PERFORMANCE OF DIAGAM IMMUNOTURBIDIMETRIC ASSAY FOR SERUM ALBUMIN ON ABBOTT ARCHITECT C16000 PLATFORM

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BACKGROUND: Immunoassays are the most specific methods for measuring albumin in serum (SA). Accordingly, in October 2015, we introduced the DiAgam immunoturbidimetric assay for measuring SA on the Abbott Architect c16000 platform. We however observed an average uncertainty of the measurement that was constantly higher than the minimum goal derived from SA biological variation (≤2.4%). Furthermore, we failed to reach the minimum total error (TE) goal (±6.1%) in the majority of performed EQAS exercises. Consequently, in agreement with DiAgam and Abbott, we carried out a study to evaluate the assay performance under standardized conditions.

METHODS: The study was performed between July and September 2016. The assay was calibrated three times (every 4 weeks), using calibrators from two different lots. Fresh aliquots of the 3-level DiAgam calibration control material (MPCO) were employed twice daily to check alignment of analytical runs and the fresh-frozen BioRad Liquichek Unassayed Chemistry Control level 2 (lot no. 16772), previously shown commutable for SA, was measured once a day for evaluating long-term imprecision. MPCO lots never changed throughout the study.

RESULTS: When compared with the manufacturer’s assigned target values, MPCOs showed for all the study period a changeless positive bias of, in average, +9.3%, +5.9% and +5.8% at MPCO target concentrations of 24.8, 50.4 and 68.5 g/L, respectively. Total CV on BioRad material, obtained from 72 runs, was 4.0% at a mean SA concentration of 49.7 g/L.

Three EQAS exercises performed during the study period showed a TE of +1.9% (Jul), +15.9% (Aug) and +20% (Sep), when our laboratory results were compared with the reference value derived from results of all participants (n=330).

CONCLUSIONS: Despite the strictly controlled conditions employed in this study, the performance of DiAgam immunoturbidimetric SA assay when applied to Abbott Architect c16000 platform did not fulfil the minimum quality specifications for the clinical use of this important test. In particular, it seems that two different issues are contributing to this situation: the inadequacy of calibrator value-assignment protocol by manufacturer and the unacceptably high imprecision of the measuring system.
ROUTINE-LIKE TESTING OF COBAS E 801 AT 3 EUROPEAN STUDY SITES

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BACKGROUND

The study was designed to stress test the new Roche Diagnostics immunochemistry analyzer, cobas 8000 <e 801> under routine-like conditions, thereby assessing the overall system functionality when the e 801 module was integrated in a cobas 8000 core with two or three other analytical modules.

METHODS

Three different cobas 8000 hardware configurations were used: a dedicated immunochemistry set-up at one site consisting of 2 e 801 modules plus 1x e 602 module, two consolidated clinical chemistry and immunochemistry set-ups (4-module configuration with ISE, 2 x c 702, 1 x e 602 and 1 x e 801; 3-module configuration with ISE, 1 x c 701, 1 x c 502 and 1 x e 801). The applied test menu per study system reflected those used in the site specific routine laboratory and ranged between 34 and 72 assays.

Up to eight routine workloads of ~1000 samples were replicated and re-processed on the respective site study system with cobas e 801 integrated. This process allowed the site staff to assess the features and functionality of the new module in their own lab specific environment. In addition, the measured study results per sample and assay were directly compared with the generated routine results. Of the > 100 assays applied on the study systems at the 3 sites during this experiment, 32 assays were installed and tested on the new cobas e 801 module.

RESULTS

Comparisons of routine results with those generated on the cobas e 801 for randomly selected samples were evaluated using the Bablok/Passing regression procedure. The yielded slopes were in the range 0.90 – 1.10 and Pearson correlation coefficients (r) were >0.995 in most cases. Concordance of routine results with those generated on the study system for the included serology assays was also shown.

During practicability assessment, the simplified reagent handling with seamless reagent loading in operation, long reagent and calibration stability, the speed and compact design were rated high.

CONCLUSIONS

During this stress testing, cobas e 801 proved to be fast and reliable with the ability to consolidate a high number of assays on a single platform while keeping the same test performance as the cobas e 602 module. With its ease of use, cobas e 801 promises to bring many benefits to immunochemistry lab testing.
MEDICAL ADDED VALUE OF CAPILLARY ELECTROPHORESIS DURING HB A1C SCREENING: EXPERIENCE FROM A REGIONAL HOSPITAL IN ITALY

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Background: Glycated hemoglobin (Hb A1c) is an important parameter for the screening and the diagnosis of diabetes mellitus (DM). As Hb A1c is part of the hemoglobin (Hb) fractions, it is subject to clinical variations if patients present pathologies involving Hb. Among them, hemoglobinopathies (HbP) are important causes. HbP are now common worldwide due to migration. At least 5.2% of the world population carries a hemoglobin (Hb) variant. Thus, it is important for laboratories to be able to diagnose patients carrying HbP during Hb A1c measurement and inform the clinicians accordingly in order to adjust DM diagnosis and treatment.

We evaluated the ability of capillary electrophoresis (CE) to incidentally discover HbP among patients needing Hb A1c measurement and calculated prevalence of HbP.

Methods: A comparative study was carried out over 2 months. A total of 949 patient samples were received for DM screening. All Hb A1c samples were analyzed by immunoassay (IA) on Dimension Vista (Siemens) and by CE on the CAPILLAYS 3 TERA (Sebia). Profiles showing Hb variants, Beta-thalassemia (Hb A2 > 3%) or elevated Hb F were counted up.

Results: In our hospital, we used IA for Hb A1c and CE for Hb testing. Recently, Hb A1c assay was released on CE. On the contrary of IA, Hb A1c by CE allows to highlight Hb abnormalities in clear-cut and precise profiles. Correlation between IA and CE for Hb A1c measurement is good but CE brought added value by displaying Hb profiles. A total of 50 atypical profiles were found: prevalence can be considered equal to 5.3%, which is compatible with ones previously calculated. Among them, 28 Beta-thalassemias were found, 12 profiles with elevated Hb F and 10 Hb variants. These Hb disorders have a prevalence of 2.95%, 1.27% and 1.05%, respectively. Presence of these abnormalities has been reported to clinicians with the Hb A1c value.

Conclusion: IA provides accurate measurement of Hb A1c but unfortunately there is no possibility to know if patients are carriers for HbP. Thus, separative methods allow the detection of undiagnosed HbP during Hb A1c assay, which is not rare in Italy. CE can be used advantageously for this purpose. In our laboratory around 2-5 cases of HbP are found per day, beyond the Hb A1c measurement. The incidental observation should be reported to the clinicians and must lead to further investigations (e.g. Hb testing by CE) but also to a better adjustment of DM follow-up and treatment.
COMPARISON OF TWO DIFFERENT METHODS (CHEMILUMINESCENCE AND FLUORESCENCE ENZYME IMMUNOASSAY) FOR DETERMINATION OF FAECAL CALPROTECTIN IN THE ASSESSMENT OF INFLAMMATORY BOWEL DISEASE

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INTRODUCTION
Faecal calprotectin (FC) is becoming an essential non-invasive marker for inflammation bowel disease (IBD). It may be elevated in certain disorders such as ulcerative colitis and Crohn's disease, the active celiac disease, and colon cancer. The aim of our study was to compare three different FC assays and assess their performance and ability for diagnosis and follow up of IBD.

METHODS
FC of samples obtained from 61 symptomatic patients (28 with active IBD, 9 in clinical remission, 24 with other intestinal diseases) were analysed using: LIAISON® DiaSorin (chemiluminescence), EliA ThermoFisher Scientific, Phadia100 (FEIA), and Calprest® (ELISA), the actual method in use in our laboratory. Correlation between assays was calculated by linear regression (Passing-Bablok) and difference plot (Bland-Altman) using R. The significance level was determined by the associated p-value set at <0.05. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative like-hood ratios (LR+ and LR-) were calculated.

RESULTS
Our results showed that both test for the measurement of FC showed a high sensitivity and non-significant difference between assays was observed. However, results showed a better concordance between Calprest and Liaison, compared to Calprest and EliA. Correlation coefficients ranged from 0.96 to 0.91, respectively, while the slopes and/or intercepts differed extensively, with up to 3.5-fold quantitative differences between assays. Calculated sensitivity values were 100% and 96.3% for the Liaison and the EliA assay, respectively. Specificity of 73.5% and 61.8%, PPV of 0.81 and 0.70, NPV of 1.00 and 0.95, LR+ of 4.57 and 2.66, and LR- of 0.00 and 0.05 were obtained.

DISCUSSION
The results obtained showed a better correlation for the Liaison assay compared to the obtained for de FEIA-EliA. Furthermore, the Liaison test allows automation, a wider analytical range, and a 5-fold less time for the first result. However, with respect to specificity and the absolute values in the borderline and pathological range, we found major differences for the two assays investigated. Therefore, clinical interpretation of assay results should be made with caution depending on the assay used.
FOUR SYSTEMS OF AUTOMATIC URINALYSIS COMPARED TO REFERENCE METHOD

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Background
The correct choice of the analytical system is one of the fundamental assumptions of urinalysis. The aim of this study was to compare four automated urinalysis systems: the Iris iQ200 Sprint (Iris) combined with the Arkray AUTION MAX AX 4030 (AUTION), Arkray AU 4050 (Arkray), Dirui FUS 2000 (Dirui), and Menarini sediMAX (Menarini).

Methods
Leukocytes (WBC) and erythrocytes (RBC) were compared to light microscopy (Leica DM2000) with calibrated FastRead plates, using both native and stained preparations. Digital image analysis is used in Iris and Dirui (native samples) and Menarini (sample concentrated by centrifugation); flow cytometry is used in Arkray. Strips AUTION for WBC and RBC detection were compared with light microscopy (5 semiquantitative categories both for chemical analysis and microscopy).

Results
"Almost perfect" evaluation (weighted kappa statistics 0.81 – 1.00, EUG 2000) of WBC was found for all tested systems; RBC classification was "almost perfect" for the Iris, Dirui and Arkray, "substantial" (kappa 0.61 – 0.80) for the Menarini. True classifications of leukocytes (best Arkray: 88%, worse Menarini: 85%) were better than of erythrocytes (best Dirui: 78%, worse Arkray: 72%). False negativities of WBC were 23.6, 13.9, 17.1, and 22.5 for Iris, Arkray, Dirui, and Menarini, respectively. Similarly, false negativities for RBC were 31.1, 21.6, 10.0, and 36.5 %, respectively. False positivities of WBC were 6.2, 11.0, 12.2, and 8.5 for Iris, Arkray, Dirui, and Menarini, respectively. Similarly, false positivities for RBC were 18.8, 34.6, 34.2, and 8.6 %, respectively. In the case of chemical examination of blood/RBC and WBC (AUTION) by the kappa statistic a perfect match was detected compared to light microscopy.

Conclusions
Expectations with regard to reliability of the automated urinalysis seemed to be higher than was found in reality. In the future, for clinical centers it would be the best combination to use a flow cytometer with subsequent digital image analysis, allowing more accurately to determine the number of elements and bacteria and recording the image data.
USEFULNESS OF THE ICTERUS INDEX FOR BILIRUBIN ESTIMATION

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INTRODUCTION

Bilirubin (BIL) is originated from the degradation of hemoglobin. Its concentration in serum or plasma is usually increased in diseases such as cirrhosis, hemolytic anemia, colelithiasis and hepatitis. In clinical laboratories, BIL levels are often measured by molecular absorption spectrometry after diazocompound formation. However, a direct absorbance measurement of the sample (Icterus Index, II) may also estimate diazo-based test results. Broken red blood cells (Hemolysis Index, HI) and the presence of triglyceride in the sample (Lipemic Index, LI) might be potential factors affecting the relationship between BIL and II.

METHODS

A total of 209,208 patient samples were analyzed during Jan-Oct 2016. BIL quantification was performed on an Architect platform (Abbott). The indices II, HI and LI were analyzed directly on samples by bichromatic spectrophotometry at 500/524nm, 572/604nm, 628/660nm, 524/804nm (Architect, Abbott) and calculated using specific algorithms. II and BIL below the limits of quantification (LoQ) were removed for the correlation analyses. Analytical imprecisions of every magnitude were assessed by means of the interday coefficient of variation (iCV). Correlations between BIL and II were assessed using the Pearson's coefficient. Type α error was set at 0.01.

The false-negative rate was evaluated by calculating the number of samples with pathological BIL values, defined as >1.2 mg/dL (20.5 µM) with II below the LoQ.

RESULTS

The values of iCV for BIL and II were 4.2% and <1%, respectively. The coefficient of correlation between both magnitudes was r=0.97 in samples with low HI and LI. Correlations in samples with HI>50 or LI>20 yielded r=0.97 and r=0.96 respectively. No statistical difference was detected with samples with low HI and LI (p>0.01). If II<1.0, a pathological BIL value was found in only 0.44% of samples.

CONCLUSIONS

II is an excellent approach for bilirubin quantification, as this simple technique might be able to robustly predict the diazo-based test results. Therefore, the measurement of II in every sample is recommended as screening strategy before performing BIL tests, whether accompanied with HI and LI or not. As a result, this approach could improve laboratory demand management and improve patient care.
Analytical technologies and applications

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TRACEABILITY IN LABORATORY MEDICINE: A DRIVER FOR ACCURATE PATIENT RESULTS

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The between-method variability of many laboratory medicine analytes presents a risk to patient safety; requires variable reference intervals; compromises the application of evidence-based clinical practice guidelines; and restricts the ability to create a single electronic patient record. Global assay standardization, based on metrological traceability, supports the production of equivalent patient test results across space and time.

The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was founded to facilitate this activity by BIPM (Bureau International des Poids et Mesures), IFCC (International Federation for Clinical Chemistry and Laboratory Medicine), and ILAC (International Laboratory Accreditation Cooperation). JCTLM has a growing number of member organizations that are committed to support equivalence of measurements in the clinical laboratory through metrological traceability to appropriate reference materials and methods. Standardization is achieved when all routine assay results for a test are traceable, with an unbroken metrological chain of comparisons, to reference materials and methods of a “higher order,” with a sufficiently small uncertainty such that results may be validly compared.

The JCTLM has developed a database of such higher order reference materials and methods and reference measurement services (www.bipm.org/jcltm/) Entry in the database is determined by review by experts using ISO standards and approval by the JCTLM Database Working Group and Executive Committee. In 2016 the database contained listings for 298 materials, 180 methods and 146 reference measurement services.

Implementation of traceability requires action by many bodies: national metrology institutes and other organizations that prepare materials and develop methods; reference measurement service laboratories; IVD manufacturers that prepare calibrators/trueness controls for field assays following appropriate traceability chains and provide traceability information to users; clinical laboratories that select and use traceable assays; EQA/PT providers that confirm claimed traceability; and guideline committees that base recommendations on traceable results. The importance of traceability in laboratory medicine is not always appreciated by these stakeholders. The JCTLM WG-TEP promotes these activities by producing freely available educational materials that demonstrate the value of traceability in laboratory medicine (www.jctlm.org).
IMPACT OF THE INTRODUCTION OF ACL TOP 750 LAS FOR HAEMOSTASIS TESTING ON THE WORKFLOW OF A TOTAL AUTOMATION LABORATORY

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BACKGROUND: To try to improve throughput and pre-analytical sample checks in our total automation core-lab (TLA), in December 2015 two new ACL TOP 750 LAS systems replaced the previous instrument version (TOP 700) dedicated to haemostasis testing. The main goal was to relieve the two TLA Architect c16000 platforms of automatic detection of potential sample interferences by measuring haemolytic, icteric and lipemic indexes in tubes for haemostasis directly on ACL. In this study, we assessed if this change could affect the turnaround time (TAT) of released results and the qualitative evaluation of interference detection.

METHODS: We divided the study into 3 phases, each lasting 3 months: 1) interference indexes (II) measured only by Architects; 2) II detected by both types of instruments, but Architect II values employed only; 3) II measured only by ACL 750. On average, our 24-hour/7-day TLA service receives 700 requests/day of first-line haemostasis tests, namely PT, aPTT and D-dimer (DD), for a total of 370 tubes, and 3800 chemistry test requests on 850 tubes.

RESULTS: In the 3 study phases, we carried out 41,322, 31,391 and 38,844 haemostasis tests, respectively. The median TAT values (expressed as the time from the sample check-in to the result made available to the clinical wards) for haemostasis tests were 39.1, 40.9 and 37.8 min in the 3 phases, respectively; corresponding average TATs for Architect biochemistry tests were 43.6, 43.6 and 45.8 min, respectively. As expected from the high interference thresholds, the number of rejected samples for requested haemostasis tests was low. However, in the third phase ACL 750 nullified for interfering icteric index 12 samples for PT and 20 samples for aPTT in comparison with none in the previous phases using Architect II. Interfering samples also increased for DD from 1-3 in phases 1-2 to 7 samples in phase 3. Haemolysis detection reflected the same trend, with 19, 18 and 7 rejected samples for PT, aPTT and DD, respectively, in phase 3 in comparison with 5-6, 4-6 and 3-1 in previous phases.

CONCLUSIONS: In our TLA setting, the direct II estimate on ACL 750 slightly improved TAT for haemostasis tests. A specific investigation should compare the accuracy of II detection by Architect and TOP 750.
Rapid low level quantification of plasma 1,25 dihydroxyvitamin D by LC-differential mobility spectrometry-MS3

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Introduction

Analysis of 1,25 Dihydroxyvitamin D, the active form of the vitamin involved in calcium homeostasis, presents challenges due to the very low (pmol/L) endogenous levels. Not only that, the metabolism pathways give rise to other dihydroxy-isomers of varying functionality & significance. Often time consuming extended chromatography, costly immunocapture methodologies and labour intensive derivitization are required to separate these, as conventional MRM alone may not be sufficient. Differential Mobility Spectrometry (DMS) and unique QTRAP® scans such as MRM3 offer the ability to reduce runtime and improve selectivity for these challenging compounds, while simple chemistries such as Amplifex™ give significantly increased sensitivity without the pitfalls of traditional derivitization.

