, while nurses had highest rate of hemolysis, which may be due to nurses having to deal with more difficult veins. Higher rates after office hours could be due to sicker patients having more difficult phlebotomies. Phlebotomists, doctors and nurses’ phlebotomy training are done separately, and further studies looking into how venepuncture techniques and phlebotomy education influence hemolysis rates can be done.
Th126

Typical patterns of analyte covariance seen in routine biochemistry requests

A Dodd1,2
1Bristol Royal Infirmary, Biochemistry, Bristol, United Kingdom, 2East Kent Foundation Hospital Trust, Biochemistry, Ashford, United Kingdom

The clinical validation of results produced by the laboratory relies heavily on pattern recognition by the person signing off the results. Knowledge of typical patterns of covariance of results seen in routine practice can therefore be useful to aid decision making. An understanding of analyte covariance is also critical to select criteria for the filtering of results for reference interval investigations.

Data for all results for alkaline phosphatase, alanine transaminase, bilirubin, calcium, creatinine, glucose, glycated haemoglobin, parathyroid hormone, and 25-hydroxy vitamin D were already extracted from the laboratory database to aid selection of paediatric alkaline phosphatase reference intervals. These data were trimmed to only include adults and processing scripts written to determine centiles of analyte A at various concentrations of analyte B for multiple pairs of analytes within the selection described. These were then plotted graphically for interpretation. Many relationships are as would be expected, for example increasing alkaline phosphatase with bilirubin and with glucose, or with abnormal calcium. The data presented here describes the strength of the association between analytes, and also the points at which an abnormality in one analyte is likely to also indicate an abnormality in another analyte. Additionally, the occasional unusual association is seen, such as the association of a low creatinine with a high alkaline phosphatase in adults. These relationships provide useful data for exclusion criteria to define a reference population, and aid recognition of both common and therefore also unusual patterns of result abnormalities seen in clinical validation. A typical laboratory produces a vast amount of data which can be easily and cheaply mined for potentially useful information, even if via such a crude method as examining covariance between analytes. The trend towards greater connectivity between databases and towards larger laboratories can only increase the potential power of informatics analysis of these data.

Th127

Clinical usefulness of ELF index in the assessment of non alcoholic fatty liver disease

I Cebreiros López 1, F Guzmán Aroca 1, J A Noguera Velasco 1, C Ramirez Ruiz 1, M Martínez Villanueva 1, I De Miguel Elizada 1, A Martínez Ruiz 1, MD Frutos Bernal 1, JA Lujuan Mompean 1, A Bas Bernal 1
1University Hospital Virgen de la Arrixaca, Clinical Analysis Service, Murcia, Spain, 2University Hospital Virgen de la Arrixaca, Radiology Service, Murcia, Spain, 3University Hospital Virgen de la Arrixaca, Surgery Service, Murcia, Spain, 4University Hospital Virgen de la Arrixaca, Pathology Service, Murcia, Spain

Background: There is a wide spectrum of liver histology in Non alcoholic fatty liver disease (NAFLD), ranging from steatosis to steatohepatitis (NASH), fibrosis and cirrhosis. Steatosis usually remains stable but patients with NASH or fibrosis have a higher risk for complications. Liver biopsy is the standard for the diagnosis of NAFLD but has risks and limitations, so that non-invasive diagnostic tools such as serum biomarkers and imaging methods have been developed.

ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase 1. The result becomes a score without units that indicates the level of fibrosis.

Acoustic Radiation Force Impulse (ARFI) is a imaging technique that provides a quantitative measure of the tissue elasticity and correlates with the degree of fibrosis.

We aimed to assess feasibility of ELF to differentiate NAFLD from NASH and fibrosis in morbidly obese before bariatric surgery using liver biopsy as a reference standard.

Methods: We selected 55 morbidly obese patients who were to undergo bariatric surgery and were classified according to their biopsy findings: group A: normal liver or simple steatosis; group B: inflammation and/or fibrosis. All the patients were evaluated with ARFI (Acuson S2000, Siemens) before surgery and ELF test (ADVIA Centaur, Siemens) was calculated.

Results: Significant differences in Elf results were found between the two groups (p< 0.005). The area under the ROC curve for differentiating patients NASH or fibrosis from those with normal liver or simple steatosis using ELF was 0.741. The cut-off value was 8.72 (71.4%Sensitivity; 74.1%Specificity).

Conclusions: A proper hepatic assessment enabling NAFLD to be differentiated from NASH or fibrosis would be fundamental for establishing a risk population. Our results show that ELF is a useful diagnostic tool for differentiating this in morbidly obesity patients.

Th128

Significant seasonal variations of 25-OHD blood levels reflecting sun exposure in the Czech Republic

K Greplova 1,2, D Valik 1,2
1Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic, 2Masaryk Memorial Cancer Institute, Regional Centre for Applied Molecular Oncology, Brno, Czech Republic

Background: Vitamin D plays an important role in a number of physiological functions including calcium absorption, bone metabolism, immune function, muscle function and cellular regulation. Numerous clinical studies have shown that vitamin D has significant protective
effect against the development of cancer. We studied changes in 25(OH)D serum concentrations in healthy individuals in the spring and autumn to assess difference between late spring and early autumn period in our geographical location.

Materials and methods: The serum aliquots were derived from blood of healthy individuals who visited Masaryk Memorial Cancer Institute (MMCI) to receive preventive health check up focused on early cancer detection. This cancer prevention program is available at MMCI and is not reimbursed by health insurance system. All subjects gave prior written consent to use their biological material. Out of this cohort, 437 blood samples in March and April and 508 blood samples in September, October were taken in sample tube without anticoagulant. Separation serum was stored in short term storage repository of biobank at -30°C. Subsequently, 25(OH)D serum concentrations were measured using Architect i2000sr (Abbott) analyzer.

Results: In the spring group median of 25(OH)D concentration was 42.7 nmol/l (95% confidence interval 21.9-90.1 nmol/l). In the autumn group median of 25(OH)D was 70.8 nmol/l (95% confidence interval 37.0-137.5 nmol/l).

Conclusions: We observed fundamental differences in 25(OH)D concentrations between late spring and late summer which is in our latitude due to exposure to sunlight during the summer. This finding may have substantial implications for populational health. However, our data may be slightly biased by the type of a source population that may carry more active lifestyle than the true population average.

This study was funded by European Regional Development Fund and State budget of the Czech Republic (RECAMO: CZ 1.05/2.1.00/03.0101) and by Ministry of Education, Youth and Sports (BBMRI: LM2010004).

Th129

Systemic inflammatory response syndrome, sepsis: which biomarkers predict an outcome?

H Brodská1, K Malicková1, R Pelínková1, J Valenta1, Z Stach1, R Šach1, T Zima1
1Institute of Medical Biochemistry and Laboratory Diagnostic of the General University Hospital and the First Faculty of Medicine of Charles University, Prague, Czech Republic, 2Department of Anesthesiology and Intensive Care First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

Objective: To analyze inflammatory markers in patients with sepsis and with systemic inflammatory response syndrome (SIRS) after the extracorporeal circulation (EC) to predict severity of status and mortality 28 days in both groups.

Patients and methods: A total of 170 measurements of 60 patients from Intensive Care Units were examined, 29 with SIRS and 31 individuals with sepsis. Procalcitonin (PCT), C-reactive protein (CRP), presepsin (PRE), leukocyte count (WBC), WBC differential (WBC DIFF) and lactate (LACT) were analyzed during 48 hours (onset sepsis, resp. after EC). SOFA score was determined daily in all individuals. Statistical analysis of obtained values was performed with the use of Statistica 9.0 CZ (Statsoft).

Results: As expected, septic patients have shown significantly higher SOFA score compared to individuals with SIRS (p < 0.0001), significantly higher PCT (p< 0.0001), CRP (p< 0.0001), presepsin (p < 0.0001), higher WBC (p=0.049) and lower monocyte counts (p=0.0026). Interestingly, only two survival predictors examined have shown different behavior in septic and SIRS cohorts: high CRP and PRE were biomarkers of imminent non-surviving only in septic group (p=0.002 and p=0.05, respectively), whereas other biomarkers did not differ between cohorts in relation to survival time prediction. Markers to estimate severity of status (correlations with SOFA) were in septic patients PRE(r=0.39),CRP(r= 0.35), in SIRS patients PCT(r= 0.49) in both group LAC(r= 0.582 resp. 0.563).

Conclusion: We noticed different behavior of the above mentioned markers in both defined groups. High CRP and PRE were biomarkers of non-surviving only in septic group. Predilection of severity of status in septic cohort show PRE and CRP unlike PCT in SIRS cohort. This could be helpful in right interpretation of inflammatory markers in ICU patients.

Acknowledgements: Supported by the research projects PRVOUK P25/LF1/2 and RVO VFN 64165/2012 of General University Hospital in Prague.

Th130

Dengue infection in Singapore: laboratory and clinical features

A Pajarillaga, L Ong, S Saw, S Sethi
NUHS, Singapore, Singapore

Introduction: In 2013, there was a rise in dengue infection in Singapore with 20,000 reported cases and 8 dengue-related deaths. We looked at dengue testing in our hospital, and the laboratory and clinical parameters associated with dengue infection.

Materials and methods: Clinical parameters (age, gender, race, days of fever and final diagnoses) and laboratory results (presence of leucopenia, thrombocytopenia or transaminitis) for patients presenting for dengue testing between December 2013 and January 2014 were reviewed. Dengue virus NS1 antigen, IgG and IgM antibodies were performed using SD Bioline Dengue Duo rapid test (Standard Diagnostics, Inc, Korea).

Results: 285 patients underwent dengue testing, with patient mean age of 39, and female: male ratio of 1.23. There were 67% Chinese, 12% Malays, 28% Indians and 11% of other ethnic groups.
175 patients had primary dengue infection (NS1 Ag ± IgM positive), while 43 patients had secondary infection (NS1Ag and IgG ± IgM positive). The median number of days of fever at diagnosis of dengue infection was 4, with 65% having leucopenia and 59% having thrombocytopenia at diagnosis. The mean nadir of platelets was on day 5 of fever, with normalisation by day 7. 35% had transaminitis with up to 3 fold increase. Patients with dengue infection had lower platelets, lower total white count and higher albumin levels compared to those without dengue infection. There were higher platelet and albumin levels in primary infection compared to secondary infection. The mean hematocrit was higher in secondary infection, and there was no association between albumin levels and hemocrit.

Conclusion: 218 patients were diagnosed with dengue infection during our two-month study period, with majority being primary infection. Thrombocytopenia and leucopenia were common with lower platelet levels in secondary infections. Further studies looking at dengue with inflammatory markers may help to explain the higher albumin in dengue infection.

Th131

Cost-effectiveness of alpha-1 antitrypsin deficiency case-finding in secondary care

L Dron1, S Whiting1*, D Thorburn1*, M Pinzani1*, B Gooptu1*, D Lomas1*, J Hurst1*
1 Royal Free London NHS Foundation Trust, Clinical Biochemistry, London, United Kingdom, 2 Basildon and Thurrock University Hospitals NHS Trust, Basildon, United Kingdom, 3 The London Alpha-1 Antitrypsin Deficiency Service, London, United Kingdom, 4 Royal Free London NHS Foundation Trust, London, United Kingdom, 5 University College London, Medical School, London, United Kingdom

Background: Screening for alpha-1 antitrypsin (A1AT) deficiency at our Institution involves initial measurement of serum protein concentration, with subsequent phenotyping if serum A1AT is < 1.1g/L. The newly established multidisciplinary respiratory and hepatology London A1AT deficiency service wished to re-examine the cost-effectiveness of our current diagnostic algorithm.

Method: We examined all unique patients who had had a serum A1AT request made in the three year period 1/1/2010 through 31/12/2012.

Results: Of 4660 A1AT requests between 2010-2012, 240 (5.4%) had serum A1AT < 1.1g/L. Of these, phenotyping was not available in 33 and of the remaining 207 the following phenotypes were present at the following frequencies (n, %): MM (75, 36%), MZ (89, 43%), MS (29, 14%), ZZ (6, 3%), SZ (6, 3%), SS (1, 1%), GM (1, 1%). In error, 28 patients had phenotyping performed when A1AT > 1.1g/l and of these 27 were PiMM and 1 PiMS.

Using the 2010-2012 data, our mean annualised spend on A1AT serum, phenotyping and genotyping was £3,642 £3,170 and £376. £994 was spent on phenotyping patients with an A1AT > 1.1g/L. The highest A1AT serum recorded in the ZZ and SZ patients were 0.4 and 1.0g/l respectively suggesting that the ≤ 1.1g/l cut-off is appropriate. The specificity, sensitivity and positive predictive value of this cut-off are 26.5%, 100% and 6.5% respectively.

Conclusion: 5.4% of serum A1AT results in our Institution are ≤ 1.1g/l. Of these, just 6% are ultimately found to represent the SZ and ZZ variants at increased risk of clinically relevant lung and/or liver disease (0.27%, 1 in 372 requests). The ≤ 1.1g/l cut off was 100% sensitive but only 26.5% specific for detection of clinically relevant deficiency alleles. The 1.1g/l cut off could be lowered with improved specificity and cost saving if the desire was only to detect ZZ patients.

Th132

The establishment of a reference interval for sweat testing in a healthy adult population

M Egan, N Montgomery
1 Sligo Regional Hospital, Sligo, Ireland

Introduction: Cystic Fibrosis (CF) is a genetic metabolic disease that affects the lungs, gastrointestinal system, the pancreas and more. Ireland has the highest incidence of CF in the world; approximately 3 in 10,000 people are born with the condition, and 1 in 19 people are carriers of a mutation in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene [1]. Mutations in the CFTR gene may result in a mild phenotype that may not be detected until adulthood [2]. The sweat test is a useful laboratory procedure that supports the diagnosis of CF. The aim of this study was to establish a reference interval for the measurement of sweat conductivity in adults, as the current reference interval is not specific for adults. Wescor® found that as people age, the variance between a normal and abnormal sweat conductivity result becomes more difficult to define [3].

Materials and methods: The sweat test was performed on 56 adults (40 females and 16 males) using the Webster sweat Inducer, Macroduct sweat collection system and the Wescor Sweat Chek sweat conductivity analyser.

Results: 10 individuals were found to produce sweat conductivity results that fell into or above what is currently defined as the intermediate range of 60-80mmol/l. The new reference interval was established by calculating +/- 2 standard deviations of the mean, producing a range of 18mmol/l- 78mmol/l. Comparing genders, it was found that there is a significant difference between males and females (p-value= 0.012).
Conclusion: The interval established here eliminates the confusion of an intermediate concentration range. The significant difference between males and females may warrant the establishment of gender specific reference ranges. However, as the sample population was relatively small, further study with a larger sample size is required to confirm these findings.

Th133

Design and implementation of a pre-analytical quality system to assess and subsequently reduce pre-analytical errors

JA Atherton, ID Watson, AM Wootton
Aintree University Hospital & Liverpool Clinical Laboratories, Department of Blood Sciences, Liverpool, United Kingdom

Introduction: The majority of errors in the test:request:report cycle occur before samples reach the laboratory. Whilst areas of analytical performance are tightly controlled with IQC and EQA, pre- and post-analytical errors are not formally monitored despite their subsequent impact on patients, the laboratory and the clinical service; problem areas remain unidentified and issues unaddressed. This study was designed to determine the quality assessment of these parts of the cycle and the recognition by service users of their role in the process.

Methods: A quality system aligned to Key Incident Monitoring and Management Systems (KIMMS) criteria was designed and implemented to capture error information directly from the Laboratory Information System (LIS). The data was grouped by location and type before feedback to service users.

Results: Over a 2 year period an average of 2960 pre-analytical errors/month were recorded; an error rate of 5.71%. Not receiving a required sample type was most frequent (38.9%) followed by haemolysis (36.0%). The number of labelling errors, insufficient and haemolysed samples were significantly reduced.

Conclusions: Building error data collection into standard laboratory working practice with the LIS ensured that continuous data quality was of high integrity. Data collection was shown to be much improved when automated systems were used. This work illustrated the need for total quality systems to minimise errors and improve patient safety with a collaborative approach to identify and address areas of concern. The impact of electronic requesting was assessed and shown to be beneficial. This project highlighted issues for pre-analytical error benchmarking such as error classification, recording and proportion calculation and reiterated the need for standardisation before benchmarking can be implemented. The literature is deficient in the laboratory application of quality improvement techniques from other disciplines, and this is an area for further development.

Th134

The biobanking research infrastructure BBMRI_CZ as a critical tool to enhance translational cancer research—an interim report 2014

K Greplova1,2, D Valik2, P Holub1,3, D Knoflickova4, R Nemutl1, R Hájduch5, T Zima6, K Jirsova6, A Ryska7
1Masaryk Memorial Cancer Institute, Regional Centre for Applied Molecular Oncology, Brno, Czech Republic, 2Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic, 3Masaryk University, CERIT-SC, Institute of Computer Science, Brno, Czech Republic, 4Masaryk Memorial Cancer Institute, Department of Pathology, Brno, Czech Republic, 5Palacky University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic, 6Charles University, Ist Faculty of Medicine, Prague, Czech Republic, 7Charles University, Faculty of Medicine, Hradec Kralove, Czech Republic

Background: We introduce the national research biobanking infrastructure, BBMRI_CZ. The infrastructure has been established under governance of the Ministry of Education and became a founding member of the European biobanking infrastructure BBMRI-ERIC. It is designed as a network of individual biobanks where each biobank stores samples obtained from associated healthcare providers.

Methods: The constructed system of biobanks at BBMRI_CZ consists of two types of storage for patient samples-long-term storage (LTS) repository, and short-term storage (STS) repository. The LTS repository collects various types of tissues (tumour, metastases, non-tumour) classified by diagnosis, serum at surgery, genomic DNA and RNA. This part of the biobank is filled with low frequency, typically at the moment of the patient’s primary surgery. STS repository contains sera only and is iteratively updated at each patient visit to the hospital when the blood specimen is taken for the determination of tumour markers. The STS serum repository thus stores leftovers of tumour marker patient material for a period of up to one year.

Results: In the years 2000-2013 biobank in MMCI archived in LTS repository the following numbers of biological material: 33513 tissue aliquots from 8345 patients, 14210 serum aliquots from 4868 patients, 2272 DNA samples. In the course of 2013 the STS repository archived 43014 serum samples.

Conclusions: The unique design of storing not only the tissue material but also longitudinal strings of sera enables access to patient-derived material during the course of the complex patient treatment, thus reflecting pathophysiological and treatment-induced changes in the course of the disease. Designed this way, the research Biobanks will become truly critical tools to enhance translational cancer research. This study was funded by European Regional Development Fund and State budget of the Czech Republic (RECAMO: CZ.1.05/2.1.00/03.0101) and by Ministry of Education, Youth and Sports (BBMRI: LM2010004).
Th135

The importance of education in a world of profile testing

T Staughton, A Purvey
Royal Berkshire NHS Foundation Trust, Biochemistry, Reading, United Kingdom

Introduction: Profile testing is commonly used by laboratories to reduce unnecessary requests and aid a diagnosis. Chest pain profile is used by the emergency department. The chest pain profile in this laboratory does not include troponin. Clinical guidance is provided for the appropriate use of troponin. If chest pain profile is requested without knowledge of the tests included or clinical guidance would MI go undiagnosed?

Methods: A month audit of emergency department chest pain and troponin requests were collected. For troponin results >50ng/L creatine kinase (CK), clinical information and follow up treatment and/or diagnosis were recorded.

Results: 488 patients had a chest pain profile and troponin requested. 59 of these patients had a troponin >50ng/L, 33 with a CK within the laboratory reference range. 19 of these patients had a cardiac related event. Of the 26 patients with elevated CK and troponin >50ng/L 17 had cardiac events.

Conclusions: Troponin >50ng/L was used as a cut off as clinical guidance indicated this was more likely to be abnormal, possibly representing an MI.

If profiles were followed with little education of the tests included or clinical input life changing diagnosis could be missed. Chest pain profile alone would not have initiated investigations on 19 patients that had cardiac events. These patients may have presented early, prior to a rise in CK. Information provided advocates the use of a second troponin to aid rule in/out MI, this was used successfully in 4 patients with troponin >50ng/L with raised CK to rule out MI, and 10 patients with a troponin>50ng/L with normal CK, 7 of which were ruled out.

This underlines the importance of communication between laboratory and clinical staff so tests are used effectively and exploited for a good clinical outcome.

Th136

Unequal utilization of screening tests in primary health care centers in Sweden-should laboratory professionals be involved?

G Schedvin, C Hedberg, K Roback
Linköping University, Department of Medical and Health Sciences, Linköping, Sweden

Introduction: Large differences exist in utilization of laboratory tests among Primary Health Care Centers (PHCC) in Sweden. The National Board of Health and Welfare provides recommendations for the use of laboratory test for population screening. The aim of this study is to compare and evaluate the use of three different tests performed by PHCCs in relation to national recommendations and disease prevalence in relevant populations, Ferritin-testing among fertile women, Faecal occult blood testing (FOBT) among elderly persons and PSA-screening among men. Estimated prevalence in Sweden of depleted iron stores is about 30%, colorectal cancer 1% and prostate cancer 3%.

Method: This is a registry study of test use in 40 PHCCs in a Swedish county. Study populations are limited to women 18-40 years old in the case of ferritin testing (recommendations non-existent); 60-74 years old for FOBT (should be offered according to national recommendations); and men 50-70 in PSA-screening (should not be actively offered). The number of tests ordered by each PHCC during 2013 was extracted from the Östergötland County Council laboratory Medicine information system.

Results: The proportion tested, in the defined populations, varied considerably among PHCCs. With respect to estimated prevalences and current recommendations, we found a general underuse of Ferritin and FOBT-testing, with variations of 1,6-12% and 1-3,6%, respectively, and an overuse of PSA-testing, with a variation of 5-18%.

Conclusions: National recommendations appear to be inadequate as a means of harmonizing test use. Laboratory feedback is one way of combating inappropriate testing. Further research should be initiated to strengthen the role of the laboratory and citizen involvement in test utilization. In the case of ferritin-testing, where no recommendations exist, we suggest a “test and counselling” strategy to empower the citizens and increase the proportion of women tested.