Materials and Methods

Sample Preparation:
Extraction was achieved by Liquid/Liquid extraction using 200µL of plasma

HPLC Conditions:
Short chromatography was provided by a C18 column and a gradient of acidified acetonitrile/water

MS/MS Conditions:
Sciex QTRAP® 6500+, equipped with SelexION™ DMS Technology, operating in Low Mass Positive MRM

Results

• DMS Technology incorporated into the method has been shown to remove interferences and other isomeric forms that can lead to misinterpretation of the results
• MRM3 scans show significantly reduced background and enhanced S/N over conventional MRM for 1,25 DHVD.
• Amplifex derivitisation chemistry shows approximately a 20fold increase in sensitivity over non derivatized methods, with minimal modification to extraction procedures.
• Results and statistics in plasma collected so far show good Accuracy (85 – 115%) Precision (<15%) and Linearity (>0.99) with sensitivity below 10pg/ml (S/N >50:1)

Conclusions

• We present here a proof-of-concept analysis for the use of DMS, MRM3 and Amplifex™ for the analysis of 1,25 DHVD with simple sample processing and short chromatography
• The proposed method offers advantages over conventional HPLC/derivitisation chemistries in terms of sample processing and throughput and overall pre-analytical complexity
• Sensitivity of the assay is sufficient to accurately quantify low circulating levels required
Robust metrological traceability has been incorporated into the design, development and manufacture of the Waters MassTrak™ Vitamin D kit*. The kit metrological traceability has been designed and developed to meet the requirements of ISO 17511:2003. The robust metrological traceability of the MassTrak™ Vitamin D kit as well as a review of the kit performance will be presented.

**Metrological Traceability**

Metrological traceability of the MassTrak™ Vitamin D kit to NIST SRM2972 has been established. Primary calibrators were prepared using certified reference materials and assigned 25(OH)D2 and 25(OH)D3 concentrations using reference measurement procedures at the University of Ghent which are traceable to NIST SRM2972. All MassTrak™ Vitamin D kit calibrators and quality control lots are value assigned using primary calibrators. The concentrations assigned to the MassTrak™ Vitamin D Kit are verified in a value confirmation process using independently assigned QC materials.

**VDSCP**

To further assess the accuracy of the metrological traceability and performance of the MassTrak™ Vitamin D kit assay, Waters enrolled in the CDC Vitamin D Standardisation Certification Program (VDSCP) for 25(OH)D in serum, which assesses bias and precision of assays relative to reference measurement procedures. The MassTrak™ Vitamin D assay achieved a mean bias of 0.6% from the VDSCP reference values and a mean imprecision of 4.9%, therefore meeting the certification performance criteria.

**MassTrak™ Vitamin D Performance Characteristics**

Precision, sensitivity, linearity, potential interferents and carryover were all assessed during the development of the MassTrak™ Vitamin D kit and found to meet the performance criteria specified for the kit.

**Conclusion**

The MassTrak™ Vitamin D kit calibrator materials are traceable to NIST SRM2972 via a documented unbroken chain of calibrations. The accuracy of this traceability to NIST SRM2972 has been verified through participation of the Vitamin D Standardisation Certification Program (VDSCP) in which the MassTrak™ Vitamin D Assay achieved a mean % bias of 0.6% from the VDSCP reference values and an imprecision of 4.9%.

*MassTrak™ Vitamin D kit is not available for sale in the United States.
OVERCOMING THE INTERFERENCE OF DARATUMUMAB WITH IMMUNOFIXATION ELECTROPHORESIS (IFE) USING AN INDUSTRY-DEVELOPED DIRA TEST: HYDRASHIFT 2/4 DARATUMUMAB


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Background: Detection of monoclonal component by serum protein electrophoresis (SPE) and immunofixation (IFE) are essential for response evaluation in multiple myeloma (MM) according to the International Myeloma Working Group (IMWG) criteria. Recent clinical trials on daratumumab, an IgG Kappa anti-CD38 monoclonal antibody, have shown impressive results with deep responses. However daratumumab may be detected on SPE and IFE: differentiating daratumumab from endogenous monoclonal protein can be challenging when both co-migrate. The availability of a specific, anti-daratumumab antibody has provided the opportunity to overcome this interference. Indeed, McCudden et al in collaboration with Janssen developed the Daratumumab Interference Reflex Assay (DIRA) test. Given the need for a commercially available automated and standardized test, we evaluated a new commercial DIRA kit test being developed by Sebia (Lisses, France): the Hydrashift 2/4 daratumumab.

Methods: The Hydrashift 2/4 daratumumab assay was prepared by Sebia using the anti-daratumumab antibody produced by Janssen and modified to allow a migration of daratumumab/anti-daratumumab complexes toward the α-globulin fraction on IFE. IFE technical procedures were performed on the standard Sebia Hydrasys platform, with the HYDRAGEL 4 IF kit. In addition to the regular procedure, an additional applicator to apply the anti-daratumumab antibody was used. Analytical performances were assessed on 99 samples from ongoing daratumumab clinical trials. Results: The Hydrashift 2/4 daratumumab assay showed excellent concordance (100%) with the laboratory developed test on 51 samples tested (i.e. 28 negative DIRA, 14 positive DIRA and 9 doubtful DIRA). Daratumumab/anti-daratumumab complexes were detected with a sensitivity of 200 mg/L. Daratumumab was shown to be completely removed from the gamma globulin fraction for all tested patients. For 48 samples tested on diagnosis, the anti-daratumumab specifically shifted daratumumab with no effect on the patients’ M-spike. Conclusion: The Hydrashift 2/4 daratumumab test provides the opportunity to automate and standardize the displacement of daratumumab interference and to improve IFE interpretation for evaluation of complete response in MM.
Background: In multiple myeloma (MM), detection and quantification of monoclonal component (M-spike) by urine protein electrophoresis (UPE) and immunofixation are essential for diagnosis and response evaluation according to the International Myeloma Working Group (IMWG) criteria. Up to now, quantification of urine M-spike was only possible on electrophoretic profile with Hydragel High Resolution (HR) (Sebia). Recently, Sebia has developed the possibility to quantify M-spike from the electrophoretic track on urine agarose gel immunofixations (Urine Profile [UP] and Bence Jones [BJ]) with the Phoresis software 8.63. The purpose of this study was to evaluate these two methods of quantification and to determine if they could be used alternatively from HR.

Methods: We included 90 urine samples in this study, with several profiles: Bence Jones proteinuria only, Bence Jones with glomerular or tubular proteinuria or both, Bence Jones with several degrees of polymerization. Finally, 67 of the 90 samples exhibited a quantifiable M-spike. Concordance of UP and BJ quantification with the referent method HR was evaluated, using a correlation graph and a Bland and Altman diagram with deviants analysis (using SFBC recommendations).

Results: Concordance of M-spike quantification between UP and HR, and BJ and HR respectively was excellent (correlation coefficients 0,98 for both). Only one deviant was observed for BJ-HR comparison and two for UP-HR comparison. Differences were due to the resolution characteristics in proteins separation (degrees of polymerization for Bence Jones proteinuria, Bence Jones and tubular microproteins) according to each gel.

Conclusion: This study shows that UP, BJ and HR are overall comparable for urine M-spike quantification. Availability of a quantification tool with UP and BJ gels provides the opportunity for many laboratories to measure urine M-spike with accuracy methods as long as the MM follow-up. For only a few samples, profiles can be slightly different between HR and UP/BJ leading to the classical recommendation in clinical biology, to mostly follow-up disease with the same method.
EFFECTS OF GLUCOSE CONCENTRATIONS ON INTRACELLULAR NAD/NADH IN CITRIN-DEFICIENCY HEPATOMA CELLS

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Nicotinamide adenine dinucleotide (NAD) is an essential coenzyme for mitochondrial oxidative phosphorylation, and plays a critical role in nonalcoholic fatty liver disease (NAFLD) development and progression. Citrin, a component of malate-aspartate (MA) shuttle, transports cytosolic NADH-reducing equivalents into mitochondria. Citrin deficiency is associated with NAFLD. Carbohydrate diets may increase cytosolic NADH production and should be avoided in patients with citrin deficiency. In this study, we aimed to establish a liquid chromatography-tandem mass spectrometry method to measure intracellular NAD, NADH and taurine, and to investigate whether citrin-deficiency and/or low-glucose culture medium would change NAD/NADH ratio leading to mitochondrial dysfunction. NAD, NADH and taurine were separated over a C18 column in a mobile ammonium formate-50% methanol/50% acetonitrile linear gradient. Then, each compound was ionized and detected in the positive multiple reaction monitoring mode of triple quadrupole tandem mass spectrometry. Linear response was found from 1 to 100 µM for NAD and NADH, and 4 to 160.0 µM for taurine. The imprecisions (CV %) for all analytes were less than 10%. HepG2 cells, when cultured under low concentrations of glucose, had a decrease of intracellular NAD and NADH levels and NAD/NADH ratio, compared with 25 mM glucose. Low glucose-containing medium decreased the cell viability about 21% in HepG2 cells. Citrin knockdown was achieved by infecting cells with shRNA lentivirus with about 90% citrin-knockdown. Citrin deficiency caused intracellular NAD decreased and NADH increased, compared with HepG2 cells. Low glucose treatment increased the intracellular NAD and decreased the NADH in citrin-deficiency HepG2 cells. To sum up, a method for measuring NAD/NADH ratio by LC-MS/MS was established. Our results indicate that low-glucose supplement or citrin knockdown caused change of NAD/NADH ratio. Interestingly, this phenomenon was alleviated in citrin-deficiency cells under low concentrations of glucose.
NEW IF FAST PROGRAM OF SEBIA MAKES IFE 1.5 X FASTER, YIELDING SAME PERFORMANCES THAN CLASSICAL IF

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Background: Monoclonal gammopathies are plasma cell dyscrasias, which are characterized by the presence of a monoclonal protein (M-protein) in the serum or in the urine of patients. At screening, immunofixation (IFE) is used to confirm monoclonality and to identify the heavy-chain (gamma, alpha, mu, delta, or epsilon) and/or light-chain type (kappa or lambda) of an M-protein initially detected by protein electrophoresis (PE). In the follow-up, IFE is required when the M-protein is no longer visible on PE, moreover it allows assessing response to therapy; for instance a negative IFE confirms complete response.

In order to improve labs workflow, Sebia has implemented a new IFE program “IF Fast” in its Hydrasys system that shortens the overall turnaround time (TAT) by 1.5. The aim of the study was to assess the analytical performances of IF Fast compared to the classical version.

Methods: IFE were carried out on the Hydrasys Focusing® system with both IF and IF Fast programs and the Kit hydragel 4 IF dynamic mask (Sebia, France) according to manufacturer’s instructions. Visual interpretation has been done independently by several experts. For establishing the limit of sensitivity, 3 serum samples containing respectively a M-protein were serially diluted into normal serum and into a serum with hypogammaglobulinemia to reach the concentrations: 2, 1.05, 0.25, 0.125, and 0 g/L. Specificity was tested with serum samples (n=25) collected from: 1) patients with a known gammopathy, 2) patients with M-spike at SPE and unknown symptoms, 3) patients with abnormal kappa/lambda ratio and non-conclusive with the PE.

Results: The three M-proteins were detectable in both conditions up to 0.125 mg/L. For a given dilution, the band intensity and migration position were identical with both programs. The 25 samples yielded identical IFE patterns with both programs, confirming the presence or absence of M-proteins as well as the presence of oligoclonality and hypergammaglobulinemia.

Conclusions: Overall results obtained with IF Fast program, which decreases the TAT around 20 minutes, demonstrate an equivalent sensitivity and specificity to the classical Sebia IF assay.
PERFORMANCE EVALUATION OF NANOPIA KL-6 ASSAY IN INERSTITIAL LUNG DISEASES

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BACKGROUND: Krebs von den Lungen-6 (KL-6), which is a mucin-like glycoproteins excreted from type II alveolar pneumocytes when these cells are injured. KL-6 has been reported to serve as a sensitive marker for monitoring disease activity and predicting the prognosis of interstitial lung diseases (ILD). The aim of the present study was to evaluate the analytical and clinical performance of Nanopia KL-6 assay (Sekisui Medical, Japan) based on latex-enhanced immunoturbidimetry method.

METHODS: From March to October 2016, 260 patients diagnosed with ILD were enrolled in this study. All patients with ILD underwent HRCT and pulmonary function test (PFT). We used 113 samples and 200 samples for disease and healthy control, respectively. The evaluation consisted of determination of the precision, linearity, method comparison with ELISA kit (EIDIA, Japan), sensitivity and specificity and correlation with HRCT findings or PFTs. The HRCT findings were graded on a one to six scale based on the classification system.

RESULTS: The total CV for low and high level quality control materials were below 2% at each concentration. Acceptable linearity was observed in their respective reportable ranges. Correlation analysis of KL-6 indicated that results of the Nanopia KL-6 assay were comparable to ELISA [correlation coefficients (r) = 0.979]. Using a ROC curve, the optimal cutoff point of KL-6 was 350 U/mL with a sensitivity and specificity of 73.9% and 98.0%, respectively, and the area under the curve was 0.953. Serum KL-6 levels was positively correlated with the extent of involvement, traction bronchiolectasis and ground-glass attenuation on the HRCT. In the comparison of all ILD patients' subgroups, significantly higher levels of KL-6 were determined in the idiopathic pulmonary fibrosis (IPF) or connective tissue diseases-related ILDs (CTD-ILD) than other groups. KL-6 levels were negatively correlated with PFTs [FVC, DLCO, TLC and 6MWT]. In IPF patients, there were statistically significant correlations with all PFT results, but in nonspecific interstitial pneumonia and hypersensitivity pneumonitis groups, some of the test measurements showed a good correlation with KL-6.

CONCLUSIONS: The overall analytical and clinical performance of Nanopia KL-6 assay is acceptable for the monitoring of disease progression in clinical practice. Therefore, KL-6 serve as useful non-invasive biomarker to assess the disease severity in patients with ILD.
The determination of thyroid hormones is essential for diagnosis and management of thyroid diseases. The laboratories are recommended to determine their own local reference intervals for the clinician to avoid missed diagnosis and unnecessary treatment.

In our laboratory we measured thyroid hormones by immunoassays on two analyzers: Siemens Advia Centaur XP and Roche Cobas e601. We use the reference intervals provided by the manufacturers. It was observed, by reviewing laboratory data, in the occasions when all thyroid tests were within reference interval, in about 7% of cases, the determination of thyroxine (T4) using Centaur method gave high values. So, the aim of this study was to compare the T4 values from two analyzers and to verify Centaur reference interval for T4.

The comparison analysis showed good correlation of T4 values measured by Centaur and Roche methods ($r = 0.994$); there was no significant difference between values from two analyzers for measuring range of 63.1-208.5 nmol/L. However, T4 values from Centaur were higher than values from Cobas in range of 124.7-172.0 nmol/L ($t$-test, $p < 0.05$). The 95% reference interval (normal distribution) for Centaur T4 values, obtained from 160 apparently healthy subjects (73 men and 87 women, aged 21-79 years) was 73.5-149.3 nmol/L, with mean value of 108.3 nmol/L. This interval didn’t fall completely within the reference interval provided by the Centaur manufacturer (58.1-140.6 nmol/L), and the mean was significantly higher than the manufacturer’s mean (90.3 nmol/L). Slightly elevated values determined by Centaur method better agreed with the reference interval provided by Roche manufacturer (66-181 nmol/L).

Our data suggest the reference interval of 73.5-149.3 nmol/L for determination of T4 using Advia Centaur XP analyzer.
REPORT OF A FULLY AUTOMATED MASSIVELY PARALLEL SEQUENCING (MPS) LIBRARY PREPARATION FOR GERMLINE BRCA1/2 MUTATIONS TESTING BY OMNIA LH100 AND LH75 (MASMEC) SYSTEMS: EXPERIENCE OF A REFERENCE LABORATORY

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Introduction. We perform about 1500 BRCA1/2 samples/year by MPS. In this context, library preparation for MPS is one of the most critical, on bench and time-consuming steps our pipeline. In addition, automation of library preparation phase is the best option to avoid the risk of human-introduced error. Our objective was to report a rapid, automated solution to prepare Multiplicom-NGS based protocol for Illumina MiSeq® sequencer for BRCA1/2 genes and to analyze the performance of MPS in terms of number of sequences/run, coverage uniformity and number of variants detected. A total of 120 samples were used to evaluate the automated preparative process.

Methods. Complete protocol for BRCA1/2 genes amplification by the BRCA MASTR Dx kit (Multiplicom, Niel, Belgium) was performed using OMNIA LH 100 and OMNIA LH 75 automated workstations designed and produced by MASMEC Biomed (Modugno, Bari). The LH 100 was equipped with a robot (X-Y-Z), 8 independent pipette channels and a layout with two racks for reagents and DNA samples, 9 deck positions for 96 well plates and different size tips and two heating-cooling units for controlled temperature steps. The LH 75 was prepared with a single pipette and a magnetic tool for analyzing 12 samples at the same time and 6 deck positions for 96 well plate and different size tips. Both workstations were controlled by MASMEC Framework software and were provided with UV lamp for decontamination to reduce the risk of cross-contamination.