Th137

Serum ADA in diagnosis of sputum negative pulmonary/extra-pulmonary tuberculosis

J Kaur1, C Prashanth2, R Katyal3, H Kaur4, D Aggarwal2, V Saini2
1Govt. Medical College & Hospital Chandigarh, Biochemistry, Chandigarh, India, 2Govt. Medical College & Hospital Chandigarh, Pulmonary Medicine, Chandigarh, India

Introduction: The global tuberculosis epidemic results in nearly two million deaths. Definitive diagnosis of tuberculosis requires demonstration of mycobacterium tuberculosis which needs investigations like simple sputum examination, invasive investigations like bronchoscopy,
biopsy and FNAC or culture. Also extrapulmonary tuberculosis (EPTB) is a growing problem worldwide. Approximately 75% of EPTB are smear negative and the diagnosis is often difficult and delayed. Adenosine deaminase (ADA) is an enzyme which contributes in the propagation and differentiation of lymphoid cells, especially T-cells. Activity of ADA increases in TB patients. However limited data is available in sputum negative and EPTB.

**Aim:** To evaluate the role of serum ADA in diagnosis of EPTB

**Method:** In a cross sectional study 130 patients were recruited who were admitted in pulmonary medicine ward. Along with other required investigations serum ADA was done by enzymatic method (Diazyme kit on Modular P 800 Rosh hitachi)

**Result:** Out of 130 patients 19 were AFB positive, 26 were sputum negative pulmonary or EPTB and 85 patients were suffering from Non-tubercular respiratory diseases. The mean value of serum ADA in sputum positive TB patients was 26.89 ±10.42 IU/L, in sputum negative pulmonary/ EP TB cases it was 40.73±18.44 IU/L and in patients with other respiratory diseases it was 16.29±5.48 IU/L. There was statistically significant difference in mean ADA levels between the sputum positive and negative groups (p< 0.005) as well as between TB and non-TB patients (p< 0.0001). Highest levels of ADA were found in EPTB.

**Conclusion:** Use of serum ADA levels as a diagnostic marker in sputum negative pulmonary/ EPTB shows promising results. Determination of serum ADA is cheap and simple. Studies with large population group is required to validate its result as a routine diagnostic investigation.

**Th138**

**Pre-analytical errors and electronic requesting: has anything improved?**

*R Jennings, JW Jones, P Williams, J Billington, V Mishra*

Royal Liverpool & Broadgreen University Hospitals NHS Trust, Clinical Biochemistry and Metabolic Medicine, Liverpool, United Kingdom

**Aim:** The use of electronic requesting through systems such as the Integrated Clinical Environment (ICE) system has been suggested as a possible solution to pre analytical errors in the laboratory. This retrospective audit aimed to assess the impact that the ICE system has had on pre analytical errors.

**Methods:** In order to assess the impact of ICE on pre analytical error rates, the in-patient samples received by Blood sciences laboratory that contained a pre analytical errors were gathered from the LIMS system (TelePath) at two time points; prior to implementation of ICE in 2006(PREICE) and once ICE has been implemented in 2012(POSTICE).

**Results:** The major pre analytical issue PREICE was the proportion of errors due to incorrect sample selection and this continued POSTICE. The total number of unsuitable samples received PREICE and POSTICE, remained similar. The total number of errors received has not improved since POSTICE and remains approximately 1.5% on a monthly basis. This indicates that there has been little improvement in the number of pre analytical errors being made despite additional information provided by ICE. Whilst the ICE system appears to have had little effect on pre analytical errors rates it has resulted in major benefits in laboratory errors and the reporting of results.

**Conclusions:** Introduction of ICE failed to produce a significant improvement in pre analytical errors. This highlights the need to educate our users in using ICE for test requesting.

**Th139**

**Leonardo da Vinci partnership project: enhance it**

*T Šálek1, J Martin2, V Gasljevic3, A Horvath4, C Borg5, Z Meštrić Flegar6, M Jakovic3, J Šilhavik1, A Adonics4, Z Szlamka3, I Brincat5, A Adkins7, T Bennett8, K Rice9, Y Taylor10*

1Tomas Bata Regional Hospital Inc., Zlín, Czech Republic, 2Wolverhampton University, Wolverhampton, United Kingdom, 3Croatian Metrology Society, Zagreb, Croatia, 4Horvath and Dubecz Consulting Ltd, Budapest, Hungary, 5Mater Dei Hospital, Msida, Malta, 6Clinical Immunology Service, University of Birmingham, Birmingham, United Kingdom, 7Shrewsbury and Telford Hospital Shrewsbury, Shrewsbury, United Kingdom, 8New Cross Hospital, Wolverhampton, United Kingdom, 9Birmingham Children’s Hospital, Birmingham, United Kingdom

**Aim:** The aim of this Partnership project is to share and develop good practice in continuing professional development for biomedical scientists and from this to collaboratively develop an EU-toolkit for delivery of high quality continuing professional development activities provided by European hospital laboratories.

**Methods:** University of Wolverhampton (UK), Department of clinical biochemistry of Tomas Bata hospital in Zlín (CZE), Pathology department of the Mater Dei Hospital of Malta, Croatian Metrology Society and Horvath and Dubecz Consulting, Ltd., Budapest (Hungary) are participants of the project. The project is divided into five parts:

- identify core elements of good practice by the comparison of approaches to CPD used within partner organizations and countries
- define European quality standards and criteria for accreditation and evaluation of local hospital CPD activities
- develop a framework for inclusion of reflective practice in CPD activities
- produce guidelines for European hospital laboratories on managing and organizing quality CPD opportunities for laboratory staff
- devise exemplar hospital laboratory CPD activities for provision on a new European hospitals CPD providers Community of Practice network.

**Results:** The first exemplar activity and first project part has been completed, the common website www.enhanceit.eu has been created. Part 1 of the toolkit, including its checklist, has been completed to general positive acceptance of all participants-both trainers and trainees.

**Conclusion:** The project has successful progress and we hope it improve CPD practice in European Union.

**Th140**

Cryoglobulins and autoantibodies: anti-nuclear antibodies detection in cryoprecipitates of hepatitis C virus-positive patients

U Basile, F Gulli, E Torti, N de Matthaeis, L Colacicco, P Cattani, GL Rapaccini
Catholic University of the Sacred Heart, Department of Laboratory Medicine, School of Medicine, Rome, Italy

Anti-nuclear antibodies (ANAs) are immunoglobulins (Igs) specific for self-antigens contained within the nucleus of cells. ANA levels are especially elevated in a subset of autoimmune diseases. ANAs are also found in HCV-positive (HCV+ve) patients, indicating an association between HCV infection and the onset of several autoimmune diseases. Moreover, Mixed Cryoglobulinemia (MC) often accompanies autoimmune diseases and HCV. Despite recent advances in research, early discrimination of HCV+ve patients at risk of autoimmune disease development is not straightforward. Our study therefore aims at comparing ANAs in cryoprecipitates of HCV+ve versus RA-positive patients and the differential content of Ig subclasses in cryoprecipitates from each cohort, in order to assess their predictive value for the onset of extrahepatic diseases in HCV affected individuals.

**Materials and methods:** 40 HCV+ve patients with no symptoms of autoimmune diseases were recruited along with 50 HCV-negative controls with Rheumatoid Arthritis (RA). All patients had Type III MC. Samples were processed at 37°C and serum was transferred to Wintrobe tubes for storage (15 days at 4°C). An aliquot was retained for RF testing (AXA Diagnostics, Italy). Supernatant and resuspended cryoprecipitate were tested for ANA by Indirect Immuno Fluorescence (IIF) on HEp-2 cells (INOVA, USA), and analysed by microscopy. Ig subclasses were assessed by Immunofixation (Sebia, France).

**Results and conclusions:** Differential IIF patterns suggest Ig subclasses in cryoprecipitates from HCV+ve patients differ from RA controls (Fig. 1). Moreover, 26/40 HCV+ve patients were IgG3 positive: of these, 25 were ANA positive (Fig. 2, 3). IgG3 are autoreactive clones which are not specifically directed to the viral capsid but can activate several cell clones: this implies they are likely to constitute the decisive factor for activation of autoimmune mechanisms These results may be a valid diagnostic tool for early detection of autoimmune onset in HCV-affected patients.

**Th141**

Procalcitonin in an intensive treatment unit (ITU): an economic evaluation

A Boldger1, J Davies2, A Johnson3, M Wheeler4
1Wye Valley NHS Trust, Dept Histopathology, Hereford, United Kingdom, 2Wye Valley NHS Trust, Blood Sciences, Hereford, United Kingdom, 3Wye Valley NHS Trust, Dept Microbiology, Hereford, United Kingdom, 4Wye Valley NHS Trust, A&E, Hereford, United Kingdom

**Aim:** This was a retrospective study to assess the cost effectiveness of procalcitonin (PCT) in an ITU setting to guide antibiotic treatment.

**Method:** Patients meeting the inclusion criteria with suspected sepsis admitted to ITU were recruited into the study. All patients followed their normal treatment regimes and samples for PCT were taken from the routine bloods collected on day admissions and days 2, 3 and 5. Samples were frozen for PCT measurement at the end of the study. Details of antibiotic treatment at the time of PCT measurement were obtained from the patient notes. Of the PCT was < 0.5 ng/mL, it was judged there was no evidence of bacterial infection and the patient could have been taken off antibiotics.

**Results:** Full data of antibiotic treatment was available for 24 patients. Only 3 patients had a PCT < 0.5 and on this basis could potentially have not started on antibiotics; no patients could have had their antibiotic treatment stopped on the basis of the PCT result. The cost of treatment in these patients ranged from £31.01 to £34.26; this compared to a cost of PCT £27 per sample (including quality control but not allowing for kit wastage).

**Conclusion:** The of PCT in an ITU setting may be a cost effective tool in deciding whether or not antibiotic treatment should be started. It may also be a valuable in limiting unnecessary antibiotic usage.
The global scenario of lab medicine: a SWOT analysis

KS Sehravat
Kalawati Saran Children's Hospital, Department of Lab Medicine, New Delhi, India

Background: Lab Medicine is a branch of medical science deals with the performance of various clinical investigations by various scientific tools and techniques for an early and accurate diagnosis, treatment and management of diseases. It is also known as Medical Laboratory Technology-MLT, Clinical Laboratory Science-CLS, Biomedical Laboratory science-BLS etc. Medical laboratory professionals, who perform this scientific task, are known globally by various names i.e. clinical laboratory Scientist, Biomedical Laboratory Scientist, Medical Technologist-MT, Lab Supervisor, Laboratory Manager, Lab Superintendents, Laboratory Technologist and Lab Technicians etc.

Historically, medical laboratory work has been the part of medicine itself, if we look in the earlier days of medical science it was not an independent discipline as of today. Many investigations were neither available nor affordable.

Objectives: To present a global SWOT analysis of Lab Medicine Profession & its Professionals for dealing with upcoming professional challenges.

Method: Relevant data related to existing regulatory & administrative framework in major countries for medical labs were collected from various professional associations/institutions worldwide and examined in detail.

Results: After a detail SWOT analysis as per available information form different part of the world, the strength, weakness, opportunities and possible threats have been recorded.

Conclusion: Due to emergence of new technology and revolutionary developments in medical sciences, Lab Medicine is also undergoing a sea change and has exponential growth. It is the need of hour for Lab professionals’ community worldwide to think outside the box to meet the emerging professional challenges and optimize the Lab services for better patient care.

Dry ice-stability and length of storage

U Nxamngoxa, MZ Warasally, MJ Turzyniecka
National Health Laboratory Service-Inkosi Albert Luthuli Central Hospital and University of Kwazulu-Natal, Chemical Pathology, Durban, South Africa

Background: Dry ice is a solid form of carbon dioxide (CO₂) and serves as an efficient coolant. It is used for transportation of biological samples to ensure their stability. At -78.5 °C it undergoes sublimation to CO₂ thus dry ice amount decreases during its storage. This has serious implications for the laboratories that do not have their own dry ice makers and resort to purchasing and storing dry ice.

The aim of this study was to determine the stability of dry ice at different temperatures during its storage.

Methods: 3 blocks of dry ice weighing 947, 1015 and 1086 grams respectively were purchased from a supplier. They were placed in the separate cooler boxes and immediately transported to the laboratory where they were weighted in their container and stored in -80, -40 and -20 °C freezers. The boxes were weighed twice daily at the same time until the dry ice completely evaporated. The percentage decrease in weight of dry ice over time was estimated.

Results: The weight of dry ice stored at -80, -60 and -20 °C decreased by 10%, 61% and 71% respectively within the first 24 hours of storage. After further 40 hours of storage the weight of dry ice stored at -40 and -20 °C decreased further by 28% and 90% respectively. The weight of dry ice stored at -80 °C in comparison to baseline decreased by 26% at 48 hours, 51% at 96 hours, 61% at 120 hours, 73% at 144 hours, 86% at 168 hours, 94% at 192 hours (day 8) and by 97% at 216 hours.

Conclusions: The temperature of dry ice storage has a significant effect on its stability. Use of -80 °C freezer allows effective storage dry ice up to 4-6 days.

An audit of requests for specialised liver function tests following the introduction of a new protocol-a demand management initiative

PS West
North Middlesex University Hospital NHS Trust, Biochemistry, London, United Kingdom

Introduction: In our Trust, doctors often requested a ‘liver screen’ with the more specialised expensive tests requested as well as the more routine ones.
As a result, the Consultant Biochemist and Lead Consultant for Gastroenterology devised a liver blood test protocol which described details of both the routine and the more specialised tests and their clinical significance. For alpha 1 antitrypsin, copper and caeruloplasmin it stated that these should only be requested by or following discussion with a Gastroenterology Specialist Registrar or Consultant and for alpha 1 antitrypsin phenotyping after discussion with or requested by either a Gastroenterology or Respiratory Specialist Registrar or Consultant. Requests for alkaline phosphatase isoenzymes were sent if specifically requested by a Gastroenterology Consultant. Requests for copper were additionally accepted for the Consultant Haematologist where levels were useful in both his Hodgkin’s disease and Non Hodgkin’s Lymphoma patients.

**Aim of the audit:** To see what cost savings could be made following introduction of the liver blood test protocol.

**Method:** All requests for the specialised tests were vetted over a nine month period by the Consultant Biochemist. Appropriate comments referring to the protocol were entered onto the reports for those requests not deemed relevant and samples kept for one month in case of any query.

**Results:** Over the audit period there were 172 requests for alpha 1 antitrypsin with 20 sent, for alpha 1 antitrypsin a single request which was sent, for copper 223 with 82 sent, for caeruloplasmin 193 with 64 sent and for alkaline phosphatase isoenzymes 31 with 6 sent. There were no calls to discuss any non processed requests.

Over the nine months, £5890 was saved.

**Conclusion:** In the current climate in the NHS where Pathology is being asked to make savings, biochemists should work with their clinical colleagues in similar demand management initiatives.

**Th145**

**“Add-on” test requests: which analytes are suitable for delayed analysis?**

_cite_ BL Kift, C Byrne, R Liversidge, F Babington, C Knox, J Binns, JH Barth_  
_Leeds Teaching Hospitals NHS Trust, Blood Sciences, Leeds, United Kingdom_

**Introduction:** “Add-on” tests are often requested for samples already in the laboratory. This avoids further venepuncture, but how stable are samples in modern laboratory environments, which now involve sample managers attached to robotic tracking systems? At Leeds General Infirmary, samples are stored uncapped in a sample manager module post-analysis, sometimes for up to 16 hours until they are removed for cold storage, leaving the samples susceptible to evaporation. This study was carried out to assess the stability of common analytes in samples stored in such conditions.

**Methods:** 20 samples from GP surgeries containing ∼2 mL serum were selected and analysed immediately following their arrival in the laboratory. The samples were then split into two sets of 10 to evaluate two different storage conditions:

- **Set 1-Samples were stored in the sample manager module for 16 hours post-analysis, re-analysed, stored at 4 °C for three days, and analysed a third time.**
- **Set 2-Samples were stored at 4 °C immediately following analysis for a period of four days, and then re-analysed.**

The total storage time was four days for both sets of samples.

**Results:** Significant differences in concentration between baseline and final measurement in set 1 were observed for several analytes, including sodium, potassium, chloride, total protein, albumin and adjusted calcium. For many of these analytes, significant differences were also observed between baseline and final measurement in set 2, indicating that storing the samples at 4 °C immediately following analysis may not maintain sample integrity for all analytes. However, the data suggest that some analytes are stable four days after collection when samples are stored at 4 °C immediately following analysis.

**Conclusions:** “Add-on” tests can only be safely performed on a proportion of routine analytes. For some analytes, storage conditions affect their suitability for delayed analysis.

**Th146**

**An audit of immunology requests in a district general hospital**

_cite_ PS West, S Moochhala, I. Flanders, S Duncan  
_North Middlesex University Hospital NHS Trust, Biochemistry, London, United Kingdom, North Middlesex University Hospital NHS Trust, Renal, London, United Kingdom_

**Introduction:** Cost savings are a priority within the NHS with Trusts looking at ways to reduce expenditure. Pathology has an important contribution to make.
Biochemistry in our Trust was receiving a large number of requests for the highly specialised immunology tests and questioning whether all of these were justified.

**Aim of the audit:** To assess the appropriateness of requests with the aim to produce a guideline following the audit.

**Method:** A list of all the highly specialised tests over one year and source of requests were sent to the renal team to follow up.

**Results:** There were 8712 requests which were stratified according to Consultant and department. The high number of requests from the renal and rheumatology departments was unsurprising. However, there were a large number of requests from accident and emergency, many asking for four or more tests. As some tests take a week to process, an investigation was made to see how patients presented, whether they were admitted and whether there was any follow up of results.

Clinical details of requests from accident and emergency included weakness, headache, weight loss and shortness of breath. In those patients in whom four or more tests were requested, 19% were not admitted and of these, 48% had no follow up.

Individually the cost of these investigations is relatively low but due to the large volume performed, the total cost over the year was significant at £20,955.32.

**Conclusion:** Educating medical professionals about appropriate indications for requesting specialised tests is important and therefore a guideline was produced by the Consultant Biochemist together with clinical colleagues emphasising that indications for these tests be documented on the request form and with which specialty it was discussed or the test would not be processed. It is intended to perform a subsequent audit to gauge the effect of the guideline.

---

**Th147**

An audit of the effect of recommending HbA1c for diagnosing type 2 diabetes on requests for the oral glucose tolerance test in a district general hospital

*PS West*

North Middlesex University Hospital NHS Trust, Biochemistry, London, United Kingdom

**Introduction:** Until 2011 the oral glucose tolerance test (OGTT) was the gold standard for diagnosing Type 2 diabetes but despite this had its limitations.

In 2011 the WHO and Diabetes UK recommended using HbA1c with a cut off of 48 mmol/mol for its diagnosis and listing situations where it could not be used.

Our Trust still experienced a high number of OGTT requests from primary care so in July 2013 the Consultant Biochemist issued a memorandum to general practitioners based on the recommendation and additionally placed on the local Clinical Commissioning Group website.

**Aim of the audit:** To see the effect of issuing the memorandum on the number of OGTT requests.

**Method:** The number of requests were audited for 8 months following and compared to the number 8 months prior to its issue.

**Results:** There were 670 requests before and 79 after, a reduction of 89%.

61 of the 79 showed a non diabetic response (normal, impaired fasting glycaemia or impaired glucose tolerance) and 18 a diabetic response to the OGTT.

Of the 79, 29(37%) additionally had an HbA1c either at the time of the OGTT or shortly before. In one patient this was 3 weeks after.

All those showing a non diabetic response had an HbA1c below 48 mmol/mol.

All but one showing a diabetic response had an HbA1c above this value.

The one exception with a value of 45 mmol/mol had HbA1c 3 weeks after the OGTT so may have commenced treatment.

**Conclusion:** The audit had a massive effect on requests for the OGTT.

This was beneficial to the patient who did not have to book the test nor fast, for the phlebotomist who could attend other patients, for pharmacy in savings for the lucozade and for biochemistry in a reduction in the number of glucose tests performed.

---

**Th148**

An audit of requests for serum HCG sent in early pregnancy to biochemistry in a district general hospital

*PS West1, E Kirk2, K Creswell2, P Corson2*

1North Middlesex University Hospital NHS Trust, Biochemistry, London, United Kingdom, 2North Middlesex University Hospital NHS Trust, Obstetrics and Gynaecology, London, United Kingdom

**Introduction:** Biochemistry in our Trust was experiencing a vast number of requests sent for serum HCG from both Accident and Emergency and the Early Pregnancy Assessment Unit for assessing early pregnancy and questioned whether all these were appropriate.
As a result, the Consultant Biochemist contacted one of the Consultants in Obstetrics and Gynaecology explaining the problem and following this, this was sent by her to relevant staff explaining that ultrasound should be the primary diagnostic test for all women presenting with pain and bleeding in early pregnancy with a serum HCG only sent in women with inconclusive scans, ultrasound visualised ectopic pregnancies and with ultrasound suspected molar pregnancies and not in those with a negative urine pregnancy test.

Additionally a laminated flow chart giving indications when to and when not to send a serum HCG was placed in both locations.

**Aim of the audit:** To assess the effect of both the memorandum and laminated flow chart on the number of requests for serum HCG.

**Method:** The relevance of 100 serum HCG requests taken before and after their issue were evaluated by the Obstetric and Gynaecology team using a software programme called Viewpoint which was used to identify the date of the scan and outcome.

**Results:** Prior to issuing the memorandum and flow chart, 84% of the 100 requests were deemed to be inappropriate.

Following their issue this number was reduced to 59%.

Most of the inappropriate requests were from Accident and Emergency.

**Conclusion:** Although there had been some reduction in requests it was again emphasised to Accident and Emergency that serum HCG should only be requested via the Early Pregnancy Assessment Unit or by a gynaecologist.

The audit will be repeated in three months to see whether the number of inappropriate requests has been further reduced.

**Th149**

**How do members of the public respond to a vitamin D result?**

**RL Shea, J Berg**  
City Hospital, SWBH NHS Trust, Clinical Biochemistry, Birmingham, United Kingdom

**Introduction:** A direct-to-the-public dried blood spot (DBS) vitamin D testing service was launched by SWBH in 2011, and has been very successful. There are conflicting messages regarding vitamin D supplementation and testing available to the public so we were interested to see how they responded to an initial vitamin D result by looking at people who had repeat vitamin D tests.