Results. OMNIA platforms were accurately customized to set up libraries preparation and purification process of 12 patients simultaneously per run in only 8-9 working hours (respect to 1-2 working days for manual execution). Because the workstations require very little hands-on time, 12 consecutive samples were prepared for the next automated amplification cycle while the first 12 samples were purified. Thanks to perfect setting of ratio between magnetic beads and DNA amount, a high-quality of BRCA1/2 libraries and MPS performance were obtained.

Discussion. This study describes a new automated solution for fast and reproducible BRCA1/2 library preparation for MPS using a robotic workstation. Automated library preparation and MPS performance were comparable to a standard manual library preparation. The throughput of our pipeline was high positively improved by introducing these machines in our routine workflow.
HARMONIZATION OF SERUM THYROID-STIMULATING HORMONE BY THE IFCC COMMITTEE FOR
STANDARDIZATION OF THYROID FUNCTION TESTS (C-STFT) MAKES THE USE OF A COMMON REFERENCE INTERVAL POSSIBLE.

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BACKGROUND
The clinical community is convinced that assay-specific reference intervals (RIs) should be abandoned in favor of common ones. They allow adopting modern public health standards, such as clinical practice guidelines quoting fixed decision limits and electronic patient records. A requisite is that routine assays are harmonized. In view of this, the C-STFT developed and applied a harmonization approach for serum thyroid-stimulating hormone (TSH) measurements. It is anchored to the statistically inferred all-procedure trimmed mean (APTM) from a multi-assay method comparison using a panel of clinically relevant samples. We completed the technical recalibration of 14 immunoassays from globally operating manufacturers, and subsequently performed a pilot RI study. We investigated whether harmonization makes the use of a common RI feasible.

METHODS
After evaluation of the efficiency of recalibration, all participating manufacturers measured 120 samples from presumably healthy donors. From each assay’s dataset and that representing the APTM (which serves as reference), we estimated the RI with a non-parametric bootstrap procedure. The hypothesis that harmonized assays can use a common RI was verified with a Cochran’s Q-test.

RESULTS
The mean bias of each assay to the APTM (concentration range: 0.002 to 193 mIU/L) met the 7.8% bias specifications (inferred from biological variation). The reference lower limit (LL) (± 90% CI) was 0.56 ± 0.13 mIU/L, the upper limit (UL) 4.27 ± 1.42 mIU/L. All assays had their RIs’ percentiles within the CIs around the reference ones (ranges: LL: 0.47 to 0.59 mIU/L; UL: 3.97 to 4.74 mIU/L). The Cochran’s Q-test confirmed that after harmonization a common RI applied (p-values for homogeneity testing: LL: 0.9982; UL: 1.0000).

CONCLUSIONS
This study showed that harmonization results in a much more uniform array of RIs. However, this is not an endpoint, because “common RI” does not mean “one size fits all-RI”. It is the ideal starting point for manufacturers to verify accordance of their individual RIs for TSH with accepted consensus standards, and establish, if needed, high quality reference ranges, accounting for between-population, clinical or biological differences.

*http://www.ifcc.org/ifcc-scientific-division/sd-committees/c-stft/
Hemoglobin A1c (HbA1c) assay is accepted as the most useful marker to determine the long-term glycemic control of diabetic patients. This marker has also been recommended for the diagnosis of diabetes mellitus when HbA1c levels are above 48 mmol/mol (6.5%). Accurate HbA1c results are essential for monitoring and appropriate treatment of diabetic patients. The aim of this study was to compare analytical performances of two HbA1c methods: Sebia capillary electrophoresis and Siemens Advia turbidimetric immunoassay. Imprecision studies at increasing whole blood HbA1c concentration, intra-assay and interassay CVs ranged: 0.9%-1.1% and 1.2%-1.5% for Sebia method, respectively. In imprecision studies intra-assay and interassay CVs ranged: 1.0%-1.5 and 2.9%-3.1% for Siemens method, respectively. The correlation of HbA1c values, determined with these methods, was evaluated using least-square regression analysis and absolute difference plot according to Bland and Altman. Obtained correlation coefficients (slope, intercept) were: Sebia vs Siemens method, 0.9906 (0.9775, 0.2626). Mean (SD) absolute difference from Bland-Altman plot was: -1.879 (2.165) for comparison of Sebia and Siemens method, respectively. The results of this study showed that there was good agreement among HbA1c values determined with these two methods.
PERFORMANCE EVALUATION OF IMMATURE GRANULOCYTES ON CELLAVISION DM96.

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Background. CellaVision™ DM96 is a cell image analysis system that automatically perform a preliminary differential on peripheral blood smears. The analyzer pre-classifies the white blood cells and to authorize the results skilled technologists perform a re-classification by a subsequent modification and verification of the pre-classified cells. The aim of this study was to confirm the previous data of accuracy obtained by the CellaVision™ DM96 image method with respect to Immature Granulocytes (IGs).

Methods. EDTA-anticoagulated blood samples (408 samples) with WBC related flags were selected. Blood smears were prepared in duplicates and stained with May-Grünwald-Giemsa stain. All smears were examined by conventional microscopy, and by the CellaVision pre-and re-classification feature. The accuracy of the CellaVision pre- and re-classified cells were evaluated by comparing the results to conventional microscopy.

Results. The AUCs were 0.88 (95% confidence interval [CI], 0.85-0.92) for the pre-classified results and 0.91 (95% CI, 0.88-0.94) for the reclassified results. Sensitivity for detection of myelocytes/promyelocytes ≥0.5%, and/or ≥1.0% metamyelocytes in manual count were 0.71 for pre-classification and 0.75 for reclassification feature. Respective specificities were 0.94 and 0.96. The mean difference (bias) between the methods was -0.8 (95% CI, -0.5- -1.0) and -1.1 (95% CI, -1.4 - -0.8) for the IG pre-versus re-classified counts and the pre-classified versus the manual counts. For samples with IG counts 1% or less, the IGs were overestimated by the pre-classification method compared to the re-classification method and the manual microscopy. For values 4% or higher, the IGs were underestimated by the pre-classification method and the difference tended to increase with increasing levels of IGs.

Conclusions. The measures of diagnostic accuracy are comparable for the pre-classification and the re-classification feature. A small proportional bias judged to be clinical irrelevant, was found. The data support the previous suggestion that the pre-classified IG count performed by the CellaVision may be reported without subsequent re-classification by a technologist.
Monoclonal antibodies (mAbs) have emerged as a major class of therapeutics used to treat life-threatening diseases such as cancer, inflammation or autoimmune diseases. There are currently more than 70 approved mAbs and related products on the market and they are considered as the fastest growing class of therapeutics with sales grown from $39 billion in 2008 to $91 billion USD in 2015. Currently over 470 therapeutic mAbs are tested in clinical trials. Such, the interest for robust qualitative and quantitative analyses for mAbs increased for clinical and pharmaceutical laboratories. Ligand-binding-assays (LBAs), such as ELISA, offer a high degree of specificity, sensitivity and throughput. However, LBAs suffer from lengthy assay development, can be affected by matrix effect and is antibody dependent with poor multiplexing possibility. Over the past 10 years, Liquid chromatography-mass spectrometry coupling in Selected Reaction monitoring mode (LC-MS-SRM assays) have increased capacity and selectivity compared with LBAs. Moreover, LC-MS-SRM assays allow multiplexed quantification within a single analysis. However, in clinical studies, mAbs are mainly quantified in very complex matrices such as plasma or serum and the selectivity of LC-MS-SRM technology could not be enough. In this study, we investigate the use of MRM3 for the multiplexed quantitation of 3 FDA and EMA approved mAbs within a 20 min analysis without immune-precipitation. We reach sub 1 µg/mL on LOQ for the 6 mAbs.
Analytical technologies and applications

Cod: M045

READYBEADS: A NEW TOOL TO IMPROVE ROBUSTNESS AND RELIABILITY IN LC-MS ANALYSES.

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Mass spectrometry coupled to Liquid Chromatography (LC-MS) is a powerful tool for the characterization and the quantification of large molecules in complex biological matrices. To obtain robust analyses that can be transferred, throughout each step of the development of new drugs or during validation of biomarkers, sample normalization with internal standards are essential. However, getting reproducible internal standard solutions, notably when several molecules are needed, is complex and requires several validation steps. Indeed, biomolecules might not be stable in solution and might need storage in a compatible container, at a specific concentration and temperature. Moreover, generating dozens or hundreds solutions at a defined concentration is time consuming and leads to poor reproducibility, especially between operators.

In order to solve these issues, we have developed an innovative technology called READYBEADS. This technology is based on a water soluble biopolymer, that does not interfere with mass spectrometry analysis, where internal standards are coated and can be released at a controlled concentration.

This innovative technology presents the key property to be stable through time at room temperature, and allows an easy way to prepare reproducible complex mixture in a minute apart from the operator. This solution allowing ready-to-use customized standards will be presented in this study through the implementation of READYBEADS for various applications.
INTRODUCTION

Alpha-fetoprotein (AFP) is one of the largest glycoproteins in fetal plasma, though its main usefulness in clinical practice is as a marker of non-seminomatous hepatocellular and germ cells carcinoma.

The analytical method used is chemiluminescence; recently, due to a problem in the supply of reagent, we were forced to use a different autoanalyzer, passing from Centaur XP® to Dimension Vista 500®, being necessary to carry out a correlation before applying the change.

MATERIAL AND METHODS

A total amount of 57 samples were analyzed consecutively by both analyzers. Previously, aliquots which were freezed at -20°C until the moment of being processed, had been made. Levels 1 and 3 of Tumor Marker Immunology® by Biorad were used as a control.

RESULTS

The statistical analysis was made with MedCalc® software, and it consisted on the calculation of the equation of the regression line by the nonparametric method of Passing-Bablok, in order to assess the degree of substitutability between both samples.

The correlation has the following equation:

\[ y = 0.2 + 1 \times x \]

where Y corresponds to the instrument DIMENSION VISTA 500 and X to the ADVIA CENTAUR XP. The correlation coefficient was \( r = 1 \), with an IC95% for the slope of 0.9333 to 1.0254, and for the intercept, of 0.0466 to 0.4067. The values of AFP were expressed in both cases in ng/mL.

CONCLUSIONS

The calculated parameters show that there exists a good correlation between both methods (\( r = 1 \)).

The intersection includes the 0 and the slope includes the 1, so it’s not necessary to apply the correction given by the above-mentioned formula, therefore, the methods are interchangeable and their results are transferable.

The change of analyzer led to the modification of the reference values of AFP (from < 8.1 ng/ml with ADVIA CENTAUR XP to < 8.00 ng/ml with DIMENSION VISTA 500), being notified to the ordering doctors, and registered through a cutline in the informatic system, in order to take account of it in the patient follow up.

Furthermore, at the operational level, the necessary time for the analysis has been reduced to 10 minutes, being specially important for those samples requiring dilution.
OPTIMIZED ACE-ACTIVITY ASSAY FOR THE ACCURATE DIAGNOSIS OF SARCOIDOSIS

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Sarcoidosis is a multisystem granulomatous disease. Diagnosis of sarcoidosis is often difficult because of the lack of gold standard tests. Measurement of serum angiotensin-converting enzyme (ACE) activity may contribute to the early diagnosis. We aimed to optimize a fluorescent kinetic assay of ACE, to reveal interfering factors and to eliminate the distorting effects of endogenous ACE-inhibitors. Our goal was, in addition, to determine the genotype depending ACE-activity reference values and test its diagnostic power in a clinical set-up.

Abz-FRK(Dnp)P-OH was used as a substrate for ACE-activity measurements. 201 healthy individuals (control) and 46 presumably sarcoidotic patients, who underwent diagnostic mediastinoscopy with biopsy from hilar lymphatic nodes, were enrolled in the study. ACE-activity and insertion/deletion genotype of the ACE gene was determined.

ACE-activity is significantly influenced by the endogenous ACE-inhibitor, serum albumin. To diminish its effects, the serum should be diluted at least 35-fold during measurement. Interference analysis showed that intense hemolysis (above 0.35 g/L cell free hemoglobin) and icterus (above 64 µM total bilirubin) significantly decreases the ACE-activity, while lipaemia does not alter it (up to 16 mM triglyceride). Genotype depending serum ACE-activity reference values were determined, II: 3.78–11.25 U/L (n=39), ID: 5.22–11.59 U/L (n=90), DD: 7.19–14.84 U/L (n=72). If the I/D genotype of the patient was unknown, the 4.85–13.79 U/L (n=201) range was used. In 7 patients, serum samples were not suitable for analysis due to ACE-inhibitor treatment. Sarcoidosis was confirmed by histopathology in 29 patients, tissue samples of 10 patients were negative. Only 6 patients had higher ACE-activity in the serum when genotype independent reference range was used (Sens: 21%, Spec: 100%, PPV: 100%, NPV: 30.3%), while 9 patients showed higher ACE-activity when genotype specific reference values were used (Sens: 31%, Spec: 100%, PPV: 100%, NPV: 33.3%).

A rapid fluorescent kinetic assay was optimized to measure serum ACE-activity. It might be an alternative of invasive biopsy for setting the diagnosis in one third of all sarcoïdic patients.
Analytical technologies and applications

Cod: M048

TRANSITIONING FROM SERUM TO LITHIUM-HEPARIN PLASMA: EVALUATION OF BD BARRICORTM, A NEW BLOOD COLLECTION TUBE WITH A “MECHANICAL” SEPARATOR

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Background

There are several blood collection tubes available with and without separator gels. Recently, BD launched a new lithium heparin tube: the BD Barricor plasma blood collection tube, containing a mechanical separator rather than the conventional gel separator. The aim of this study was to evaluate the transition from a predominant serum workflow to a lithium-heparin plasma workflow using the new BD Barricor tube for our routine 24/7 chemistry and immunochemistry tests. In addition, analyte stability in the lithium-heparin plasma of the BD Barricor tube was evaluated.

Methods

After informed consent, two additional blood specimens were collected from 40 patients visiting the outpatient clinic of our internal medicine department. Tubes were processed according to the manufacturers specifications. The lithium-heparinized plasma (BD Barricor) and serum (BD SSTII) samples were assayed simultaneously for 66 clinical chemistry and immunochemistry tests using Roche Cobas 6000 and 8000 analyzers, and results were statistically analyzed using Passing-Bablok regression analysis. Analyte stability in lithium-heparin plasma was evaluated after 2 and 4 days of cold storage (4°C).

Results

Overall, the serum vs. plasma comparison was good. The minimum and maximum relative bias observed was 0.94 (myoglobin) and 1.05 (estradiol) respectively. Of the 66 analytes, 54 were within the relative bias of the Passing-Bablok 95% confidence interval (95% CI). The remaining 12 analytes fell outside the 95% CI range. However, the observed relative bias was well within the total allowable error margin of those analytes. For all 66 analytes, no significant differences were found for the absolute bias. Furthermore, the BD Barricor tube showed good stability results. With exception of the angiotensin converting enzyme (ACE), plasma stored in the centrifuged primary BD Barricor tubes showed good stability for all other analytes up to 4 days of cold storage (increase/decrease of analyte concentration <10%).

Conclusions

The results of the lithium-heparin plasma from the new BD Barricor tube showed good comparison with serum from the BD SSTII. With the exception of ACE, analyte stability in 4°C cold-stored lithium-heparin plasma in primary centrifuged BD Barricor tubes is guaranteed for up to 4 days. We conclude that when transitioning from a predominantly serum to a lithium-heparin plasma workflow for routine 24/7 chemistry and immunochemistry tests, the Barricor tubes can be used.
METHOD EVALUATION OF THE ADVANCED® MODEL 2020 OSMOMETER

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Background: The Advanced® Model 2020 (Automated Instruments Inc, Mass, USA) is a multi-sample osmometer which takes up to 20 samples with unattended automated sample processing using freezing point technology. Compared to single-sample osmometers, the Advanced® Model 2020 Osmometer provides a 75% increase in productivity for laboratories processing more than 15 samples per day, improving laboratory efficiency and throughput.

Objectives: To evaluate the performance of the Advanced® Model 2020 Osmometer using serum and urine samples.

Methods: The linearity study was performed using an osmolality linearity set (Advanced Instruments, Inc) comprising of 5 levels of concentration (100, 500, 900, 1500 and 2000 mOsm/kg). The imprecision (within-run and between-run) study was done using the MAS UrichemTRAK urine osmolality controls (Thermo Scientific) and Protinol serum osmolality controls (Advanced Instruments, Inc). Three levels were used for the serum controls (240, 280 and 320 mOsm/kg) and two levels for the urine controls (391 and 832 mOsm/kg). The accuracy was assessed by comparing patient sample results from the Advanced® Model 2020 to those obtained with the Advanced Micro-Osmometer Model 3320 (single sample osmometer).

Results: The linearity was acceptable for the concentration range that was studied. Least squares linear regression equation was y=0.9983x - 1.2236 r = 1 and recovery at the different levels was between 99.2% – 100%. The within-run imprecision (coefficient of variance, CV %) was 0.49, 0.61 and 0.44% for the serum control levels and 0.41 and 0.38% for the urine control levels, respectively. The between-run CV% was 0.90, 0.97 and 0.70% for the serum control levels and 2.95 and 2.30% for the urine control levels, respectively. The least squares regression analysis equation from the comparison study was y=0.9873x+8.1618 and r=0.975 for serum and y=1.0301x-6.1034 and r=0.99 for urine.