**Methods:** Data was gathered from 01/05/2011 until 31/03/13 for DBS vitamin D results. Duplicate records were identified by first name, surname and date of birth. If parameters did not agree then the record was ignored. Age, sex, date samples were received in the laboratory and results were recorded. Vitamin D status was defined in the following way:

- **Severely Deficient** = 10.3-14.9nmol/L
- **Deficient** = 15-30nmol/L
- **Insufficient** = 30.1-50nmol/L
- **Adequate** = 50.1-220nmol/L
- **High-to-Toxic** = 220.1-500nmol/L

**Results:** In total, 5,534 samples were received with 1,136 repeat samples identified. 476 people sent in repeat samples, ranging from 2-10 samples. Mean time to repeat testing was 132 days (range 1-526). Repeat tests showed a vast improvement in concentration and status. 4.6% were severely deficient on initial test and only 0.2% were severely deficient on first repeat testing. Median concentration went from 51.3nmol/L to 82.7nmol/L. The proportion of adequate patients went from 49.2% to 82.6%. Only 7.6% found that their status decreased after retesting, however several people needed to decrease their status from high-to-toxic and did so by becoming adequate. Therefore only 5% actually saw a deterioration in their status.

**Conclusion:** Members of the public are very adept at interpreting their vitamin D results and managing their therapy with Vitamin D. Very few people saw a deterioration in concentration/status and nearly everyone that needed to increase their levels did. When the public has a personal interest in achieving a health outcome, such as monitoring their own status, they can clearly achieve this for Vitamin D.

**Haematology**

**Th150**

**The prevalence of low vitamin B<sub>12</sub> status assessed using holotranscobalamin with methylmalonic acid versus stand alone serum vitamin B<sub>12**

**A Sobczynska-Malefora<sup>1</sup>, B Witchlow<sup>2</sup>, DJ Harrington<sup>1</sup>**  
<sup>1</sup>Viapath, Nutristasis Unit, Haemostasis & Thrombosis, London, United Kingdom, <sup>2</sup>Viapath, Laboratory Informatics, London, United Kingdom

Low vitamin B<sub>12</sub> status is common in patient populations, especially in the elderly (as a consequence of malabsorption), vegetarians/vegans (restricted intake) and pregnancy/neonates (increased requirement). The timely detection and correction of low vitamin B<sub>12</sub> prevents anaemia,
To identify patients with ID in clinical practice, the Thomas-plot should be used in combination with ferritin as single marker.

**Conclusion:**

With functional ID from patients without functional ID for the parameters sTfR, ferritin and Delta-He were 0.84, 0.73 and 0.72, respectively. Whereas the Thomas-plot did not indicate latent or functional ID. The area under the curve (AUC) for the discrimination between patients with functional ID [quadrant 3 and 4], whereas the ferritin-value was $<30 \text{ ng/ml}$. Conversely, 5.6% (25/445) had a ferritin-value $\geq 30 \text{ ng/ml}$. In total 34.4% (n = 153) presented ID with the Thomas-plot. Of them 40.5% (62/153) had latent ID (quadrant 2) and 59.5% (91/153) had functional ID (quadrant 3 and 4). If ID was defined as a ferritin-value $<30 \text{ ng/ml}$, only 23.5% (105/445) were functional ID. Delta-hemoglobin (Delta-He) was calculated as CHr-MCH. ROC-curves for the parameters ferritin, sTfR and Delta-He were calculated to compare the ability to discriminate patients with functional ID from patients without functional ID.

**Results:**

In total 445 hospitalized adult patients were studied. The CHr and sTfR/log ferritin ratio were used in form of the Thomas-plot (quadrant 1-4). Functional ID was defined as a CHr $<28 \text{ pg.}$ Using ferritin as single marker, a plasma-level of $<30 \text{ ng/ml}$ was defined as ID. Delta-hemoglobin (Delta-He) was calculated as CHr-MCH. ROC-curves for the parameters ferritin, sTfR and Delta-He were calculated to compare the ability to discriminate functional ID from patients without functional ID.

**Background:**

This study was conducted to compare the results of the Thomas-plot (reticulocyte hemoglobin content [CHr] and soluble transferrin receptor [sTfR]/log ferritin ratio) with the classical single marker ferritin in identifying patients with iron deficiency (ID). Furthermore, Delta-hemoglobin (Delta-He) as potential marker for the discrimination of patients with functional ID from patients without functional ID was evaluated.

**Methods:**

In total 445 hospitalized adult patients were studied. The CHr and sTfR/log ferritin ratio were used in form of the Thomas-plot (quadrant 1-4). Functional ID was defined as a CHr $<28 \text{ pg.}$ Using ferritin as single marker, a plasma-level of $<30 \text{ ng/ml}$ was defined as ID. Delta-hemoglobin (Delta-He) was calculated as CHr-MCH. ROC-curves for the parameters ferritin, sTfR and Delta-He were calculated to compare the ability to discriminate functional ID from patients without functional ID.

**Results:**

In total 34.4% (n = 153) of all the patients (n = 445) presented ID with the Thomas-plot. Of them 40.5% (62/153) had latent ID (quadrant 2) and 59.5% (91/153) had functional ID (quadrant 3 and 4). If ID was defined as a ferritin-value $<30 \text{ ng/ml}$, only 23.5% (105/445) were identified as ID. A total of 16.6% (74/445) presented ID with the Thomas-plot (44.6% [33/74] had latent ID [quadrant 2] and 55.4% [41/74] had functional ID [quadrant 3 and 4]), whereas the ferritin-value was $\geq 30 \text{ ng/ml}$. Conversely, 5.6% (25/445) had a ferritin-value $<30 \text{ ng/ml}$, whereas the Thomas-plot did not indicate latent or functional ID. The area under the curve (AUC) for the discrimination between patients with functional ID from patients without functional ID for the parameters sTfR, ferritin and Delta-He were 0.84, 0.73 and 0.72, respectively.

**Conclusion:**

To identify patients with ID in clinical practice, the Thomas-plot should be used in combination with ferritin as single marker.

---

**Th151**

**Laboratory tools and markers for the diagnosis of iron deficiency**

D Enko, R Stolba, E Rezanka, F Wallner, G Halwachs-Baumann

Central Hospital Steyr, Department of Laboratory Medicine, Steyr, Austria

**Background:** This study was conducted to compare the results of the Thomas-plot (reticulocyte hemoglobin content [CHr] and soluble transferrin receptor [sTfR]/log ferritin ratio) with the classical single marker ferritin in identifying patients with iron deficiency (ID). Furthermore, Delta-hemoglobin (Delta-He) as potential marker for the discrimination of patients with functional ID from patients without functional ID was evaluated.

**Methods:** In total 445 hospitalized adult patients were studied. The CHr and sTfR/log ferritin ratio were used in form of the Thomas-plot (quadrant 1-4). Functional ID was defined as a CHr $<28 \text{ pg.}$ Using ferritin as single marker, a plasma-level of $<30 \text{ ng/ml}$ was defined as ID. Delta-hemoglobin (Delta-He) was calculated as CHr-MCH. ROC-curves for the parameters ferritin, sTfR and Delta-He were calculated to compare the ability to discriminate functional ID from patients without functional ID.

**Results:** In total 34.4% (n = 153) of all the patients (n = 445) presented ID with the Thomas-plot. Of them 40.5% (62/153) had latent ID (quadrant 2) and 59.5% (91/153) had functional ID (quadrant 3 and 4). If ID was defined as a ferritin-value $<30 \text{ ng/ml}$, only 23.5% (105/445) were identified as ID. A total of 16.6% (74/445) presented ID with the Thomas-plot (44.6% [33/74] had latent ID [quadrant 2] and 55.4% [41/74] had functional ID [quadrant 3 and 4]), whereas the ferritin-value was $\geq 30 \text{ ng/ml}$. Conversely, 5.6% (25/445) had a ferritin-value $<30 \text{ ng/ml}$, whereas the Thomas-plot did not indicate latent or functional ID. The area under the curve (AUC) for the discrimination between patients with functional ID from patients without functional ID for the parameters sTfR, ferritin and Delta-He were 0.84, 0.73 and 0.72, respectively.

**Conclusion:** To identify patients with ID in clinical practice, the Thomas-plot should be used in combination with ferritin as single marker.

---

**Th152**

**The inappropriate requesting of haemochromatosis genetic testing instigated by high ferritins**

A Patel1, S Francis2

1Royal Wolverhampton NHS Trust, Haematology Department, Wolverhampton, United Kingdom, 2Royal Wolverhampton NHS Trust, Wolverhampton, United Kingdom

Genetic testing (HFE) for haemochromatosis is recommended in the presence of high ferritin and transferrin saturation (TS) greater than 50%. We reviewed all requests for HFE genotyping in one year at a large district hospital.

87 patients had HFE testing. Median age was 56.4 years with 58 males. 46 requests were from gastroenterology, 37 from haematology and 4 from general physicians. 40/46 gastroenterology requests were for high ferritin and abnormal liver function, 5 for isolated high ferritin and 1 for family history of HCT. 32/37 haematology requests were for high ferritin and five for related siblings. Remaining four from general physicians was for high ferritin.

Average ferritin was 1408.1 (range 20.5-6375ng/ml). Only 25/87 patients (28.7%) had TS greater than 50%. Six patients had no TS requested, and none had fasting TS. Only 3/78 patients were homozygous for the C282Y mutation while eight had C282Y/H63D compound heterozygosity. Those with mutations had average TS of 64.3% (range 33-83%). One patient with TS of 33% was identified following family testing. All eleven patients had normal ultrasound scans.
27 patients in this study had liver disease/fatty liver with average ferritin of 1705ng/ml, 15 patients had cancer with a ferritin 2844ng/ml, 5 patients had rheumatological disorders with a ferritin of 703ng/ml, and 29 patients had high ferritins due to infection/inflammatory conditions with ferritins of 635ng/ml.

HFE testing is commonly triggered by high ferritins, but this approach identified only 11/78 cases with GH. Ideally high ferritin should be followed by TS estimation prior to genetic mutation analysis. When TS >50% is used as the screening method for HFE testing, there is a >90% sensitivity. Patients with haemochromatosis are presenting with biochemical evidence of iron overload but are otherwise asymptomatic. They have ferritins less than 1000ng/ml at diagnosis. Patients with ferritin>1000ng/ml may need to investigated for cancer.

Th153

Heterozygous hemoglobin G-Waimanalo [alpha 1 or alpha 2 64 (E13) Asp-Asn] does not cause overestimation of the HBA1C proportion in routine HPLC

Ic Vella Ramirez, D Beneitez Pastor, M Gaona Palomo, A Blanco Alvarez, J Fernández Castro, S Casais
1Hospital Universitario de Burgos, Burgos, Spain, 2Hospital Vall d’Hebron, Barcelona, Spain

We report here the findings in a case of hemoglobin G-Waimanalo [alpha 1 or alpha 2 64 (E13) Asp-Asn] that do not cause overestimation of HbA1C in our routine HPLC analysis. A 63-years-old woman, a well-controlled and free of complications type 2 diabetes patient. The blood glucose level was 132 mg/dL and the HbA1c by HPLC was 5.9%, and this results matched with the expected value taking in account her previous glucose results two months before, and the the equation that translate Hb A1C into average blood glucose. HbA1C by HPLC was performed on an AdamsTM A1c HA-8160, a Menarini Diagnostics full automated system, showing a Hb peak of 18.9% labeled as “variant”, that had an electrophoretic mobility on agarose gel at pH 8.6 migrating like Hb S and on citrate agar gel at pH 5.6, not separated from Hb A1. These characteristics matched with an alpha globin variant of Hb, like Hb Stanleyville-II, Hb Russ or others variants. We perform the molecular characterization of the variant using standard methods Sequence analysis revealed the substitution GAC®AAC at codon 64, corresponding to the amino acid replacement Asp-Asn at position 64 (E3) of the alpha 1 globin gene, as the Hb G-Waimanalo or Hb Aida (alpha 64 Asp-Asn), but the mutation was detected in alpha1 gen [alpha 1 64 (E13) Asp-Asn]. One proposita’s daughter exhibited the same mutation that her mother.