Conclusion: Based on these results the Advanced® Model 2020 performance is found to be acceptable for use in serum and urine samples.
Background: Procalcitonin (PCT) is a well established biomarker that facilitate the decision of whether to use antibacterial agents in patients with systemic bacterial infections and when antibiotics can be safely stopped. The aim of our study is to compare the original reference method BRAHMS PCT on Kryptor CompactPlus© assay with 3 other methods on different analyzers (Lumipulse©G1200, Vidas PC©and Cobas© 8000).

Methods: The study was based on 83 heparinized plasma of patients, selected on the basis of their PCT results to cover the analytical range of 0.02-32ng/mL. The samples were aliquoted and measured simultaneously on each analyzer with BRAHMS PCT on Kryptor CompactPlus© (Time resolved Amplified Cryptate Emission method), with Elecsys BRAHMS PCT on Cobas© 8000 (Electrochemiluminescence method) and on the Lumipulse©G1200 (Chemiluminescent Enzyme Immunoassay method). Amongst the 83 samples, 54 were also measured on the VIDAS PC© (Enzyme-Linked Fluorescence method). The correlation with Kryptor was evaluated with a Passing-Bablok (PB) regression and a Bland-Altman plot. Clinical concordance between the methods with disease state was assessed using a Cohen's \( \kappa \)-test. Therefore, the population was divided into 3 categories (PCT <0.5ng/mL, PCT=0.5-2 ng/mL, PCT >2ng/mL).

Results: When we compared Kryptor with Cobas, from the PB analysis we found a slope of 0.94 (95% confidence interval (CI) of 0.92-0.97) and an intercept of 0.014ng/mL (95% CI of 0.007 to 0.022). The Bland-Altman Comparison (BAC) showed -1.1% differences between methods and the Kappa (K) value was 0.98 (discordance 0.46 vs 0.52ng/mL). When we compared Kryptor with Lumipulse, from the PB we found a slope of 1.10 (95% CI of 1.03-1.14) and an intercept of -0.18ng/mL (95% CI of -0.028 to -0.004). The BAC showed -6.9% differences between methods and the K value was 0.98 (discordance 0.5 vs 0.44ng/mL). When we compared Kryptor with Vidas, from the PB we found a slope of 1.08 (95% CI of 1.00-1.17) and an intercept of -0.055ng/mL (95% CI of -1.80 to 1.80). The BAC showed -8.4% differences between methods and the K value was 1.

Conclusions: When compared to the original method BRAHMS PCT on Kryptor, we showed a good concordance between the different methods but a slight overestimation of the Lumipulse and the Vidas. We also showed a slight underestimation of the Cobas. The strength of agreement between the methods was considered to be very good or excellent in the range of 0.02-32ng/mL.
Analytical technologies and applications

Cod: M051

COMPARATIVE STUDY OF TWO ANALYZERS OF URINE STRIPS.

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Background: Urine analysis is a basic and very useful test to assess the existence, severity and development of kidney and urinary tract diseases. It consists in a macroscopic examination, a physical-chemical test, a microscopic examination of sediment and an urine culture if necessary. The objective of this study is to compare the results of the automatic analyzer Aution Max AX 4030 (recently implanted in our emergency laboratory), with the results of the semi-automatic analyzer Aution Eleven AE-4020 (to replace) for Glucose, Protein, Blood, Nitrite and Leukocytes parameters, and to check the matching between these results from both analyzers.

Methods: A total of 150 received samples of isolated urination in our emergency laboratory during 3 consecutive days were included for a complete analysis. The samples were processed at the time of reception. For the physicochemical study, they were introduced in two Analyzers. The Aution Sticks 10EA test strips were used. Both analyzers employed the same methodology (double wavelength photometric reflection), the same ranges of analysis for the different parameters and the same test strips. Undoubtedly, the most significant difference between both instruments was the automaticity that typically characterized the Aution Max analyzer.

Results: Because is a semi-quantitative analysis, we calculated the Cohen's kappa coefficient (k), the 95% confidence interval (95% CI) and the concordance of the results of the two scanners for each parameter. We noted that there is a very good concordance for proteins (0.877k, 0.810-0.944 95% CI) and nitrites (1.000 k 1.000-1.000 95%CI), good for glucose (0.630 k, 0.430-0.831 95% CI) and blood (0.759 k, 0.664-0.855 95% CI) and a moderate for leukocytes (0.525 k, 0.403-0.647 95% CI).

Conclusions: With this study, we evidence that Aution Max Analyzer is more accurate than Aution Eleven, because it considerably reduces errors, especially taking of aliquot. The Aution Eleven process is manual and depends on the manipulator (aliquot amount, time while the Strip is dampened, and time that it takes to analyze), while Aution Max being automatic taking of the aliquot is constant.
Analytical technologies and applications

Cod: M052

ANALYTICAL EVALUATION OF A NOVEL LIPASE ACTIVITY MEASUREMENT REAGENT FROM SHINO-TEST CO. USING DGGMR AS SUBSTRATE

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BACKGROUND
Elevation of pancreatic enzyme activities is one of the important diagnostic evidences. Most clinical laboratories can routinely analyze amylase, pancreatic amylase, or lipase. Especially pancreatic lipase has been reported to be superior to amylase and pancreatic amylase at the point of diagnostic sensitivity and specificity for acute pancreatitis. In general lipase activities are measured by using either color rate method which employed a fat emulsion named 1,2-diglyceride (DG) as substrate, or synthetic chromogenic substrate, 1,2-O-dilauryl-rac-glycero-3-glutaric acid (6-methylresorufin)-ester (DGGMR). Here we compared basic performance of a new DGGMR reagent kit developed by Shino-Test Corporation (Japan) with other 3 reagents.

METHODS
Cygns Auto LIP (DGGMR; Shino-Test, hereinafter S method), Liquitech® Lipase Color II (DGGMR; Roche, R method), Lipase Color Auto Test Wako (DG; Wako, W method), Iatro LP Rate II (DG; LSI Medience, L method) and each reagent’s calibrator were used in this experiment. All measurements were performed on a Hitachi LABOSPECT 008 using the following test articles and conditions: (i) Within-run reproducibility (N=20); (ii) Linearity; (iii) Interference (ascorbate 50 mg/dL, Bil F 50 mg/dL, Bil C 50 mg/dL, hemoglobin 500 mg/dL, chyle 3000 FTU); (iv) Commutability (377 serum samples); (v) Correlation using 4949 serum samples.

RESULTS
All reagents are similar in precision and correlated well but varied in linearity range and interference. Negative interference by chyle in use of S, positive interference by hemoglobin in use of R, negative interference by Bil and positive interference by hemoglobin and chyle in use of W, and negative interference by Bil and positive interference by chyle in use of L were observed. Correlation coefficients between S method and the other reagents were 0.958 to 0.996. Further correlation analysis between S and R methods revealed 10 of 4949 samples produced white turbidity during the second reaction. Biochemical analysis suggested that some of them contained M-proteins.

CONCLUSIONS
The analytical performance of the newly developed serum lipase activity measurement reagent from Shino-test is considerably precise and convenient. This reagent could be useful in diagnosis of acute pancreatitis.
Colorectal cancer (CRC) is constantly increasing in incidence both worldwide and at the national level. Chemotherapeutic agents often prescribed in CRC are such as Capecitabine (CCB) and 5-Fluorouracil (FU). CCB is activated to FU in a three steps reaction giving 5'-deoxy-5-fluorocytidine (DFCR), followed by 5'-deoxy-5-fluorouridine (DFUR) to yield finally FU, the active form, which is later deactivated to 5,6-dihydro-5-fluorouracil (DHFU). Patients exhibited variable responses and adverse events in response to CCB therapy, despite being treated with the same dose. This could be explained by the presence of different possible enzyme SNPs that can occur along the CCB activation and deactivation pathways. This study aims at developing a new method of analysis of CCB and its metabolites using HPLC-UV, to determine the plasma concentrations of CCB and its metabolites DFCR, DFUR, FU, DHFU and 5-Chlorouracil (CLU; the internal standard), followed by a correlation study with the toxicities occurring during therapy, to become a predictive method for toxicity, away from the exhausting genotyping process. A new superior analytical method is presented using computer-assisted method development, which achieved full separation of the six analytes during the least possible gradient time, eluting the compounds at 2.8, 3.2, 4.4, 5.2, 5.8 and 9.9 minutes for DHFU, FU, CLU, DFCR, DFUR and CCB, respectively. The method showed accuracy, precision and robustness upon validation. Clinical results showed a positive correlation between the DFCR concentration and mucositis, as well as, between the DFUR concentration and Hand-Foot Syndrome, confirming that this technique could be used for predicting such toxicities in CRC patients.
ROUTINE IFCC-STANDARDISED CDT MEASUREMENT (CDT/IFCC) BY THE SEBIA CAPILLARYS CDT ASSAY

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BACKGROUND
Carbohydrate-deficient transferrin (CDT) is a glycoform profile of serum transferrin that increases in response to sustained high alcohol intake. When used clinically as an alcohol biomarker, the range of CDT measurement procedures using different reference limits hampers comparison of results. The IFCC therefore founded a working group on CDT standardisation (WG-CDT), aiming to define the measurand (% of disialotransferrin to total transferrin), select a reference measurement procedure (RMP), assign values to calibrators, and setting a reference interval. This study presents the results of a routine application of IFCC-standardised CDT measurement (CDT_{IFCC}) by the Sebia CAPILLARYS CDT capillary electrophoresis assay.

METHODS
The study involved the serum samples submitted for routine CDT testing at the Karolinska University Laboratory. CDT was measured by the Sebia CAPILLARYS CDT assay on a CAPILLARYS 2 FLEX PIERCING multicapillary system, equipped with a Phoresis CORE software that automatically generated CDT_{IFCC} results. External comparison was made with the % disialotransferrin results obtained by the HPLC RMP. In case of abnormal peak profiles in the CAPILLARYS CDT assay, samples were re-measured by the RMP. The CDT samples treatment solution (Sebia) was not used.

RESULTS
The CAPILLARYS CDT assay generated lower % disialotransferrin values compared with the HPLC RMP. However, following method standardisation according to the WG-CDT recommendation, the automatically generated CDT_{IFCC} results by the CAPILLARYS CDT agreed well with those obtained by the RMP (slope 1.02, intercept 0.06; r^2=0.984). In <3% of routine samples, re-measurement by HPLC was considered necessary, due to genetic transferrin variants (~75%), “di/tri-bridge” samples (~5%), or other analytical interferences (~20%), all of which were flagged by the software. In most cases, the HPLC analysis provided quantification, or at least estimation (i.e. normal or elevated value), of the CDT level.

CONCLUSIONS
These results indicated that a laboratory setup for routine measurement of IFCC-standardised CDT values (CDT_{IFCC}), using high-throughput multicapillary CE analysis with a confirmatory HPLC analysis option, will combine rapid quantitative and qualitative workflow with enhanced analytical safety.
EVALUATION OF THE PERFORMANCE OF OPTILITE SYSTEM FOR THE DETERMINATION OF FREE LIGHT CHAINS IN SERUM

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BACKGROUND: Determination of serum immunoglobulin κ and λ free light chains (FLC) and κ/λ ratio calculation is recommended for evaluation and management of plasma cell disorders. However, some analytical issues persist in these measurements, among which a too large imprecision seems to be the main challenge. In this study, we evaluated the new Optilite system (The Binding Site) for FLC determination, by comparing its performance with specifications for bias and imprecision (CV) derived from biological variation of FLC.

METHODS: We collected data during a 5-month period of routine use, employing two different reagent lots. The bias estimation was performed using the two-level (L and H) liquid Optilite control material by comparing the obtained long-term experimental means (n=74, both levels) with the manufacturer’s assigned values. The protocol for CV evaluation employed the liquid-frozen BioRad Liquichek Unassayed Chemistry Control Level 2, measured in each performed run for 71 runs. Goals at desirable (DG) and minimum (MG) quality levels for bias and CV were <±4.1%/6.1% and <4.0%/6.0% for κFLC and <±7.1%/10.6% and <3.5%/5.3% for λFLC, respectively. CV for κ/λ ratio should be <2.3%/3.4%.

RESULTS: Average cumulative bias [-2.8% (L) and -5.4% (H) for κFLC, and 4.6% (L) and 1.3% (H) for λFLC, respectively] fulfilled the DG, except for κFLC H fulfilling MG. However, the change of reagent lot positively influenced the bias of κFLC H (from -6.3% for lot 405569 to -3.4% for lot 401356). Overall CV resulted within the MG for κFLC (5.8%, mean 11.6 mg/L), while CV for λFLC (7.4%, mean 14.8 mg/L) and κ/λ ratio (7.1%, mean 0.8) failed to reach MG. Differences in variance results (F-test) provided by different reagent lots were statistically significant for λFLC (P <0.001), but not for κFLC (P=0.13).

CONCLUSIONS: Considering our previous experience with the SPAplus system (Biochim Clin 2013;37:370), the Optilite solution seems to offer a better method alignment. However, the variability of λFLC measurement is still too high to fulfil the performance specifications needed for a suitable clinical application.
EVALUATION OF A NEW AUTOMATED METHOD FOR GLYCOXYLATED HEMOGLOBIN ON THE ABBOTT ARCHITECT C8000

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Introduction
The objective of this study was to verify the analytical performance of HbA1c on Abbott Architect C8000 Chemistry System, a fully automated and high-throughput enzymatic assay. Its performance was compared to another enzymatic HbA1c method on Roche Cobas.

Methods
The analytical performance of the instrument was verified for imprecision, linearity and accuracy. Imprecision was carried out by measuring 2 levels of QC material (Biorad Diabetes Control) in triplicate over five days in accordance to CLSI EP5-A2 guidelines. The linearity and accuracy were analysed in triplicate for 5 linearity material specimens ranging between 4.0% to 15.0% HbA1c.

Results
The within-laboratory imprecision is satisfactory; coefficient variation of less 1.1% was obtained for 5.4% HbA1c concentration and less than 1.5% for 10.0% HbA1c concentration. NGSP stated the HbA1c assay is designed to have an imprecision of <2.0% within-laboratory %CV for samples targeted to 6.5% HbA1c and ≤3.5% within-laboratory %CV for samples >7.0% HbA1c. Abbott HbA1c assay is linear across the manufacturer’s claimed measuring range of 4.0% to 15.0% with the slope obtained is 1.002 and intercept of -0.11. Abbott HbA1c assay also passes the accuracy test. The maximum deviation for a mean recovery from 100% was 3.1%.

Conclusion
The imprecision of the Abbott HbA1c assay is well within the NGSP targets of <2.0% CV for samples targeted to 6.5% HbA1c and ≤3.5% CV for samples over 7.0% HbA1c. There was close agreement between the Abbott and Roche A1c assays. The new Abbott HbA1c assay offers clinical laboratories an additional choice for HbA1c measurement.
PERFORMANCE EVALUATION OF THE SIEMENS ADVIA CENTAUR PLGF AND SFLT-1 ASSAYS


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BACKGROUND: Preeclampsia (PE), a disease found in pregnant women, is characterized by hypertension and proteinuria. Rapid, accurate diagnosis of PE is essential for best clinical practice and maternal/fetal care. PlGF and sFlt-1, both produced by the placenta, regulate fetal angiogenesis. The sFlt-1/PlGF ratio becomes elevated in PE; therefore, this ratio is valuable in helping diagnose PE. Siemens Healthineers (Tarrytown, NY, U.S.) has developed ADVIA Centaur® PE assays* for detecting PlGF and sFlt-1 in serum and plasma to be used in tandem for a clinically relevant ratio of diagnostic value. This study evaluated performance of the Siemens ADVIA Centaur PlGF and sFlt-1 assays.

METHODS: The assays were evaluated on Siemens ADVIA Centaur Immunoassay Systems for precision, method comparison, linearity, limit of detection (LoD), limit of quantitation (LoQ), calibration interval, onboard stability (OBS), hook effect, and endogenous interferences. Clinical agreement with Roche ELECSYS PE assays with >150 clinically relevant samples was also evaluated. Also, sensitivity and specificity of the PE cutoff ratios were evaluated in ≤34 and >34 weeks gestation from normal and physician-diagnosed PE patients.

RESULTS: The PlGF and sFlt-1 assays have respective ranges of 10–10,000 and 30–85,000 pg/mL with acceptable sensitivity and specificity within early and late gestational windows. The precision results were <8% CV, and both were linear within each assay’s range, with no observed hook effect. The LoD and LoQ were <10 pg/mL (PlGF) and <30 pg/mL (sFlt-1). The calibration interval and OBS for the Centaur PlGF and sFlt-1 assays were >14 and >28 days, respectively. <10% endogenous interference was observed for all tested interferents for both assays. In addition, the Centaur and ELECSYS PE assays showed strong positive (98.2%) and negative (98.8%) agreement by clinical outcome

CONCLUSIONS: These studies indicate that the ADVIA Centaur PE assays are precise and sensitive for measuring PlGF and sFlt-1 across a wide range of concentrations.

*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.
Background: Bence-Jones proteinuria (BJP) is detected by monoclonal light chains into the urine. When light chains are in excess, they make histological and functional lesion to tubules, glomerulus and vessels. The identification of urinary light-chains excretion represents 15% to 20% of cases of multiple myeloma (MM). The guidelines recommend monitoring these patients with 24-hour urine collections. The aim of our study was to investigate the significance of BJ proteinuria in the diagnosis of MM.

Methods: We studied 22 patients with a possible diagnosis of MM from Pathological Clinic of AHEPA University Hospital (13 females and 9 males). BJ proteinuria was tested by immunofixation electrophoresis. Serum urea, creatinine and protein in 24 hour urine samples were measured by electrochemilumine-scence.