To our knowledge, the case reported here is the first reported in alpha1 gene (Globin Gene Server Home Page, http://globin.cse.psu.edu/)) and the second case of Hb G-Waimanalo reported in Caucasian people in the world, and does not cause any hematologic symptoms and does not present abnormalities in the Hb functional properties. The other few cases described were reported in people from Asia, and all of them affecting the alpha2 chain.

Th154

Demand optimization: as clear as blood

I. Russell
NHS Tayside, Dept of Blood Sciences, Dundee, United Kingdom

Introduction: Demand optimization is increasingly vital in the current climate of restricted budgets and increased demand for testing within NHS laboratories. An area of particularly high demand in our laboratory is protein electrophoresis involving time consuming and expensive analyses requiring expert interpretation. One putative contributor to increased testing volumes was identified as addition of an automated comment to reports with elevated plasma viscosity (PV ≥2.2mPa.s.); this study aimed to verify the affect of this comment on follow-up testing and outcomes.

Methods: The origins of test requests, follow-up testing and outcomes were obtained via scrutiny of laboratory information systems and clinical portal. Samples from patients with known monoclonal gammopathies were excluded. All other PV requests from two 6-month periods pre- and post-comment addition was included.

Results: Common sources of protein electrophoresis requests were identified as immunology (for consideration with immunoglobulin measurement), haematology (suspicion/exclusion of multiple myeloma) and bone (unexplained fractures and lesions). The audit of the addition of the automated comment showed similar number requests for plasma viscosity measurement were made in two 6-month periods pre- and post-comment addition (30,561 and 32,705). Follow-up rates were also similar at 56% and 59%, while the percentage of samples with high viscosity rose from 209 (0.7%) to 366 (1.1%) and the resultant rate of positivity of testing decreased from 11% to 4.3% (accounting for 13 and 9 new cases, respectively).

Conclusion: This study demonstrates the complexities of demand optimization and identifies some of the barriers to implementation of measures to ensure appropriate testing (e.g. local non-agreed protocols). Addition of the automated comment did not improve multiple myeloma detection rates or significantly alter clinician testing patterns. It was unclear whether the small increase in the percentage of results with PV ≥2.2mPa.s. was due to natural variation or a significant bias associated with an analyzer upgrade.
Microbiology

Th155

A novel host-immune protein signature for diagnosing bacterial infections and guiding antibiotic treatment

K Oved1, O Boico1, A Cohen1, R Navon1, T Friedman1,2, I Etshtein1,2, O Kriger1, E Bamberger1,2, Y Fonar1, R Yacobov1, R Wolchinsky1, I Kulbatski1, G Denkberg1, Y Dotan1,2, A Hochberg1, Y Reiter1, M Grupper1,2, I Srugo1,5, P Feigin11, I Chistyakov3,5, R Dagan12, A Klein4, I Potasman3,10, E Eden1

1MeMed Diagnostics, Tirat Carmel, Israel, 2Rambam Medical Center, Haifa, Israel, 3Technion-Israel Institute of Technology, Rappaport Faculty of Medicine, Haifa, Israel, 4Hillel Yaffe Medical Center, Department of Pediatrics, Hadera, Israel, 5Bnai Zion Medical Center, Department of Pediatrics, Haifa, Israel, 6Technion-Israel Institute of Technology, Faculty of Biology, Haifa, Israel, 7University Health Network, Toronto, Canada, 8Applied Immune Technologies, Haifa, Israel, 9Bnai-Zion Medical Center, Department of Internal Medicine, Haifa, Israel, 10Bnai-Zion Medical Center, Infectious Diseases Unit, Haifa, Israel, 11Technion-Israel Institute of Technology, Department of Industrial Engineering and Management, Haifa, Israel, 12Soroka Medical Center, Pediatric Infectious Disease Unit and Clinical Microbiology Laboratory, Beer-Sheva, Israel

Objectives: Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Traditional host-proteins such as procalcitonin, C-reactive protein, and interleukin-6 can help determine infection etiology, but their performance is negatively affected by inter-patient variability. Our goal was to develop and validate a host-immune signature that measures both novel and traditional viral- and bacterial-induced proteins, and computationally combines them into a predictive score that distinguishes between bacterial and viral etiologies.

Methods: We prospectively recruited 1002 hospitalized and emergency department patients with acute infection, and controls with no apparent infection. Patients underwent comprehensive clinical and laboratory assessment, and the final diagnosis was determined by a panel of three independent experts. We quantitatively screened 600 circulating host-proteins and developed a multi-parametric signature using logistic-regression on half of the patients, and validated it on the remaining half.

Results: The cohort included 319 bacterial, 334 viral, 112 control and 98 indeterminate patients (139 were excluded). The best performing signature had an area under the curve (AUC) of 0.92±0.02. It consisted of the following novel viral-induced and traditional bacterial-induced soluble proteins: TNF-related apoptosis-inducing ligand, Interferon gamma-induced protein-10, and C-reactive protein. The signature was superior to any of the individual proteins (P<0.001), as well as routinely used clinical parameters and their combinations (P<0.001). The signature was robust across different physiological systems (respiratory, urinary and systemic), times from symptom onset (0-12 days), and pathogens (56 species), with AUCs between 0.87 and 1.0.

Conclusions: The present host-signature based assay provides valuable information over routinely used clinical variables and is readily usable on blood samples drawn as part of routine care. It has the potential to improve the management of patients with acute infections and reduce antibiotic misuse.

Th156

Urinary tract infection: evaluation of an automatized test to obtain results in 4-hours

LN Martins, H Brizido, C Ribeiro, LM Lito, J Melo-Cristino

Centro Hospitalar Lisboa Norte-Hospital Santa Maria, Clinical Pathology, Lisboa, Portugal

Introduction: Due to the high prevalence of urinary tract infections, both in outpatient and inpatient settings, the bacteriological urine exam is one of the most requested exams to the microbiology laboratory. Additionally to the need of starting an empirical treatment in symptomatic patients, the high frequency of negative results for this exam (approximately 80%) makes it mandatory to obtain a quick result.

Objectives: The authors compared the cultural method and the automatized test HB&L (Alifax®), by assessing its sensitivity and specificity, as well as the advantages and disadvantages in using both methods.

Material and methods: 250 urine samples were simultaneously analyzed by the traditional and the Uroquick methods (culture in liquid media). Each urine sample sent in a boric acid container was seeded with a 10 μL calibrated loop in blood gelose and MacConkey gelose, and incubated in 37°C during 18 to 24hours. In parallel, the same samples were seeded in the bottle of Uroquick liquid media and incubated in the Alifax®/HB&L equipment.

Results: From the 250 samples universe, by using a cutoff of 1000cfu/mL, 225 were included (25 contaminated), of which 178 had agreeing results and 47 had contradictory results (7% false negative). By assessing the specific sensitivity for each group of germs, the sensitivity of the automatized method reached 92% for Gram negatives, 71% for Gram positives and 50% for fungi.

Conclusions: Even though Alifax® allows us to report negative results in just 4 hours and present a high sensitivity in identifying the main agents responsible for urinary tract infections, the low sensitivity for Gram positive agents and fungi revealed by the automatized system limit its use as a screening method when applied in a hospital setting.
**Th157**

**Evaluation of ELF index as non-invasive marker of liver fibrosis in patients with hepatitis C**

I Cebreiros López, F Guzmán Aroca, JA Noguera Velasco, C Ramirez Ruiz, M Martínez Villanueva, I De Miguel Elízaga, A Martínez Ruiz, A Vargas Acosta, ML Ortiz Sánchez

1University Hospital Virgen de la Arrixaca, Clinical Analysis Service, Murcia, Spain, 2University Hospital Virgen de la Arrixaca, Radiology Service, Murcia, Spain, 3University Hospital Virgen de la Arrixaca, Hepatology Service, Murcia, Spain

**Background:** Evaluation of liver fibrosis in patients with chronic hepatitis C is essential for establishing prognosis and indication of treatment. The standard is biopsy but has risks and limitations, so that non-invasive diagnostic tools such as serum biomarkers and imaging methods have been developed in recent years.

ARFI is a radiological technique that provides the speed (m/s) at which an acoustic pulse crosses the liver parenchyma. This is a quantitative measure of the tissue elasticity and correlates with the degree of fibrosis.

ELF is a diagnostic algorithm of liver fibrosis that combines three serum direct markers: hyaluronic acid, procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase 1. The result becomes a score without units that indicates the level of fibrosis.

We aimed to evaluate the utility of ELF to discriminate degrees of liver fibrosis obtained by ARFI in a group of patients with hepatitis C and its correlation with it.

**Methods:** 105 patients with chronic hepatitis C were evaluated with ARFI (Acuson S2000, Siemens) to determine the degree of liver fibrosis. According to result were classified into five groups: F0-F4.

In serum of all the patients was determined ELF (ADVIA Centaur, Siemens)

**Results:** Significant differences in Elf results between groups were found (p < 0.001).

ELF was significantly correlated with ARFI, both the speed expressed in m/s (p < 0.001) as with the degree of fibrosis obtained (p < 0.001).

To evaluate the effectiveness of ELF we elaborated ROC curves considering pathological a degree of fibrosis ≥ F2. The area under the curve was 0.856, p < 0.001.

**Conclusions:** ELF test difference accurately and correlates with the degree of fibrosis determined by ARFI. It allows stratification and assessment of fibrosis in patients with hepatitis C so could be useful in hospitals where there isn't this imaging technique.

---

**Th158**

**PI3Kγ regulates expression of MMP in microglia by control of cAMP dependent signalling**

A Frister, R Findeisen, C Schmidt, N Schneble, R Bauer, R Wetzker

1Oberlausitz-Kliniken gGmbH, Institute for Laboratory Diagnostic, Microbiology and Hospital Hygiene, Bautzen, Germany, 2Friedrich Schiller University Jena-University Hospital, Institute of Molecular Cell Biology, Jena, Germany

**Background:** Septic encephalopathy (SE) is a frequent complication of sepsis and is associated with increased morbidity and mortality. A characteristic during the development of SE is an impaired function of the blood brain barrier (BBB). Strong hints suggest microglial activation and thereby enhanced secretion of matrix metalloproteinases (MMP) to be involved in the degradation of BBB components. Recent results indicate a PI3Kγ dependent suppression of cAMP signalling by enhancing PDE activity as a critical regulatory element of microglial phagocytic activity and activation. Therefore, we analyzed microglial MMP secretion in dependence on PI3Kγ mediated suppression of cAMP signalling.