Results: MM was diagnosed in 5/22 (~23%) of our patients, in which BJ proteinuria was detected (3 free-k chains and 2 free-l chains). Interestingly their serum urea levels were (122 ± 36,7 mg/dl), serum creatinine levels were (1,26 ± 0,63 mg/dl) and protein levels in 24h urine samples were (2548,5 ± 1160,28 mg/24h). The other patients with no Bence Jones proteinuria 17/22 (~77%), had serum levels of urea (40,14 ± 8,34 mg/dl), serum levels of creatinine (0,84 ± 0,15 mg/dl) and protein levels in 24h urine samples were (112,33 ± 34,09 mg/24h), (p<0,01, p=0,21 and p=0,022 respectively).

Conclusions: Detection of BJ proteinuria can give clinical benefit through earlier diagnosis and hence treatment earlier in the patients’ disease. Impaired renal function is detected as 24h proteinuria, uremia and elevated serum creatinine levels. These parameters could be used as possible prognostic indicators for the further investigation of MM.
Analytical technologies and applications

Cod: M059

**CLINICAL LABORATORY PARTICIPATION IN THE PROFICIENCY TESTING PROGRAM FOR LUMINEX BEAD-BASED CYTOKINE ASSAYS (EQAPOL)**

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**Background and aims**

Since 2006 our laboratory has been intensely using Luminex systems (Luminex 100IS, in 2013 we switched to MagPix / Luminex Inc., USA/) for clinical research purposes. Our research is focused mainly on serum or plasma protein (including cytokines) levels in relation to different malignancies and other diseases and searching for new biomarkers. Since 2014 we have been participating in the Proficiency Testing Program for Luminex Bead-based Cytokine Assays (EQAPOL Luminex) organised by Duke University (US). Our aim is to maintain and improve our lab performance thank to the feedback we receive.

**Methods**

Twice a year the Luminex EQA testing is performed: the organiser sends out a reagent kit and a set of 21 spiked human serum samples to 23 participating sites over the world. The participating laboratory has to determine the levels of five cytokines (IFN gamma, IL-2, IL-6, IL-10 and TNF alpha) following the given protocol and report results via a web-based system. Additionally, the laboratory can perform a site-choice testing with site’s own reagent kit on EQAPOL provided samples. The organiser evaluates several criteria; most valued are accuracy to the consensus and precision of the assay.

**Results**

Our laboratory has already participated in four cycles (9th to 12th) including site-choice testing. In the cycle 9 we were assessed as Excellent (92.7 %), in the cycle 10 as Good (85.1%) due to impaired precision. Since we adopted appropriate measures, our next testing was again rated as Excellent (94.3%). In the meantime we were invited to participate in a teleconference with the organisers about the improvement of EQA testing.

**Conclusion**

The EQAPOL Luminex is an useful and well-organised tool to follow our Luminex assay performance.
VALIDATING CAPILLARY BLOOD SAMPLE KIT ON SEBIA CAPILLARYS 3 TERA

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Validating capillary blood sample kit on Sebia Capillarys 3 TERA

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Introduction:
Since the pediatricians on Aalborg University Hospital use capillary HbA1c blood tests for monitoring children with type 1 diabetes, it is important to assess a new method comprehensively. The purpose of this study was to validate the Sebia capillary blood sample kit (microtubes) for analyzing HbA1c on Sebia Capillarys 3 Tera. Quality goals were a combined imprecision < 3,7% (CV) and a mean bias < 2,8%, relatively to a certified reference material (National Danish Standard).

Methods
The accuracy and precision of HbA1c on Sebia Capillarys 3 Tera using the Sebia capillary blood sample kit was determined from samples of certified reference material in three levels analyzed in duplicate on five consecutive days. Furthermore, one hundred patient samples from corresponding K2-EDTA tubes and capillary blood sample kit were analyzed in duplicate. Range for patient samples: 25-160 mmol/mol.

Results
Certified reference material:
On Level one (48.0 mmol/mol) we found a CV% on 2,5% and a bias on -2,0%. On Level two (62.9 mmol/mol) the results were CV% 1,5% with a bias on -2,0%. Finally level 3 (77.3 mmol/mol) we achieved a CV% 1,5% with a bias on -0.4%
Blood samples:
We found complete linearity between microtubes and samples from K2-EDTA (Y=0.979x + 0.026) and R2 = 0.997
Mean differences between the microtubes and samples from K2-EDTA tubes: 1.4 mmol/mol

Conclusion
The Department of Clinical Biochemistry at Aalborg University Hospital participates in an internal as well as an external quality control program for the Sebia Capillaries 3 Tera. We didn’t find any pre- or post-analytical aspects to consider. We concluded that SEBIA capillary blood sample kit for analyzing HbA1c on Sebia Capillaries 3 Tera meets the quality criteria set in this study. The mean difference between K2-EDTA and Sebia capillary blood sample kit hasn’t any clinical relevance in diabetes monitoring. Therefore, the Department of Clinical Biochemistry at Aalborg University Hospital has implemented the Sebia capillary blood sample kit for routine analyzing capillary samples.
DESIGN VALIDATION OF A NEW IMMUNOCHEMISTRY ANALYZER COBAS E 801 AT 3 SITES IN EUROPE AND THE US

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BACKGROUND

With the continuous need to further improve and optimize laboratory testing efficiency, we took the opportunity to extensively evaluate the new high throughput immunochemistry module from Roche Diagnostics, cobas e 801, with 48 reagent positions and a throughput of 300 tests per hour. Here we report on the outcome of this testing.

METHODS

The analytical performance and overall system functionality was tested at 3 study sites using 15 assays (TSH, FT4, Anti-TSHR, NT-proBNP, CEA, CA 15-3, E2, Testosterone, Folate, RBC Folate, HBsAg Quant, Anti-HAV IgM, Anti-HCV, HIV DUO, CMV IgG Avidity) covering all assay characteristics represented within the Elecsys assay menu. Two different hardware configurations were used, a single cobas e 801 module at 1 site and a double module configuration at 2 sites. Testing protocols included precision studies based on guidance from CLSI EP5-A3 over 21 days, method comparisons over ≥5 days, an inter-laboratory survey using ring trial material, randomized testing under simulated routine conditions and usability testing.

RESULTS

A total of ~100,000 results were generated over the 4 month evaluation period. 84% of the 336 CVs for repeatability (within-run) and intermediate (within-lab) precision over all assays and measured materials were ≤3.5%. Evaluating method comparisons versus a cobas e 601 module using the Bablok/Passing regression procedure yielded slopes 0.92 - 1.11 and most Pearson correlation coefficients (r) >0.995 (most Kendall’s tau > 0.96). Comparable analyte recovery in ring trial material was shown across sites. The overall system functionality, defined as the interaction between software, hardware, chemistry and biological samples was tested under routine-like conditions and proved to perform very well throughout the study.

CONCLUSIONS

The results of this study demonstrate that the cobas e 801 module showed very good analytical performance and comparability with cobas 6000 <601>, high reliability and ease of use. Its compact footprint combined with multiple features convincingly improves immunochemistry lab testing efficiency.
Analytical technologies and applications

Cod: M062

EVALUATION OF SYPHILIS TREPONEMA PALLIDUM (TP) ON THE ABBOTT ARCHITECT I-2000

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Introduction
Serological testing is the most frequently used approach in the laboratory diagnosis of syphilis. The Architect Syphilis TP (Abbott) is a chemiluminescence immunoassay (CMIA) and is considered to be a good screening test due to its high sensitivity and full automation. The objective of this study was to verify the analytical performance of Syphilis TP on Abbott Architect I-2000 for detection of antibodies to Treponema pallidum in human serum.

Methods
The analytical performance of the instrument was verified for imprecision, carryover, analytical sensitivity and specificity. Imprecision was performed in triplicate with positive and negative control samples and 2 levels of pooled sera for five days in accordance to CLSI EP5-A2 guidelines. Carryover study was conducted. Sensitivity and specificity of the CMIA were compared with Bio-Rad enzyme immunoassay (EIA) old and newly revised kit.

Results
The within-laboratory imprecision showed good performance with coefficient variation of less than 20% was obtained for negative control and less than 10% for positive control. The results of the High-Low sequence are statistically identical to the results of the Low-Low sequence with less than the acceptable Error limit. The sensitivity and specificity were 95.2% and 100% respectively demonstrated comparable performance with EIA.

Conclusion
The evaluation showed Abbott Syphilis TP has a satisfactory analytical performance. The assay is fully automated and it is easy to use and hence it could be suitable choice for high volume laboratories.
EVALUATION OF THE ISONIAZID DRIED BLOOD SPOT ASSAY USING THE ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Background
Therapeutic drug monitoring (TDM) of anti-tuberculosis (TB) drugs is a key role for proper treatment to control TB. Owing to a variety of advantages of dried blood spot (DBS), Each assay in DBSs for rifampicin, ethambutol, and pyrazinamide was already developed. However, there is no isoniazid (INH) assay in capillary DBSs due to the instability of isoniazid. We developed an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for measuring concentrations of INH in venous and capillary DBSs.

Methods
Each DBS was analyzed on an UPLC system. INH and internal standard (IS) were determined by multiple reaction monitoring in positive ion mode. Analytical performances including precision, linearity and comparison of different type specimens were evaluated. And the stability of INH capillary DBSs was tested.

Results
INH and IS were clearly separated in UPLC-MS/MS system without matrix effect. Within-run precision and between-day precision were 6.07-11.34% and 8.33-11.35%, respectively. Linearity was acceptable in the range of 0.3-4.8 µg/mL. Simple linear regression and Deming regression analysis revealed good correlations with two types of DBS and plasma. The INH concentrations on DBSs stored at -70 degrees Celsius had been stable for 28 days. However, those of DBSs stored at 60 degrees Celsius decreased after 3 days.

Conclusions
Our assay for INH DBSs showed good performance such as precision, linearity, and comparison except for stability. If the problem of stability is solved, our method may be suitable for drug monitoring during TB treatment.
A label-free DNA sensor using a field-effect transistor and PNA probe layer on a gold surface

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A quantitative polymerase chain reaction (qPCR) is one of the most common techniques in molecular biology and diagnostics of pathogens. The initial copy number of a DNA sequence in a sample can be determined using qPCR. The predominant method to do qPCR relies on the use of fluorescent dyes to monitor DNA concentration in real time. Methods based on fluorescence detection are very sensitive and reliable. However, these methods require complex and expensive instrumentation in order to obtain the fluorescence signal. These attributes have limited the use of qPCR in point-of-care applications. To overcome this problem, we report a label-free sequence specific DNA sensor using PNA oligomer as capture probe on a gold surface. Thiol-modified PNAs and 6-mercapto-1-hexanol were used to create a self-assembled monolayer on the gold surface. Hybridization of the target DNA oligomer is detected by connecting the sensor surface to the gate electrode of a commercial low-cost field-effect transistor (FET). Hybridization of the negatively charged DNA molecules onto close proximity of the gold surface alters the gate potential of the FET thus causing a change in the current passing through the transistor. Obtainable signal responses from DNA-DNA hybridization are commonly found to be modest due to counter-ion screening. The PNA probe monolayer significantly enhances the detection since efficient hybridization can be achieved in much lower salt concentration. We have routinely measured changes in the gate potential up to 80 mV when 1 µM target DNA is added to sensor in a low salt concentration buffer (1 mM Tris, pH 8, 1 mM MgCl2). Potential changes induced by 1 µM non-complementary DNA were measured to be few millivolts at most. Low ion concentration increases the sensitivity of the sensor due to decreased screening of the electrochemical effect of the DNA molecule by cations in the solution. The results indicate an important step towards wash and label free DNA detection with a very low-cost and easily scalable system.
Analytical technologies and applications

Cod: M065

IMPROVED AUTOMATIC SAMPLE RECOVERY

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Methods

Our hospital has since April 2016 a Core Laboratory with a robotic structure parallel flow. This consists of a pre-analytical module (Cobas 8100, Roche Diagnostics®) which distributes the samples (approximately 2800 samples per day), through lines bidirectional transport, three COBAS 8000 for processing samples of serum and Sysmex for hematology samples and a module post-test (Cobas p501) that allows automatic archiving of samples. The middleware system is Cobas Infinity ensure traceability of samples throughout the process.

The system allows automatic storage of samples in the Cobas the p501 and automatic recovery thereof to the analyzers via transmission lines. We wonder whether this automatic recovery managed by the software allows removal of listings samples with results pending tests and manual search.

Results

Automatic recovery from the storage module to the analyzers Cobas p501 has considerably reduced listings pending tests results but we have not achieved their complete elimination. The reasons why we find samples with results pending are several: tubes insufficient serum to complete the tests, system errors in storage or sample recovery, manual recovery of samples before the system does, samples shows that for various reasons have left the system, they have not followed the automated processing and have not been filed in the Cobas p501.

Discussion

Automatic recovery of samples represent a reduction of listings pending tests results, saving time and less technician dedication to the search and retrieval the samples by technician staff. It is necessary to identify the causes that may remain without performing tests to take appropriate corrective action.
Analytical technologies and applications

Cod: M066

PERFORMANCE EVALUATION OF THE ADVIA CHEMISTRY SYSTEM ALANINE AMINOTRANSFERASE (P5P) AND ASPARTATE AMINOTRANSFERASE (P5P) ASSAYS*

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Background: The ADVIA® Clinical Chemistry (CC) System Alanine (ALTPLc) and Aspartate Aminotransferase (ASTPLc) assays each with ready-to-use liquid P5P (pyridoxal-5'-phosphate) are IFCC-traceable. We describe the performance of these assays.

Methods: The ALTPLc and ALTPLc assays are an adaptation of the IFCC reference methods. The reactions are initiated by the addition of α-ketoglutarate as a second reagent. The concentration of reduced nicotinamide adenine dinucleotide (NADH) is measured by its absorbance at 340/410 nm, and the rate of absorbance decrease is proportional to the analyte concentration.

Results: The ALTPLc and ASTPLc assays use a 25 µL sample volume of serum or plasma with an analytical range of 9–1000 U/L and 8–1000 U/L respectively. With dilution, extended range is 6000 U/L for each assay. Time to first result is 10 minutes for each assay. Precision was evaluated per CLSI EP05-A2 using serum pools and commercial quality control materials. Repeatability and within-lab precision were ≤3.6% CV and ≤4.9% CV for ALTPLc and ≤1.1% CV and ≤2.1% CV for ASTPLc, respectively, across the assay range.

Patient sample method comparison studies versus two different systems:

ADVIA CC ALTPLc = 0.96 * IFCC Reference Assay – 1.6 U/L (r = 1.00, n = 101)
ADVIA CC ALTPLc = 1.03 * ADVIA CC ALTP_c + 0.2 U/L (r = 1.00, n = 104)
ADVIA CC ASTPLc = 1.03 * IFCC Reference Assay – 1.6 U/L (r = 1.00, n = 103)
ADVIA CC ASTP_c = 0.97 * ADVIA CC ASTP_c – 1.4 U/L (r = 1.00, n = 103)

Conclusions: The ADVIA CC Alanine Aminotransferase (ALTPLc) and Aspartate Aminotransferase (ASTPLc) assays with ready-to-use liquid P5P exhibit similar performance compared to the current ADVIA CC ALTP_c/ASTP_c assays.

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*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.
Background. The ADVIA Chemistry Liquid Creatine Kinase assay (CK_L) is an IFCC traceable, calibrated, liquid stable, ready to use, chemistry assay. We describe the performance of the Creatine Kinase assay.

Methods. Creatine Kinase reacts with creatine phosphate and adenosine diphosphate (ADP) to form adenosine triphosphate (ATP), which is coupled to the hexokinase-G6PD (glucose-6-phosphate dehydrogenase) reaction, generating NADPH (reduced nicotinamide adenine dinucleotide phosphate). The concentration of NADPH is measured by the increase in absorbance at 340/596 nm. The resulting reaction rate signal is proportional to the concentration of analyte in the sample. The ADVIA Chemistry CK_L assay is an adaptation of the IFCC Reference Method.

Results. The assay uses a 4.5 µL sample volume of serum or plasma and has an analytical range of 16-1300 U/L undiluted. With dilution, samples up to 7800 U/L can be tested. Results are traceable to Creatine Kinase IFCC reference assay. Time to first result is 10 minutes. Precision was evaluated per CLSI EP05-A2 using serum pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 2.0 %CV and ≤ 3.4 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus two different systems: ADVIA Chemistry CK_L = 1.05 * IFCC Reference Assay - 6.9 U/L (r = 1.00, n = 100), ADVIA Chemistry CK_L = 1.01 * ADVIA Chemistry CKNAC – 1.8 U/L (r = 1.00, n = 116). Minimal interference (≤ 10%) was observed with Hemolysate (125 mg/dL), Bilirubin (60 mg/dL), Lipemia (1000 mg/dL), Ascorbic Acid (6mg/dL), Sulfasalazine (300 mg/L), and Sulfapyridine (300 mg/L).

Conclusions. The ADVIA Clinical Chemistry Creatine Kinase assay exhibits competitive performance characteristics and shows improvement to the current Siemens CKNAC assay via calibration and ready to use reagents.

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Analytical technologies and applications

Cod: M068

OMNIPAQUETM INTRAVENOUS CONTRAST INTERFERENCE IN CAPILLARY ZONE ELECTROPHORESIS

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Introduction

The clinical laboratory plays a critical role in the screening, diagnosis and follow-up of patients with monoclonal gammopathies. Capillary zone electrophoresis (CZE) is an important method utilised for analysis of serum in these cases. Recent reports have identified certain substances which interfere with the CZE method, most notably radio-opaque intravenous contrast media. These interferences complicate interpretation and can lead to unnecessary subsequent testing.