**Method:** Microglial cells were derived from wild type C57/BL6 mice, PI3Kγ-deficient (KO) mice as well as mice carrying a targeted mutation in the PI3Kγ gene causing loss of kinase activity (KD). Cells were grown from forebrains of P0-3 animals. After treatment with LPS for 3h gene expression of the gelatinases MMP-2 and MMP-9 and the collagenase MMP-13 by qPCR and activity by zymography were quantified and content of cAMP was determined.

**Results:** A strong increase in LPS-induced MMP expression and activity in PI3Kγ KO microglial cells compared to wild type control and KD mutants was detected. The independence on PI3Kγ lipid kinase activity and an increased level of cAMP in PI3Kγ KO cells suggest the inhibitory effect of PI3Kγ on cAMP signalling to be the major cause of this enhanced MMP expression.

**Conclusion:** Our results support the hypothesis of the PI3Kγ lipid kinase independent PDE activation to be a crucial mediator of MMP expression in microglia. These data propose the moderating effect of PI3Kγ and PDE on cAMP signalling as a mediator of MMP-induced degradation of the BBB during LPS-induced SE.
**Brain/CNS Biochemistry**

**Th159**

Automated software versus laboratory staff in interpretation of CSF spectrophotometry

**JD McNeilly, A Cruickshank**  
NHS Greater Glasgow & Clyde, Biochemistry, Glasgow, United Kingdom

Increased CSF bilirubin is a sensitive but non-specific finding in the diagnosis of subarachnoid haemorrhage (SAH). In SAH, increased CSF net bilirubin absorbance (NBA) is frequently accompanied by a visible oxyhaemoglobin peak. Automated software packages using cut-off values based on revised national guidelines (net oxyhaemoglobin absorbance (NOA) >0.02AU plus NBA >0.007AU) are increasingly being adopted for interpretation, rather than interpretation by laboratory staff as recommended by the guidelines and the practice in our department.

**Aim:** To determine the added-value of interpretation of CSF spectrophotometry by laboratory staff.

**Methods:** All cases of CSF NBA >0.007AU plus NOA >0.02AU with a visible oxyhaemoglobin peak were identified retrospectively over a 5yr period (2008-12). LIMS and electronic patient records were interrogated to establish the final diagnosis. Real time interpretive comments by the reporting biochemist were reviewed to assess if these were suggestive of the correct diagnosis.

**Results:** A firm diagnosis was established in 58 patients (26 aneurysmal-SAH, 5 non-aneurysmal SAH, 12 cerebral infarction/haemorrhage, 6 meningitis, 9 “other”) all of whose spectrophotometry would be reported as “consistent with SAH” using automated software interpretation. The 31 confirmed SAH cases were reported with no modification in this comment. Modified interpretation assisting diagnosis accompanied 33% (4/12) cases of cerebral infarction/haemorrhage and 87% (8/9) cases where findings of NOA >0.02AU and NBA >0.007AU were not due to any cause of intracranial haemorrhage.

**Conclusion:** Interpretation of CSF spectrophotometry by laboratory staff reduces the incidence of inappropriate “consistent with SAH” comments. Such misleading interpretation may precipitate unnecessary and potentially invasive further investigation.

**Th160**

24 hours a day, 7 days a week-an audit of CSF Xanthochromia requests and the associated impact on patient pathways

**N Unsworth, Z Arkir**  
Viapath, Reference Chemistry, Biochemical Sciences, St Thomas' Hospital, London, United Kingdom

**Introduction:** Since the publication of “NHS Services, Seven Days a Week” in December 2013, there has been a drive towards emergency departments providing 24/7 Consultant cover. In-line with this, laboratories are coming under increasing pressure to support consistent and efficient clinical decision-making.

**Aims:** We currently operate a CSF-Xanthochromia service Monday-Friday, 09:00-16:00 with out-of-hours samples referred as required. The aim of this audit was to review the current state with regard to sample handling, requesting patterns and the associated impact on patient discharge.

**Methods:** 69 requests were retrospectively reviewed between October 2013 and March 2014 and compared to a previous audit (2012). Data was collected from request forms and electronic patient records on sample integrity, CT results, outcomes and discharge dates.

**Results:** Despite the addition of advisory notes on electronic ordering in 2012, 25% of samples were not protected from light (65% in 2012) and 14% had insufficient volume for analysis so required pooling (4% in 2012). 96% of patients were CT negative, the remainder either showed abnormalities or had no CT performed. 90% of CSF samples were reported as negative with 9% having sufficient oxyhaemoglobin to impair the ability to detect bilirubin. One sample tested positive however sepsis/meningitis was the working diagnosis. 64% of samples were received on weekdays and 36% after 16:00 on Fridays and over weekends. The median number of days to discharge from initial CT was 1.5 days (IQR 1-4) during the week and 3 days (IQR 1.7-4) at weekends.

**Conclusion:** These results show that pre-analytical issues remain problematic. A significant proportion of samples are received out-of-hours and although not clearly evident from the data, there is a definite need to formalise and improve processes both within and outside of working hours so as to enable efficient clinical decision-making and streamline patient pathways.

**Th161**

Cerebrospinal fluid (CSF) lactate: measurement of a reference range in adults and evaluation of the CSF lactate/glucose ratio as a novel marker for bacterial meningitis

**S Slack, I Holbrook**  
York Teaching Hospitals, Dept of Clinical Biochemistry, York, United Kingdom

Differentiating bacterial meningitis from viral meningitis is a diagnostic challenge. CSF lactate has been proposed as a valuable test to differentiate disease states; however its use in adults is limited by a lack of robust reference range data. Samples from 120 adults were used to derive
a CSF lactate reference range of 1.0 (95% CI 0.9-1.1)-2.2mmol/L (95% CI 2.0-2.6). The CSF biochemical profile in bacterial meningitis includes a decreased CSF glucose and increased lactate, therefore the diagnostic utility of a CSF lactate/glucose ratio was evaluated. A reference range of 0.27 (95% CI 0.26-0.29)-0.56 (95% CI 0.53-0.72) was derived for this novel parameter.

CSF biochemical profiles in 18 patients diagnosed with relevant pathology were reviewed. CSF lactate and the CSF lactate/glucose ratio were significantly increased in cases of bacterial meningitis when compared to the non bacterial meningitis group (p = 0.001 and p = 0.002 respectively). The utility of the CSF lactate/glucose ratio was investigated as a potential diagnostic test for bacterial meningitis; a ratio >1.38 demonstrated a sensitivity of 1.0 and specificity of 0.99 in this small data set. Further work is required to confirm these findings.

**Th162**

**Development of a HPLC-MS/MS method for midazolam for use in brain stem death testing**

KI Birch¹, CA Chadwick¹, J Rudge²

¹The Walton Centre, Neurobiochemistry & Neuroimmunology, Liverpool, United Kingdom, ²Phenomenex®, Macclesfield, United Kingdom

**Background:** Midazolam is a short acting benzodiazepine used as a sedative in intensive care. In the UK, a previously sedated patient cannot be classed as brain stem dead until the clinician is confident that their lack of consciousness is not due to this drug.

The aim of this project was to develop a HPLC-MS/MS assay for serum midazolam to aid the clinician in these difficult scenarios.

**Methods:** A Waters® Acquity® TQD with a Phenomenex® Kinetex® column and ammonium acetate and methanol mobile phases was used. Samples were prepared in a 96 well plate by addition of midazolam D4 in methanol with zinc sulphate. Following electrospray positive ionisation, two transitions were monitored for midazolam (325.98>244.16 and 325.98>291.23) and 1 for midazolam D4 (329.95>295.30).

**Results:** A 2.6 min method for midazolam in serum was optimised so no matrix effects were evident. The method was fully validated and gave acceptable results for lower limit of quantitation (5 μg/L), linearity (2000 μg/L), recovery (>90%) and within and between batch precision (CV< 7.8% and < 8.9% respectively). The method compared well to a HPLC method for midazolam offered at another Trust (R²= 0.9971, n=9).

**Conclusion:** A HPLC-MS/MS method was developed to measure midazolam in serum. The method was optimised for the clinical cut-off of 10 μg/L advocated in guidelines for brain stem death testing. The method has been used to monitor the serum midazolam concentrations in 2 sedated ITU patients. The levels fell as expected when infusion was stopped and matched the levels of consciousness of the patients.

Trademarks: Phenomenex and Kinetex are registered trademarks of Phenomenex. Waters and Acquity are registered trademarks of Waters Corporation.

**Th163**

**kFLC Index in cerebrospinal fluid: a valid aid in early diagnosis of MS?**

F Duranti¹, M Pieri¹, D Centonze²,³, F Buttari²,³, S Bernardini², M Dessi¹

¹Tor Vergata University Hospital, Experimental Medicine and Surgery, Rome, Italy, ²Tor Vergata University Hospital, Systems Medicine, Rome, Italy, ³IRCCS Fondazione Santa Lucia, Rome, Italy

Determination of free light chains (FLC) in the cerebrospinal fluid (CSF) could become a promising biomarker to represent intrathecal IgG synthesis in Multiple Sclerosis (MS). Although to date, the diagnosis can be made only on clinical and radiological data, evidence of intrathecal immunoglobulin synthesis is still important to exclude alternative diagnoses. In addition, intrathecal immunoglobulin synthesis is considered a risk factor for conversion to definite MS (CDMS). We measured, by nephelometric assay, kappa free light chain (kFLCs), albumin and kFLC Index in CSF/serum, in 80 patients who underwent lumbar puncture (LP) for diagnostic purposes. The patients were divided into three groups based on clinical diagnosis: group 1 (33 patients; 2.4 ± 1.7 kFLC Index) patients having diseases without inflammation, patients with inflammatory diseases other than MS group 2 (22 patients; 4.1 ± 3.7 kFLC Index) and patients with definitive MS group 3 (25 patients; 94.1 ± 110.6 kFLC Index; p< 0.001). Our last work suggests that an altered kFLC Index (>12) may improve and support the accuracy of the current criteria for MS diagnosis being able to discriminate MS from other inflammatory disease too (Duranti et al. 2013).

During the study our attention has been focused on four patients with a MS diagnosis at first hospitalization time. These patients showed classical intrathecal immunoglobulin synthesis test like IgG Index or oligoclonal bands (OCBs) as negative, but have an increased value of kFLC Index (>12). Furthermore two patients, with an initial diagnosis of Clinically Isolated Syndrome (CIS), develop MS in the follow up; some months before, they already have an altered kFLC Index with no OCBs. May be this test could play an important role in MS early diagnosis, but it has to be discussed in a larger cohort of patients and its prognostic value has not yet been evaluated.
Th164
Serum adiponectin levels in dementia patients and age-matched controls

L. Dukić1, I. Martinic-Popovic2, A-M. Simundic1
1Medical School University Hospital Sestre Milosrdnice, University Department of Chemistry, Zagreb, Croatia, 2Medical School University Hospital Sestre Milosrdnice, University Department of Neurology, Zagreb, Croatia

Aim: Adiponectin is cytokine produced by adipose tissue and involved in inflammation and insulin resistance. Since adiponectin has neuroprotective effect on hippocampal neurons, we hypothesised that patients suffering from dementia would have lower levels of adiponectin in comparison to healthy population. Aim of our study was to assess the possible difference in adiponectin concentration between patients with Alzheimer’s disease (AD), vascular dementia (VAD) and age-matched controls.

Methods: 68 patients with diagnosis of AD (median 75, range 56-94 years) and 71 patient with diagnosis of VAD (median 78, range 58-89 years) were recruited from University Department of Neurology. Control group was divided to 49 cognitively healthy individuals (median 66, range 53-83 years) and 51 individual with diagnosis of mild cognitive impairment (MCI) (median 72, range 53-81 years). Serum concentration of adiponectin was determined using immunoturbidimetric assay Adiponectin (ADPN) (Randox Laboratories Limited, Crumlin, UK).

Results: Median adiponectin concentrations for the tested groups were: AD (10.5; range 8.4-13.3 μg/mL), VAD (10.6; range 7.2-13.4 μg/mL), MCI (10.4; range 7.8-14.8 μg/mL) and cognitively healthy controls (8.8; range 5.8-13.3 μg/mL). Kruskall-Wallis statistical test showed no statistically significant differences between the tested groups (P = 0.297).

Conclusion: Serum adiponectin concentration does not differ between patients suffering from AD and VAD and healthy individuals without dementia.

Clinical Studies
Th165
The measurement of placental biomarkers in the detection of compromised pregnancies

DB. Nice1, K. Hayden1, I. Higgins2,3, E. Johnstone2,3, AE. Heazell2,3
1Central Manchester University Hospitals NHS Foundation Trust, Department of Clinical Biochemistry, Manchester, United Kingdom, 2Maternal and Fetal Health Research Centre, Institute of Human Development, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, United Kingdom, 3St. Mary’s Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom

Placental dysfunction is thought to be responsible for a significant portion of stillbirths that occur in the third trimester of pregnancy. Current measurements of fetal growth cannot be used to directly monitor the functionality of the placenta. Biochemical tests that can detect placental dysfunction offer a novel means to identify high-risk pregnancies. This study aimed to measure and evaluate the use of placental products: human placental lactogen (hPL), placental growth factor (PIGF) and progesterone in maternal plasma, serum and urine using commercially available assays to determine the most appropriate biofluid. The analytes were compared to assess whether biomarker concentration could differentiate infants of normal and small for gestational age (SGA) birthweights.

Methods: Three cohorts of 25 participants presenting after 28 weeks gestation with reduced fetal movements or SGA pregnancies were recruited alongside healthy controls, and serum, EDTA, lithium heparin and urine samples collected. hPL was measured by DRG ELISA kit, and progesterone by Roche immunoassay. PIGF was measured using three methods: Alere Triage, R&D ELISA and Roche PIGF and sFlt-1.

Results: All analytes could be measured reliably in serum, EDTA and lithium heparin plasma. Precision, accuracy, linearity and stability were assessed. There was no relationship between hPL levels in urine and serum or plasma. Serum PIGF measured by R&D ELISA was the most effective in the differentiating SGA infants (AUROC 0.85; p=0.002) and if sampling occurred before 35 weeks. Serum hPL had an AUROC 0.72 for prediction of SGA (p=0.006). Progesterone was not effective in discriminating between normal and SGA pregnancies (AUROC 0.59; p=0.25).

Conclusion: hPL and progesterone could be reliably measured in serum, EDTA and lithium heparin. Pregnancies with a fetus that was SGA were more likely to have lower concentrations of hPL and PIGF compared to appropriately grown infants.
Lipoproteins and oxidative lipoproteins levels in patients with stroke

EA Ahmed1, AH Siddig Salih2, HI Marrif3
1Qassim University, Laboratory Medicine, Burydah, Saudi Arabia, 2Qassim University, Burydah, Saudi Arabia, 3Princess Noura University, Riyadh, Saudi Arabia

Objectives: Oxysterols (cholesterol metabolites in brain) have significant role in in neuronal dysfunction and degeneration. The objectives of this pilot study is to investigate the status of oxidized lipoproteins in brain after exposure to cerebral accidents in compared to healthy controlled individuals.

Material and methods: (Patients Group I) (n = 23); patients with cerebrovascular accident were admitted to the Medical Ward at AL-Yarmouk Teaching Hospital within 24 hours of event were allocated in this study group age(40-73 years). Each patient was thoroughly examined and managed in hospital by neurologist, medical history recorded and patients signed the consent forms. Group II (control) (n = 23), healthy individuals were employed as a control. Ten milliliters of fasting venous blood sample was obtained from each subject serum processed for determination of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein and very low density lipoprotein (VLDL-C) oxidized and non- oxidized fraction the oxidation is measured by level of serum malondialdehyde (Friedwald et al. 1972). Atherogenic indices and particle sizes of LDL-C were calculated.

Results: We report here that all levels of oxidized lipid fractions were significantly (p < 0.001) higher than that of normal healthy individuals (6.061 ± 2.490 vs 0.504 ± 0.123 nmol/mL respectively).

Conclusion: Oxidized lipoproteins rather than lipoproteins themselves or lipid variables may involve in cerebral ischemic stroke. Protocols used in this study was approved by research and ethical committee of college of medicine at university of University of Al-Mustansiriya.
Author Index

A
Abdeldayem R.A.A. W167
Abdelrahman S. W134
Abeysekara A. W91
Ahrara H.D. W89
Acha-Sagredo A. Th9
Ackermans M.T. W15, W60
Adams R. W51
Adaway I. Th21, Th43
Adkins A. Th139
Adonics A. Th139
Agarwal N. W51
Agbi N.A. W89
Aggarwal D. Th137
Aguilar-Quintero M. O6 / W18
Agyeman-Yeboah F. Th12
Ahmed E.A. Th166
Ahmed F. Th47, Th117
Ahmed S.M. Th17
Alta A. Th81
Altman TJ. Th93
Akinlade F. Th110
Akyildiz E. Th52
Albaladejo Otón. Th1
Albrecht S. Th15
Alcock R. W85, W130, Th4, Th62, Th68
Allen J. W169
Allen S. W53, W152
Allison J.J. W31
Alliu N. W36
Ancaius M. W9
Anderson S. W165
Angiollilo A. Th56
Antal-Szalmas P. Th11
Antunovic T. W26, W38
Arenas M. W159, W165
Arik Z. Th123, Th160
Arbruster F.P. Th33
Armer J. W130, Th62, Th68
Armitage S. Th43
Armstrong A.E. W65
Armstrong A. Th45
Armstrong A.E. Th24
Armstrong A. W171
Arroyo M. W137
Ashby H.L. W69
Ashby J.P. Th29
Ashley E. W4
Ashton K. Th60
Aslan I. W57
Aslan M. W57
Asmoh R.H. Th12
Atif S. W134
Atherton J.A. Th133
Aud/W. W30
Awinibuno I.A.N. W151
Ayling R. Th94
Ayling R.M. Th41
B
Babbington F. Th145
Bagg E.A.L. Th93
Baglioni P. W40, W51
Bailey J. Th116
Bailey J.R. Th58
Bailey L.M. W6, W135, Th13, Th25
Baldovino S. Th89, Th102
Baldwin J. W130
Bamberger E. O11 / Th155
Bandera F. W34
Banks A. W70, Th20
Bansal H. W47
Baraldi E. Th54, Th55
Barbour H.M. W25
Barceló A. W82
Barceló B. W172
Bardou-Jacquet E. S2.2
Barlow N.L. W166
Barth J.H. W1, Th79, Th145
Bartlett W.A. W99
Barton A.L. W128, W132
Bas Bernal A. Th127
Basile U. Th140
Basso G. Th101
Bateman K. W105
Bauca J.M. W82, W172
Bauer R. Th158
Baumann N.A. W58
Bayly G. Th94, Th95
Bayly G.R. W157
Bayram E. Th52
Beardsmore A. Th4
Beardsmore A.W. W118
Beednarska-Makuruk M. O8 / Th87
Behulova D. Th6
Bell N. W105
Belli S. Th55
Beneitez Pastor D. Th153
Benes-Diez A. Th86
Benjamin N. W10
Bennett M.R. W24
Bennett T. Th139
Benton S.C. Th26, Th58, Th64, Th116
BenYounes H. W103
Berg J.D. W166
Berg J.P. PC2.1
Bergmann K. W27, W21
Bernardini S. Th163
Bernatikova H. Th6
Bernstone L. W55
Berry H. Th57, Th65
Bialas-Chromies B. W37
Bianchi V. W168
Bileck T. W57
Biljen J. W15
Billington J. Th138
Bilton D. Th57
Binns J. Th145
Birch K.J. Th162
Blake D. Th48
Blake O. W75
Blaker P. W159
Blanco Alvarez A. Th153
Blankenstein M.A. W15, W60
Blaton V. W36
Block D.R. W58
Bloom B. Th58
Boa F. Th67, Th69
Bochynska A. O8 / Th87
Bodis M. W94
Boeri A. Th102
Boerjigter S. W9
Boico O. O11 / Th155
Boldergh A. Th141
Boot C. W80
Borg C. Th139
Borgwardt K. Th53
Bosomworth M. Th19
Boudry P. W28
Bowron A. W105, W107, W111, W114
Bozovic D. W38
Bradbury S. W75
Brady S. W66
Braga F. W164, Th88
Brault D. Th71
Bray R. W59, W102
Brekke O.-L. Th100
Brincat I. Th139
Brissot P. S2.2
Brizido H. Th88, Th156
Brodska H. Th129
Brookes M. W93
Bruce H. Th26
Buccig G. Th101
Buch H.N. W69
Bukan N. W81
Bulo A. W36
Burgess E. Th34
Burgess J.C. Th57
Burke-Murphy E. W52
Busch J. Th10
Buttari F. Th163
Buttigieg D. Th139
Büttler R.M. W60
Byrne C. Th145
C
Caballero-Villarraso J. O6 / W18, W19, W20, Th86
Cabello S. W82
Camacho-Martínez P. Th10
Canbay E. Th52
Cafiete-Estrada R. O6 / W18, W19
Cafiete-Vazquez M.D. W20
Carbonell Muñoz R. Th1
Card D.J. W96
Cariani E. Th54
Carless D. Th44
Carling R. W113, W115
Carling R.S. W114, W122
Carroll R.M. Th26
Casais S. Th153
Caslake M. W153
Cassidy D. W150
Castells Sarret N. Th96
Castillo C. W137
Castro-Vega L.M. W141, W142
Cattani P. Th140
Cavey T. S2.2
Cebreiro Lopes I. Th127, Th157
Celap I. Th84
Centzone D. Th163
Cerezueta Fuentes P. Th1
Chadwick C. Th4
Chadwick C.A. Th162
Chaloner C.M. W112
Chan A.O.K. Th92
Chandrasaj D. W1, Th79
Chang W.W. W164
Chatha K. W124
Chatterjee V.K. W75, Th38
Chaudhari R. Th32
Chiang S.T. W164
Chica-Alhambra F.J. Th86
Chistyakov I. O11 / Th155
Chithiramohan A. W53, W152
Chocholova A. Th6
Chronopolou E. W107
Ciantar N. Th39
Clarke C. Th29
Clarke E. W53, W152
Clifford-Mobley O. W139, Th22
Clough M. W97
Clough V.E. Th110
Clunie I. W31