Aims

1. To determine if Omnipaque™, the radio-opaque contrast medium most commonly used by the radiology department, interferes with the CZE method
2. To characterise the observed interference with regards to the position of the interfering peak
3. Provide guidelines regarding an appropriate time interval for obtaining follow-up specimens

Methods

A total of 5 patients scheduled to undergo radiological imaging with Omnipaque™ were recruited. Venous blood samples were collected prior to imaging (baseline) and 1 hour, 8 hours and 12 hours post contrast medium administration. CZE was performed on all samples. In addition, agarose gel electrophoresis (AGE, to confirm the absence of interference on the gel method) and immunosubtraction (to confirm that the interfering peak was not of monoclonal origin) was performed on the 1 hour specimen.

Results

The interference will be demonstrated by means of 6 figures.

Discussion and Conclusion

Our study confirms the interference by Omnipaque™, which manifests as a peak in the late alpha-2 region in CZE based systems in the clinical laboratory (Fig 2 and Fig 4). This study also confirms that this interference does not occur in traditional gel-based electrophoresis systems (Fig 3). Creating clinician awareness has the potential to largely eliminate this type of interference. Furthermore, manufacturers should be motivated to include details of this expected interference in relevant product package inserts. However, in the event of interference occurring, the study suggests the most appropriate time for follow-up specimens to be more than 8 hours post contrast medium administration when the residual peak has become too small to complicate interpretation (Fig 5).
The serological measurement of anti-tetanus toxoid antibodies produced in response to vaccination with tetanus toxoid protein aids the assessment of a patient’s immune response. Here we describe the evaluation of the Anti-Tetanus Toxoid Immunoglobulin assay for use on the Binding Site Optilite analyser. The measuring range of the assay is 1.67 - 50 IU/mL at the standard 1/10 analyser dilution. Correlation to the Binding Site Anti-Tetanus Toxoid Immunoglobulin assay for the SPAPLUS® was performed using 115 plasma samples ranging from 1.74 - 47.72 IU/mL. This demonstrated good agreement when analysed by Passing and Bablok regression (y = 0.98x + 0.51). The assay also demonstrated good agreement between serum and plasma matrices using 107 paired serum and EDTA plasma samples ranging from 1.585 - 48.363 IU/mL (Passing and Bablok analysis: y = 0.98x + 0.06). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing six serum levels (2.77, 3.15, 4.27, 7.43, 8.85 and 17.04 IU/mL) on a single kit lot over three analysers and 21 days. All levels gave total precision CV values of <9%. Linearity studies were performed following the CLSI EP6-A, using a serially diluted plasma sample. The assay was linear across the measuring range (all results were within 10% of expected values). Interference testing was performed according to CLSI EP7-A2, using serum samples with anti-tetanus toxoid antibody concentrations both close to the medical decision point and at an elevated level. No significant assay interference was observed with triglyceride (1000mg/dL), Intralipid (1000mg/dL), bilirubin (200mg/L) or haemoglobin (5g/L). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and gave a limit of 0.15 IU/mL. The antigen excess capacity of the assay was determined to be equivalent to 120 IU/mL at the standard dilution.

In conclusion, the Anti-Tetanus Toxoid Immunoglobulin assay for the Optilite® analyser provides a reliable, accurate and precise method for quantifying anti-tetanus toxoid antibodies in serum and plasma.
C-reactive protein (CRP) is a nonspecific inflammatory biomarker of hepatic origin that is commonly quantified in the detection and monitoring of infection and acute phase inflammation. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. CRP concentrations reach a peak within two days on acute phase response, having a half-life of approximately 18 hours.

Here we describe the evaluation of The Binding Site CRP serum assay for SPAPLUS analyser. The assay has been validated, using a linearity study performed to CLSI EP06-A guidelines, to have a measuring range spanning 5–250 mg/L at a 1/1 analyser dilution. A limit of quantitation (LoQ) study based on CLSI EP17 confirmed a limit of 5 mg/L. Correlation to the Roche Modular P assay demonstrated good agreement using 225 clinical samples ranging from 4.66–498.23 mg/L (Passing and Bablok analysis slope y=1.02x+3.22). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on four analysers. The coefficients of variation (CVs) were as follows: 7.8% at 8.7 mg/L, 5.7% at 13.5 mg/L, 2.6% at 60.0 mg/L, 3.6% at 147 mg/L and 6.2% at 225.2 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 18 potential drug and metabolite interferents, including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.5, 63.4 & 170.0 mg/L). No significant interference was observed (maximum difference in the control samples was -9.86%).

In conclusion, the SPAPLUS CRP assay provides an accurate method for quantifying human serum CRP.
A RAPID AND FULLY AUTOMATED LC-MS/MS APPROACH TO THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESSANT DRUGS

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Therapeutic drug monitoring (TDM) is a recognized necessity for the clinical management of immunosuppressive drugs (IDs). Accuracy and rapid availability of results are equally important aspects in this type of analytical activity. Methodologies that employ immunoassays are fast enough but intrinsically suffer from accuracy pitfalls.(1) Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is an intrinsically more accurate technology but much slower since it commonly relies on manual sample preparation.

In order to overcome this practical limitation in the rapid and precise TDM of IDs, we tested a new fully automated system composed by a Zivak Multitasker (Zivak Technologies, Istanbul, TUR) coupled to an API5500 mass spectrometer (Sciex, Toronto, CAN). This system enables fast processing of whole blood primary tubes for the quantitative simultaneous analysis of tacrolimus (T), sirolimus (S), everolimus (E), cyclosporine-A (C) with typical accuracy and enhanced precision over manual preparation. Moreover, unlike other automated approaches,(2) it enables serial direct sample analysis, which is particularly valuable for urgent clinical request processing.

We fully validated the analytical performances of the new system in terms of sensitivity, specificity, accuracy and robustness, according to ISO 15189 standard indications.

We compared the performance of this novel system with an established LC-MS/MS analysis method (in use for ten years in our laboratory) based on manual extraction. We performed comparison over 1000 sample analysis over a three week activity period. Passing & Bablock and Bland-Altman data regressions confirmed a stringent correlation of method accuracy. Intra- and inter-assay precision were better with the new automatic system. This allowed us to conclude that the new automated approach based on Zivak Multitasker could be a valuable improvement for accurate, high throughput and fast TDM of IDs.


DEFINING A STANDARDISED CENTRIFUGATION PROTOCOL FOR A RANGE OF BD VACUTAINER® BLOOD COLLECTION TUBES

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BACKGROUND:
Centrifugation is a key step in the creation of high quality samples. Centrifugation conditions differ depending on the tube type e.g. gel separator based vs. non gel. With the recent introduction of BD Vacutainer® Barricor™ Plasma Blood Collection Tubes, centrifugation recommendations for BD Vacutainer® tubes now range from 1300 to 5000g and from 3 to 10min. In today’s modern accredited laboratory, a range of centrifugation conditions can lead to inefficiency by not maximizing throughput using laboratory automation or requiring manual processes for alternate conditions. One strategy is to standardize centrifugation protocols (g force, time & temperature) for all blood collection tubes. This study evaluated the use of 3000g for 5min at 20°C for a range of chemistry serum and plasma, with or without gel, and coagulation tubes.

METHOD:
Duplicate BD Vacutainer® Serum CAT, BD SST™II Advance (Serum/Gel), BD PST™II (Plasma/Gel), BD Lithium Heparin, BD Sodium Citrate & BD Glucose tubes together with a single BD Barricor™ tube were collected from 40 apparently-healthy subjects. One tube of each type was centrifuged according to BD recommended conditions and the second was centrifuged at 3000g for 5min at 20°C along with the BD Barricor™. Samples were visually inspected for quality indicators prior to testing. Sample stability was assessed by retesting after 24h at 4°C.

RESULTS:
Using potassium as a key indicator of sample quality, initial results demonstrated no statistically significant difference between recommended and standardized conditions for each tube type. BD CAT: 3000g, 5min (4.28±0.27 mmol/L) vs. 1300g, 10min (4.31±0.28 mmol/L), p=0.308; BD SST™II: 3000g, 5min (4.32±0.28 mmol/L) vs. 2000g, 10min (4.32±0.275 mmol/L), p=0.872; BD PST™II: 3000g, 5min (3.93±0.28 mmol/L) vs. 2000g, 10min (3.94±0.30 mmol/L), p=0.417; BD Lithium Heparin: 3000g, 5min (3.89±0.30 mmol/L) vs. 1300g, 10min (3.90±0.30 mmol/L), p=0.538; BD Barricor™: 3000g, 5mins (3.97±0.30 mmol/L). Further, complete barrier formation was achieved and integrity maintained in all samples.

CONCLUSIONS:
The standardized condition of 3000g for 5 mins at 20°C is acceptable for the centrifugation of BD Vacutainer® Serum CAT, BD SST™II, BD PST™II, BD Lithium Heparin, for the determination of potassium.
Analytical technologies and applications

**Cod: M074**

**SINGLE ASSAY MEASUREMENT OF ALDOSTERONE-TO-RENIN RATIO BY UHPLC-MS/MS.**

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**Background**

Hypertension or high blood pressure is a highly prevalent disease that results from complex interactions of genes and environmental factors. Secondary hypertension is less common (around 5% of hypertensive patients) and is caused by kidney or endocrine disorders. To define the cause of the secondary hypertension the measurement of aldosterone-to-renin ratio (ARR) is a useful tool, especially for screening of primary aldosteronism.

Aldosterone can be measured in 24-hour urine samples or in blood. Renin, a proteolytic enzyme, is quantified in blood by direct immunoassay or by the mean of its activity. Plasma renin activity (PRA) is assessed by measuring the amount of angiotensin-I produced in a defined time range. We present here a method that allow to measure low aldosterone levels as well as low PRA in a single sample after simple preparation.

**Methods**

Plasma (100µL) from blood collected on EDTA-K3 is buffered and spiked in duplicate with internal standards. One sample is kept at +4°C to be used a reference (t0) while the other one is incubated at 45°C for 3 hours. After incubation, both samples were precipitated with acetonitrile and filtered through a phospholipid depletion sorbent. After evaporation and reconstitution, extracts were placed on autosampler tray in polypropylene vials.

15 µL of extract was injected on a Shim-Pack GIST C18 2µm 50x2.1mm (Shimadzu Corp.) operated at a flow-rate of 0.4 mL/min. Compounds were separated with a gradient of water and methanol. Cycle time was of 7 minutes.

Multiple Reaction monitoring (MRM) acquisition was operated in positive ionization for angiotensin-I and in negative mode for aldosterone (2 transitions per compound) with polarity switching on LCMS-8060 (Shimadzu Corp.).

**Results**

Quantification of angiotensin-I was performed from 0.1 to 100ng/mL. PRA is calculated as the difference in angiotensin-I concentration between t3h and t0 divided by incubation time. Therefore, as a difference of 0.1 ng/mL in angiotensin-I was significantly measurable, PRA as low as 0.033 ng/mL/hr were reachable. Quantification of aldosterone was possible from 10 to 1 000 pg/mL. Measured concentrations were not significantly different at t0 and t3h. ARR is then calculated as the ratio of the values obtained before. All the calculations were performed automatically by the acquisition and processing software LabSolutions (Shimadzu Corp.).
Analytical technologies and applications

Cod: **M075**

**ANALYSIS OF 25-OH VITAMIN D2/D3 IN SERUM BY LC-MS/MS WITH FULL-AUTOMATED SAMPLE PREPARATION**

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**Introduction**

Vitamin D measurement has become an important component in clinical assays largely because deficiency is associated with a number of disorders, such as rickets, osteomalacia and osteoporosis.

LC-MS/MS has become an essential tool for monitoring the concentration of Vitamin D2/D3 in biological samples due to its high level of sensitivity and specificity; however, manual sample preparation often involves several complicated steps which can introduce error into the results. In this study, we investigated the ability to analyze for 25-OH Vitamin D2/D3 by LC-MS/MS (LCMS-8050, Shimadzu) using automated sample preparation (CLAM-2000, “For research use only. Not for use in clinical diagnostics” Shimadzu) to process large sample sets. The CLAM-2000 has the ability to perform a variety of steps appropriate for automated sample preparation by LC-MS/MS. This system is seamlessly integrated with the LCMS system. We validated the automated method by using a kit containing standard compounds.

**Methods**

Compounds were measured using a commercially available test kit ClinMass® LC-MS/MS Complete Kit for 25-OH-Vitamin D2 / D3, MS7000 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992 München, Germany). Calibrators, control samples, analytical column and mobile phase solvents were provided by the kit. These calibrators and controls were loaded directly into the CLAM-2000 for sample processing. The CLAM-2000 was programmed to perform protein precipitation using acetonitrile followed by filtration and sample collection. The LC-MS instrument was equipped with an atmospheric pressure chemical ionization source (APCI).

**Results**

The calibration curves showed good linearity ($R^2>0.998$) over a clinical relevant range of 4.10-68.5 µg/L for 25-OH Vitamin D2 and 4.68-77.3 µg/L for 25-OH Vitamin D3. The reproducibility (N=7) at three concentrations, including LLOQ, of each compounds was excellent (CV<6.5%). Different day reproducibility (N=7) for 3 days at three concentrations as well (CV<7.2%). Comparison of 25-OH Vitamin D3 concentration between manual sample preparation and automated sample preparation using serum from human samples who have Vitamin D intake shows good agreement. The correlation coefficient of the automated operation against the manual operation was excellent ($R^2>0.9$).
Law enforcement against illicit use of cannabis requires rapid, feasible, and reliable tools for on-site testing of cannabinoids. Notably, methods based on cannabinoid-specific antibodies enable efficient screening of multiple specimens. Thus, we investigated in vitro affinity maturation to generate a single-chain Fv fragment (scFv) that recognizes with high affinity the psychoactive cannabinoid, \( \Delta^{9} \)-tetrahydrocannabinol (THC).

A mouse monoclonal antibody against THC, Ab-THC\#33, with \( K_a = 6.2 \times 10^7 \) M\(^{-1}\) (as Fab fragment) was first established by the hybridoma technique. Then, a "wild-type" scFv (wt-scFv) with \( K_a = 1.1 \times 10^7 \) M\(^{-1}\) was prepared by bacterial expression of a fusion gene combining the \( V_H \) and \( V_L \) genes for Ab-THC\#33. Subsequently, random point mutations in \( V_H \) and \( V_L \) were generated by error-prone PCR, and the resulting products were assembled into mutant scFv genes, which were then phage-displayed. Repeated panning identified a mutant scFv with 10-fold enhanced affinity (\( K_a = 1.1 \times 10^8 \) M\(^{-1}\)) for THC, in which only a single conservative substitution (Ser50Thr) was present at the N-terminus of the VH-CDR2 sequence. Kinetic analysis revealed that a lower dissociation rate constant (\( k_d \), \( 3.2 \times 10^{-4} \) s\(^{-1}\)) for the mutant scFv (2.2 \( \times 10^{-3} \) s\(^{-1}\) for wt-scFv) mainly contributes to the enhanced affinity.

In competitive ELISA, the mutant scFv generated dose-response curves with midpoint 0.27 ng/assay THC, which was 3-fold lower than that of wt-scFv. Limit of detection was low enough (0.10 ng per assay) for detecting THC in urine. Even higher reactivity with a major THC metabolite, 11-nor-9-carboxy-\( \Delta^{9} \)-tetrahydrocannabinol, indicated that the mutant scFv will be useful for testing not only THC in confiscated materials, but also the metabolite in urine.

We expect that this mutant scFv will be useful to detect trace of THC and its polar metabolite in illicit cannabis-derived materials and/or in cannabis users.
PERFORMANCE OF CRP ASSAY FOR USE ON THE BINDING SITE OPTILITE® TURBIDIMETRIC ANALYSER

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Detection of C-reactive protein (CRP) within serum is widely regarded as the most reliable biomarker for systemic inflammation. CRP functions by readily binding damaged cell membranes and microbial polysaccharides, and is involved in the agglutination and precipitation of invasive bacteria. It is also capable of activating the complement cascade, resulting in opsonisation and phagocytosis of cell debris and bacteria. Normal serum contains <10 mg/L CRP, an increase in circulating CRP levels can be detected within 6 hours post onset of inflammation. Moderately elevated serum levels (10 – 40 mg/L) are associated with mild inflammation and viral infections whereas high levels (>40 mg/L) are indicative of acute phase inflammation and bacterial infections.

Here we describe the performance of an immunoassay for the detection and quantification of serum CRP on the Binding Site Optilite® analyser. The Optilite CRP assay displayed good agreement with the Roche Modular P CRP assay in a comparison of 193 serum samples ranging from 3.87 – 498.23 mg/L (Passing-Bablok analysis; Y=1.00x+5.56). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing five serum levels on three kit lots and three analysers over 21 days. Samples were targeted to the medical decision point, pathological concentrations, the reference interval and the minimum dilution. Total precision gave CVs of 3.9% at 9.99 mg/L, 2.9% at 22.33 mg/L, 2.8% at 63.88 mg/L, 3.1% at 146.73 mg/L and 2.8% at 258.28 mg/L. Interference testing was performed using 18 potential drug and metabolite interferents including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.50, 60.06 & 170.73 mg/L). No significant interference was observed (maximum difference from control samples was -8.69%). The assay was also observed to be linear over the measuring range of 5 – 300 mg/L at the standard 1/1 dilution using a serially diluted sample pool (recovery was ±7.4% for all samples).

In conclusion, the Optilite® CRP assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.
Quantification of total protein in serum provides a useful tool for the assessment of the synthesis and maintenance of circulating proteins. Abnormal total protein levels act as a key indicator for multiple disease states; elevated TP levels are a marker for bone marrow disorders, liver cirrhosis and inflammation. A decrease in total protein concentration can be detected in disorders associated with defective protein synthesis, impaired kidney function, malnutrition and malabsorption.

Here we describe the performance of the Total Protein assay for use on the Binding Site Optilite Analyser. The measuring range of the assay was determined as 0.12-15 g/dL. Linearity was assessed using a serially diluted serum sample, following the CLSI approved guideline EP6-A. The assay was linear across the measuring range (all results were within 10% of expected values). Correlation to the Roche Hitachi 917 assay demonstrated good agreement using 94 clinical samples ranging from 0 – 14.3 g/dL by Passing and Bablok analysis (y=1.017x-0.038). A precision study was performed over a period of 5 days. Total coefficients of variation (CVs) were as follows: 0.77% at 5.8 g/dL, 0.57% at 7.0 g/dL, and 0.54% at 10.8 g/dL. Interference testing was performed according to CLSI EP7-A2 guidelines. No significant assay interference was observed in the presence of triglycerides (1000mg/dL), L-ascorbic acid (60mg/dL), unconjugated bilirubin (60mg/dL), conjugated bilirubin (60mg/dL) and haemoglobin (500mg/dL). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and reported a limit of 0.03g/dL.

In conclusion, the Optilite Total Protein assay provides a reliable and precise method for quantifying total protein in human serum.
Analytical technologies and applications

Cod: M079

PERFORMANCE OF THE TRANSFERRIN URINE ASSAY FOR USE ON THE BINDING SITE OPTILITE® TURBIDIMETRIC ANALYSER

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Transferrin is an iron-binding negative acute phase protein found in blood plasma. Transferrin has the capacity to bind two molecules of iron which can be transported through the blood to the liver, spleen and bone marrow. The presence of transferrin in urine is a key indicator of nephrotic syndrome and glomerular damage. Quantitation of urinary transferrin, when used in conjunction with albumin results, permits an estimation of the charge selectivity of glomerular defects as both proteins are of a similar size but different charge.

Here we describe an assay designed for the quantification of human transferrin in urine on the Binding Site turbidimetric Optilite analyser. The measuring range of the assay spans 2-60 mg with a standard dilution of 1/1 and the ability to reflex up to a higher dilution of 1/10. Linearity studies were performed following the CLSI approved guideline EP6-A using a serially diluted urine sample. The assay was linear across the standard measuring range (all results were within 8% of expected values). Interference was assessed using four potential metabolite and drug interfering substances over 3 urinary transferrin concentrations. Testing with urobilinogen (45 mg/L), albumin (100 mg/L) and ascorbic acid (200mg/L) did not significantly disrupt assay performance compared to control samples (maximum difference was 9%). Within run precision studies were performed according to the CLSI approved guideline EP5-A2, using five serum levels on one kit lot. Samples were selected to include medical decision point concentrations, pathological concentrations, the reference interval and the minimum dilution. Within run precision gave CVs of 2.03% at 3.74 mg/L, 4.61% at 13.726 mg/L, 1.63% at 32.3 mg/L, 1.13% at 48.92 mg/L and 2.58% at 269.22 mg/L. A preliminary comparison to the Siemens BN™II Transferrin Urine assay resulted in good agreement over 27 samples covering the measuring range (Passing and Bablok analysis: y=0.94x-0.01).

In conclusion, the Transferrin Urine assay for the Binding Site Optilite analyser is reliable, accurate and precise, and shows excellent agreement with existing assays.
Analytical technologies and applications

Cod: M080

VERIFICATION OF BÜHLMANN FAECAL CALPROTECTIN TEST (FCAL TURBO TEST) ON ABBOTT ARCHITECT C8000 ANALYZER

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Background: Faecal calprotectin as a non-invasive marker of inflammatory bowel disease (IBD) is routinely measured in our laboratory with fluoroenzymatic immunoassay (FEIA, Thermo Fisher Scientific) on Phadia 100 analyzer. The aims of this study were: i) verification of fCAL turbo test (Bühlmann) on ARCHITECT c8000 (Abbott Diagnostics) using particle enhanced turbidimetric immunoassay (PETIA) and ii) verification of calprotectin stability specified by manufacturer in faecal extract obtained with CALEX® Cap device extraction tube (Bühlmann).

Methods: To evaluate assay imprecision, commercial quality control sample and patient faecal extract were run in triplicate for five days. Comparison of FEIA and PETIA assays was made on 40 faecal extracts with calprotectin concentration throughout entire measuring range. Carry-over was calculated by measuring calprotectin in sample with high concentration (in duplicate) followed with measurement of calprotectin in sample with low concentration (in triplicate). Verification of stability was performed by measuring calprotectin in four faecal extracts (27–1035 mg/kg) stored at 4-8°C during 6 days. Allowable bias was set at 30%, according to Labquality external quality assessment criteria.

Results: Interlaboratory imprecision for fCAL turbo test was 2.41% for mean calprotectin concentration of 123.9 mg/kg and 5.48% for 74.5 mg/kg. Passing-Bablok regression analysis showed statistically significant proportional difference \[y = 0.51(95\% CI -35.66 - 0.61) + 1.83(95\% CI 1.33 - 3.41)x\] and Bland-Altman analysis showed both proportional and constant difference between two methods. Calprotectin proved to be stable in faecal extracts kept at 4-8°C during 6 days in samples with concentration at cut off value (50 mg/kg) and higher, with mean biases 2.67–21.63%. Sample with concentration lower than cut off had mean bias 35.1% but it has no influence on clinical decision. Calculated carry-over effect was 0.10%, in accordance with manufacturer's claim.

Conclusion: fCAL turbo test has satisfactory imprecision and minimum carry-over effect. Calprotectin in faecal extract was proved to be stable according to manufacturer specification. Comparison study showed that FEIA and PETIA methods cannot be used interchangeably.
Background: Quantification of amino acids in clinical samples is important in the diagnosis and follow-up of aminoacidopathies. As inborn errors of metabolism attracts more interest in time, the necessity of new analytical methods with increased sensitivity and efficiency arises. The aim of this study was to develop, implement and validate a new method for amino acid analysis by QDa mass detector in plasma, urine and CSF samples.

Materials and Methods: Plasma, urine and CSF samples were precipitated with 10% sulfosalicylic acid and supernatants were derivatized with AccQTag Ultra. Calibrators and QC samples were prepared as clinical samples. Chromatographic separation and quantitation of amino acids was performed using Acquity UPLC equipped with Acquity Cortecs C18 column (150 mm x 2.1 mm, 1.6μm particles) and Acquity QDa Mass Detector (Waters, Manchester, UK). Method validation studies included accuracy and precision studies, method comparison with Acquity UPLC coupled with Acquity TUV detector (Waters, Manchester, UK). Statistical analyses were performed with MS Office Excel 2013.

Results: 41 amino acids in plasma, urine and CSF samples were separated with retention times varying from 2.02 to 5.40 minutes. A single run lasts about less than 9 minutes. Results of precision studies performed within day 5 times and between 6 days varied from 0.66 to 12.54 % (intra-day) and from 2.34 to 13.30 % (inter-day). Method comparison studies were performed with 20 CSF, 80 plasma and urine samples. Although results were mostly correlated, some discrepant results were found. Discrepancies were interpreted as superiority of QDa mass detection to UV detection.

Conclusions: A rapid, both sensitive and specific method was developed and validated for amino acid analysis in plasma, urine and CSF samples using UPLC coupled with QDa mass detector.
UTILITY OF GAUSSIA LUCIFERASE AS A FUSION PARTNER WITH SCFVS FOR BIOLUMINESCENT IMMUNOASSAYS TESTING CLINICAL BIOMARKERS

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Recent genetic engineering enabled generation of fusion proteins with unique characteristics. Here, we show the utility of Gaussia luciferase (GLuc), much smaller than previously found luciferases, as the fusion partner with artificial antibody fragments for developing sensitive immunoassay systems. As an example, we constructed a bioluminescent enzyme-linked immunosorbent assay (BL-ELISA) system determining the major glucocorticoid, cortisol.

The variable domains (VH and VL) of a newly elicited anti-cortisol antibody were cloned and combined to encode a single-chain Fv fragment (scFv). This scFv gene was then linked to wild-type GLuc gene or that encoding GLuc mutants reported to show improved emission kinetics, and expressed in the periplasmic space of Escherichia coli cells. We found that the wild-type GLuc fusion protein (scFv-wtGLuc) showed the most suitable luminescent properties for our BL-ELISA system, in which scFv-wtGLuc was reacted competitively with the analyte and immobilized cortisol moieties on microwells, and the bound GLuc activity was monitored with coelenterazine as the substrate. It is particularly worth noting that batch-type luminescence detection was successfully achieved using a plate reader without built-in injectors. The midpoint and limit of detection in a typical dose-response curve were 4.1 and 0.26 pg/assay, respectively, thus exhibiting much more sensitivity than conventional cortisol immunosassays. Although this sensitivity might largely be due to the high antigen-binding affinity of the scFv used ($K_a$ $1.7 \times 10^{10}$ M$^{-1}$), the advantages of GLuc were shown by the fact that the BL-ELISA was more sensitive than the fluorescent ELISA using the same scFv fused with an alkaline phosphatase variant. Serum cortisol levels (determined as the sum with cortisone due to cross-reactivity) for healthy subjects, determined without any pretreatment, were compatible with reported reference ranges. The scFv-GLuc fusion protein could be obtained from the periplasmic space of transformed E. coli with practical expression levels (>100 µg/L), and was stable over a year under storage as periplasmic extracts at -30°C or with repeated freeze-thawing.

Thus, we first used GLuc as a fusion partner with scFvs for developing standard immunoassay systems. We hope that our present results encourage the use of GLuc and enlarge its utility in in vitro biomedical assays for monitoring various biomarkers.
FAECAL CALPROTECTIN: COMPARISON OF AN AUTOMATED TURBIDIMETRIC METHOD WITH AN ELISA ASSAY

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Background: Faecal calprotectin (fCal) allows to rule-out or rule-in intestinal inflammation among patients with abdominal symptoms and to monitor the disease phase (active/remission) among patients with inflammatory bowel diseases. In medical laboratories, a significant increase in the number of fCal assays has been observed in the last decade; therefore, the availability of high-throughput and cost-effective methods is advisable. The aim of this study was to compare an automated turbidimetric method with an ELISA fCal assay.

Methods: fCal was measured in 96 faecal samples randomly selected from the laboratory routine series by means of an ELISA procedure (IDK Calprotectin, Immundiagnostik, Germany), automated on DSX ELISA processing system (Dynex Technologies) and of a turbidimetric assay (BÜHLMANN fCALTM turbo, BÜHLMANN Laboratories, Switzerland) automated on Cobas Module c501 (Roche).

Results: The upper measurement limit of IDK was 2100 $\mu$g/g, while that of fCALTM turbo was 8000 $\mu$g/g. The results obtained with the two methods were correlated ($R^2=0.787$). The Bland-Altman analysis demonstrated a bias of 119 (95%CI: -963 to 1201) $\mu$g/g, while Deming regression underlined a non-constant bias. The lack of concordance between the two methods was mainly due to samples with very high fCal levels (n=6), because the fCALTM turbo assay performs an automated dilution step for values higher than 2058 $\mu$g/g; instead, the IDK assay requires a repeated analytical set-up of manually diluted samples. After the exclusion of 6 results with values above 1500 $\mu$g/g, the Bland-Altman analysis documented a bias of 0.02 (95%CI: -195 to 195) $\mu$g/g. The Deming regression confirmed the absence of proportional or constant bias (slope 95%CI: 0.9176-1.092; intercept 95%CI: -26.80 to 25.37).

Conclusions: The two studied methods for fCal measurement have comparable performances for values covering the clinical relevant range. The fCALTM turbo assay presents the advantages of an automated turbidimetric platform, such as decision criteria for control rules before the set-up of the analytical series and the independence from any analytical batch. Moreover it is faster (about 30 minutes for 96 samples) than an automated ELISA (about 3 hours).
A NEW RECOMBINANT CALPROTECTIN ANTIGEN FOR QUALITY ASSESSMENT OF FAECAL CALPROTECTIN TESTING

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Background:
Fecal calprotectin (fCal) can be used for diagnosing and monitoring inflammatory bowel diseases (IBD). fCal testing includes the initial weighting/extraction of faecal sample, while quantification is generally performed by ELISA. The available evidences show a need of harmonization for fCal testing results, although no independent quality control material is available for the monitoring of performances in internal and external quality schemes. The aim was to verify if a new purified recombinant calprotectin antigen (PRCA) (DiaSorin Inc., USA) could be suitable for the assessment of fCal imprecision.

Methods:
A pH 8 buffer containing 50 mM Tris, 150 mM NaCl, 2.5 mM CaCL2, 3% BSA, 0.05% Tween 20, added or not with polyethylenimine (PEI) was used to prepare PRCA solutions. MALDI-TOF/MS and PhiCal Calprotectin ELISA (Immundiagnostik, Germany) were used for the analyses.

Results:
PRCA purity was confirmed by MALDI-TOF/MS analyses, showing the S100A8 and the S100A9 peaks and the resulting hetero-dimer. The two PRCA solutions at 1400 and 700 ng/mL, prepared with or without 0.025% PEI, confirmed that PEI is necessary to obtain results closer to the expected concentrations (1220 and 469 ng/mL in place of 720 and 232 ng/mL) and that the biases (-27% for high and -28% for low concentrations of PRCA) were not affected by PEI at 0.03%, 0.035%, 0.04% and 0.05%. PRCA was dissolved with 0.025% PEI at the final concentration of 840, 420 and 210 ng/L. 15 uL of each solutions were further diluted 1:25 in stool extraction buffer, to reproduce the entire pre-analytical and analytical processes. The expected ELISA results (336, 168 and 84 ng/mL) chosen were close to the 300, 150 and 50 ug/g diagnostic cut-offs. Biases were: -1.2%, -4% and +6.6% from two replicated measures for the 336, 168 and 84 ng/mL targets respectively. By using 11 independent assays in duplicate, inter-assay and intra-assay imprecisions were: CV=9% and 3% for mean=331.84 ng/mL; CV=12% and CV = 7% for mean=175.7 ng/mL; CV=13% and CV = 4% for mean=100.3, ng/mL. In parallel, two fecal samples were analyzed, being inter-assay CV 24% (mean=73.8 ng/mL) and 6% (mean=221.7 ng/mL).

Conclusions:
PRCA could be suitable as internal quality control for fCal assay and commutable with faecal samples.
THE PERFORMANCE OF THE DIAZYME PROCALCITONIN ASSAY ON THE ABBOTT ARCHITECT SYSTEM

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Background. Abbott Diagnostics has started developing the PCT assay. The aim of the study was meanwhile to evaluate the analytical performance of the available Diazyme PCT assay on the ARCHITECT. The results were compared to currently used Biomerieux miniVIDAS results to choose the best method for the routine work. The assay principles are different. Diazyme PCT assay is the determination by latex enhanced immunoturbidimetric method while VIDAS B.R.A.H.M.S PCT assay combines a one-step immunoassay sandwich method with a final fluorescent detection.

Materials & Methods. First, with the Diazyme PCT Control Set five parallel runs on five different days were conducted in two different concentration levels to evaluate the repeatability, reproducibility and variability of the test results. Secondly, the BioRad Lypochek Speciality Immunoassay Control was used for five parallel runs on two days to obtain the test characteristics at a low concentration. Besides 32 patient samples from LH plasma were analyzed with both analyzers to estimate the correlation between the methods. The results were assessed with analysis of variance (ANOVA).

Results. The imprecision of QC low, 1 and 2 on the ARCHITECT c4000:

**QC low (BioRad):** mean 0.42 ng/mL; within series CV=21.1%, SD=0.088; total CV=23.4%, SD=0.098.

**QC 1 (Diazyme):** mean 1.31 ng/mL; within series CV=5.5%, SD=0.073; total CV=5.5%, SD=0.073.

**QC 2 (Diazyme):** mean 16.28 ng/mL; within series CV=2.5%, SD=0.40; total CV=2.6%, SD=0.43.

**QC low (BioRad) results of the miniVIDAS:** mean 0.50 ng/mL, total CV=7%, SD=0.04.

The precision estimated from patient samples comparing ARCHITECT to miniVIDAS revealed r²=0.931, slope 0.75, intercept 0.46, mean bias 19.7%.

Conclusions. The performance of the Diazyme PCT assay on the ARCHITECT system was analytically unstable comparatively, especially at a concentration of 0.5 ng/mL, which is the decision limit of the test. Also the correlation between two methods was not satisfying. Therefore our laboratory didn’t take into use the Diazyme PCT assay on the ARCHITECT. We decided to await the Abbott Diagnostics original assay.
Reduction of non-specific binding of upconverting nanoparticles in bioaffinity assays

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Background: Upconverting nanoparticles (UCNPs) are photoluminescent reporters composed of inorganic lattice doped with lanthanide ions. UCNPs have an exceptional capability of converting low energy infrared radiation into high energy emission at visible wavelengths. The advantage of UCNPs over organic reporters is that autofluorescence from the sample matrix or reagents can be completely avoided enabling the development of ultrasensitive assays. However, the non-specific binding of the UCNP conjugates prevents from achieving the ultra-high sensitivity.

Methods: Reduction of non-specific binding of the UCNP conjugates was studied with non-competitive bioaffinity assays for thyroid stimulating hormone (TSH) and bovine serum albumin (BSA). For the assays, the UCNPs were functionalized with polyacrylic acid (PAA, Mw 2000) and conjugated with anti-TSH Mab or streptavidin. In the TSH immunoassay, biotinylated anti-TSH Mabs were immobilized into a streptavidin-coated microtiter well. TSH standards were detected with Mab UCNP conjugates. For the BSA assay, BSA was biotinylated and detected with streptavidin conjugated UCNPs. In both assays, UCNPs were added either in a regular assay buffer or a buffer containing PAA. The upconversion luminescence was measured from the microtiter well with a modified Chameleon plate reader using 980 nm excitation and 550 nm emission.

Results: The addition of PAA to the buffer in the reporter incubation step reduced the non-specific binding of the PAA-coated UCNP conjugates while maintaining the analyte specific luminescence intensity high. The optimal PAA concentration in the buffer was 0.5% resulting in four times higher signal-to-background ratios. The decrease in the non-specific binding of the UCNPs enhanced the analytical sensitivities of both TSH and BSA assays. The analytical sensitivities (3SD) were 0.21 and 0.76 mU/L for the TSH assay, and 0.97 and 2.7 pM for the BSA assay with and without PAA, respectively.

Conclusions: By using PAA-coating in the UCNPs and adding PAA to the buffer in the reporter incubation step, the non-specific binding can be significantly decreased and the assay sensitivity improved. The PAA in the buffer most likely blocks the empty places in the solid phase and prevents the non-specific binding of the UCNP conjugates thus reducing the assay background. This contributes to developing ultrasensitive assays and employing the full potential of the UCNPs.
Analytical technologies and applications

Cod: M087

**ACTIVOGRAPHY: A NOVEL, VERSATILE AND EASILY ADAPTABLE DIAGNOSTIC METHOD FOR MONITORING ENZYMATIC ACTIVITIES IN TISSUES**

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Most of the protein detection assays used in clinical diagnosis rely on antibody-based methods. For the many proteins that are active enzymes the currently available methods provide no information on the status of their activity that is biologically relevant to normal physiology and often its alterations are linked to disease phenotypes. This limits the value of current diagnostic procedures. Here, we describe a new histochemical, easy-to-perform, and versatile technique, which we named “activography”. The technique can be applied to map enzymatic activities in biopsy sections by use of an activity-based probe (ABPs) that binds specifically to tissue enzyme(s) only when present in active form, and visualization of the resulting conjugates with commonly used chromogens. We developed activography using as prototype B24P, a new ABP that we synthesized, which labels active serine proteases. For proof-of-concept validation we used B24P to stain normal skin biopsies prepared from wild-type mice and diseased skin biopsies from knockout mice recapitulating skin disorders characterized by elevated activities of serine proteases in the epidermis. Activography was carried out side-by-side with in situ zymography (the only available method to analyze protease activities in tissues) and shown to provide comparable spatial and quantitative information but higher selectivity. A significant advantage of this chemically-based assay is that it does not require sophisticated instrumentation, thus, it is easily adaptable by clinical laboratories. Importantly, in contrast to in situ zymography that is exclusively used for proteases, activography can be potentially applied to all different enzyme families, especially considering the remarkable dynamics in new ABP development. New ABPs with specificity for single enzymes will enable highly specific activography assays in the future. On the other hand, ABPs with broad specificity like the B24P probe are useful for monitoring multiple enzymes with similar specificities. For the first time, we demonstrate that ABPs can be exploited to quantify and spatially localize enzymatic activities by histochemical assays, which opens a new window in routine clinical diagnostics and in basic research.
PERFORMANCE EVALUATION OF THE SERUM IGG SUBCLASSES QUANTIFICATION BY SPAPLUS TURBIDIMETRIC ANALYZER

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Background: IgG consists of four subclasses; IgG1, IgG2, IgG3 and IgG4. These four subclasses have different effector functions such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, and complement activation. Therefore, changes in serum concentration of each IgG subclass reflect different clinical situation and quantification of each subclasses is important to assess patient’s clinical states. Herein, we evaluated the analytical performances of the SPAPLUS turbidimetric analyzer (The Binding Site, UK) for IgG subclasses.

Methods: Precision, linearity, comparison and reference interval were assessed according to the CLSI (Clinical and Laboratory Standards Institute) guidelines. Briefly, precision was assessed using two control materials and one pooled serum, and they were measured in duplicate per run and two runs per day for 20 days. Linearity was assessed for five levels in each IgG subclass, and they were prepared using patient’s serum and SPAPLUS sample diluent (ref. SN080.S, The Binding Site) and measured in quadruplicate. Comparison was assessed in total 169 patient samples, and the results were compared between SPAPLUS and BN II (Siemens Healthcare Diagnostics, Germany). Reference intervals were evaluated using at least 20 samples from healthy individuals.

Results: The within-run precisions ranged from 0.64% to 1.49% CV (coefficients of variation) for three concentrations in four IgG subclasses. Total precisions ranged from 1.76% to 4.79% CV. The coefficient of determination ($R^2$) was higher than 0.99 for analytical measurable range in all IgG subclasses. The correlation coefficient ($r$) between SPAPLUS and BN II was 0.946 for IgG1, 0.954 for IgG2, 0.904 for IgG3 and 0.992 for IgG4. And, no more than two results were outside in the reference intervals by manufacturer’s claim for each IgG subclass.

Conclusion: The SPAPLUS shows good analytical performance for quantification of four IgG subclasses. This test is considered to be appropriately utilized in clinical diagnosis.
SDS ELECTROPHORESIS AS A QUALITATIVE AND SEMIQUANTITATIVE TOOL FOR EVALUATION OF URINE FREE LIGHT CHAINS

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Managing of multiple myeloma requires detection and quantitative determination of monoclonal component in urine. Quantitative determination of urine monoclonal protein level procedures (usually immunoglobulin free light chains) are included in International uniform response criteria for multiple myeloma. Typical agarose gel electrophoretic procedure suffer from low sensitivity below required diagnostic level. Immunochemical methods FLC are encumbered by non-linearity, antigen excess and high imprecision.

The aim of this study was evaluation of the SDS protein electrophoresis as qualitative and semiquantitative tool for evaluation of urine free light chains

Materials and methods:
Monoclonal free light chains in urine samples collected from patients with monoclonal gammopathy were analyzed by SDS electrophoresis, Bradford method, classical immunoturbidimetric method and latex immunochemical tests (Binding Site and Siemens).

Results:
Evaluated by densitometry lowest detection limit of monoclonal free light chains on SDS electrophoretic gels were 10 mg/l reaching normal reference range values. Densitometry based concentrations of FLC obtained from electrophoretic procedure were in good agreement with protein determination by Bradford method (in absence of albumin and other interfering proteins), and classic immunoturbidimetric method. In some cases latex immunoturbidimetric methods produced false overestimated results.

Conclusions:
SDS based densitometry gives reliable semiquantitative levels of urine free light chains. Sensitivity of this electrophoretic variant covers normal and pathological ranges and is resistant to interferences affecting immunochemical methods. Electrophoretic method provides additional information concerning type of renal injury (glomerular, tubular) and appears as a useful tool for screening potential renal threat.
EFFECT OF SHAKING ON ANALYTE AND LABEL CONJUGATE BINDING DISTRIBUTION IN MICROTITER PLATE BASED BIOAFFINITY ASSAY

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BACKGROUND Defining the analyte and label conjugate distribution on microtiter well surface would provide an important tool for assay optimization but yet there is no simple method to study the surface. In contrast to organic fluorescent dyes the green-emitting upconverting nanoparticles (UCNPs) can be imaged at low quantity without the interference of autofluorescence due to their unique capability to produce anti-Stokes photoluminescence. This enables detecting few labels providing a more detailed way to examine analyte and label distribution on the well surface which is important for assays that require performing measurements from the well surface.

METHODS A two-step bioaffinity binding assay with biotinylated bovine serum albumin was performed by using streptavidin coated UCNPs as labels. The assay incubations were performed in streptavidin coated wells in three different ways: without mixing, in a rocking movement and in orbital shaking which is typically used in immunoassays. After the assay, dry wells were imaged with an anti-Stokes photoluminescence imager using 980 nm excitation and 550 nm emission.

RESULTS The distribution of the label in the well varied between different incubation methods. Despite creating a circle like pattern, the orbital shaking produced the highest overall luminescence intensity and the least label aggregates due to the most effective mixing method. Incubations without shaking resulted visually in the most uniform label distribution. The rocking movement created a darker area across the well, splitting the label enriched area in two parts. Incubating without shaking and in rocking movement resulted in up to 74 % lower overall luminescence intensity depending on the analyte concentration and 2–8 percentage units higher relative standard deviation compared to the orbital shaking.

CONCLUSIONS The mixing method has a surprisingly great impact on how the analyte and the label distribute in the well. This phenomenon can be readily studied by using UCNPs as labels. Incubating without mixing results in a uniform distribution but less assay components are bound during incubation due to slow kinetics. Shaking speeds up the kinetics but can result also in uneven distribution in the well as was detected with the rocking and orbital shaking. The studied imaging method could provide information also for the evaluation of different plate types which is relevant for developing assays that require well surface measurements.
Background: Fecal calprotectin is an important marker in distinguishing organic, inflammatory disease of the gastrointestinal tract (inflammatory bowel disease (IBD); Crohn’s disease (CD); or ulcerative colitis (UC)) from functional disease (irritable bowel syndrome, IBS), in patients with chronic abdominal pain. It also supports inflammatory status monitoring during therapy. The BÜHLMANN fCAL® turbo test is based on the immunoturbidimetric technique (PETIA) and allows quantification of calprotectin in fecal extracts. This kit was adapted in several analyzers but not on the AU640 Beckman Coulter® automate. In this present study we present steps that carried out the installation of fCAL® turbo kit on the AU640.

Method: The BÜHLMANN fCAL® turbo kit was installed in the AU640 with different protocols in order to have a correct calibration curve that lead to obtain good values of the high and low internal quality controls. The second step consisted to determine: the variation coefficient at the low and the high concentration (using repeatability tests), the linearity and the range of measurement on the AU640.

Results: First we obtained variant coefficient (CV) for low control 5.4% and the high control 3.4%. The range of measurement in this protocol was comprised between 25 µg/g to 1700 µg/g. To validate our protocol we compared results from AU640 fCAL® turbo kit with fCAL® ELISA kit, which is the reference technique for BÜHLMANN. We get a correct correlation coefficient (R² = 0.85).

Conclusion: We reached to install the BÜHLMANN fCAL® turbo kit on the AU640 with satisfying analytical parameters in term of reproducibility, linearity and precision. Finally, our protocol can be applied on all AU automates. Adaption for the modern AU analysers with reduced reaction volumes may lead to an improved economic outcome.
Analytical technologies and applications

Cod: M092

SUPERIOR PERFORMANCES OF CAPILLARY ELECTROPHORESIS (CE) VS. HPLC TO DETECT ASIALO-TRANSFERRIN, AN IMPORTANT, ALTHOUGH OFTEN NEGLECTED, COMPONENT OF HUMAN TRANSFERRIN (TF) GLYCOFORMS.

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BACKGROUND
Carbohydrate deficient transferrin (CDT) is widely accepted as the most specific biomarker of chronic alcohol abuse. CDT is the collective name of minor glycoforms of Tf including, traditionally, asialo- and disialo-Tf. Asialo-Tf is characterized by low concentrations hampering its detectability. It is expected that IFCC1 will soon recommend the exclusion of asialo-Tf from the computation of CDT to help standardization. However, in our opinion, asialo-Tf could provide precious additional information to confirm, e.g. in a forensic context, an elevated CDT result, merely based on disialo-Tf. On this basis, in view of a re-evaluation of the potential of asialo-Tf as an additional biomarker of alcohol abuse, the purpose of our investigation was to compare the two most popular separative techniques, i.e. CE and HPLC, in their ability to detect asialo-Tf in real serum samples.

METHODS
Samples: 25 serum samples from subjects undergoing screening for alcohol abuse were analyzed in parallel with CE and HPLC.

Analysis: CE and HPLC methods according to ref. 2.

Statistics: Data were analyzed by using non-parametric and parametric statistics.

RESULTS
Correlation between asialo- and disialo-Tf. CE: y = 0.311 x – 0.330, r² = 0.524 (n=25); HPLC: Scarce number of asialo-Tf detected (n=5)

Correlation between CE and HPLC to determine disialo-Tf: y = 1.177 x – 0.158, r² = 0.970 (n=25)

Detection ability for asialo-Tf. CE: 25 out 32 (range detected 0.10 – 3.78 %); HPLC: 5 out 32 (range detected 0.51 – 1.61 %)

Separation efficiency [N = 5.54 · (t_r / w_r)²]. CE: 51,000 plates; HPLC: 4,200 plates

CONCLUSIONS
Notwithstanding a comparable performance of CE and HPLC in the determination of disialo-Tf, the ability of CE to detect asialo-Tf proved clearly superior. In fact, CE offers a neatly better separation efficiency of Tf glycoforms, providing sharper and more detectable peaks. In addition, the asialo-Tf peak is located in a very flat region of the electropherogram, far from other potentially interfering peaks. The present results strongly support the use of CE in further studies exploring the potential of asialo-Tf as an additional, important biomarker of alcohol abuse.

REFERENCES
Analytical technologies and applications

Cod: M093

UPCONVERTING NANOPARTICLES IN LATERAL FLOW ASSAYS

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Introduction: Lateral flow (LF) assays with quantitative and semiquantitative results have been introduced for several clinical analytes such as biomarkers for cancer, myocardial infarction or interferon-induced gene expression. Photon-upconverting nanoparticles (UCNPs) are lanthanide doped nanocrystals which are able to produce visible emission upon excitation with near-infrared radiation unlike conventional fluorescent labels. In addition UCNPs allow permanent excitation since they do not photobleach, like traditional fluorophores, and so can be stored indefinitely. UCNPs have been introduced as promising labels for point-of-care testing because of their high detectability by optical sensors based on UCNPs low background and total elimination of autofluorescence by spectral separation. Conventionally in LF assays liquids containing the UCNPs are absorbed via glass fiber conjugate pad. In this study the release efficiency of dried UCNP-reporters from glass fiber conjugate pad was studied and the drying conditions to achieve efficient and consistent release are described.

Methods: The release of UCNPs deposited on glass fiber was studied with multiple assays including HIV-1, HBsAg and P. falciparum. Different drying conditions for optimal release of UCNPs were tested.

Results: It was demonstrated that UCNPs can be dried to glass fiber conjugate without compromising the assay sensitivity and the particles were released from the glass fiber pad without significant variation between replicate reactions. Variation between replicas was 10 ± 3 %.

Discussion: Often in LF assay development phase UCNPs are added to LF strips in solution and thus need a separate step. In this study the UCNPs were first dried to the glass fiber where they could be released for the assay. Drying reduces the need for liquid handling and makes the assay easier to perform.
SIMULTANEOUS MEASUREMENT OF WHOLE BLOOD VITAMIN B1 & VITAMIN B6 USING LC-ESI-MS/MS

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Background:
Vitamin B1 is used in several decarboxylation- and transketolase reactions and is also involved in the generation of ATP. Vitamin B6 is a cofactor in numerous biological processes that include gluconeogenesis, neurotransmitter synthesis and amino acid metabolism. Our aim was to develop a method to measure the concentration of the biologically active forms of vitamin B1 (thiamine pyrophosphate, TPP) and vitamin B6 (pyridoxal phosphate, PLP) in EDTA whole blood with LC-ESI-MS/MS and compare this new procedure with established homemade methods for total thiamine and PLP.

Methods:
A stable isotope (TPP-d3 & PLP-d3) was added to the samples, followed by deproteinization with 10% TCA. After centrifugation, 20 µl of the supernatant was injected into the LC-ESI-MS/MS. Reversed phase chromatography was performed on a UPLC system, using a Waters™ Symmetry C18 column, with a gradient of 0.1% formic acid in methanol. TPP and PLP were measured on a tandem MS with respective mass transitions of 425.1>121.85 and 247.9>149.9.

Results:
The chromatographic run lasts 2 minutes. The method is linear from 0-300 nmol/L. The intra-assay and inter-assay precision are 5.5% and 10.4% respectively for TPP and 3.8% and 5.5% for PLP. The matrix effect (absolute: TPP 107%, PLP 101% and relative: TPP 97%, PLP 93%), recovery (TPP 99%, PLP 94%) and lower limit of quantification (TPP 12 nmol/L, PLP 6 nmol/L) are acceptable.

The comparison of the new LC-ESI-MS/MS method for TPP with our current HPLC-FI method for total thiamine yields the following equation: LC-MS/MS=0.97 [0.86-1.10] x HPLC - 10.61 [-27.77-2.70] (r²=0.94). The comparison of the new LC-ESI-MS/MS method for PLP with our current LC-ESI-MS/MS method results in LC-MS/MS new=1.01 [0.98-1.04] x LC-MS/MS old – 1.58 [-4.04-0.67] (r²=0.99).

Conclusion:
This LC-MS/MS based method is characterized by simple sample processing and a short run time. Comparison with the current methods is excellent. The new LC-MS/MS method is an appropriate method to determine TPP and PLP in whole blood for both clinical routine and research applications.
INFLUENCE OF CREATININE MEASUREMENT METHODS ON MELD-SCORE IN ICTERIC PATIENTS

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Background:
In patients with end-stage liver disease the MELD score is used to predict survival, and is subsequently applied as a tool for the allocation of transplant organs. The MELD score is calculated from serum creatinine, bilirubin and the PT-INR. This raises a challenge in strongly icteric patients, since elevated bilirubin levels interfere with the measurement of creatinine.

Methods:
In this study, the extent of interference by bilirubin was examined in 198 samples from 39 clinical patients with an icteric index higher than 300 (total bilirubin ≥237 µmol/L). The total bilirubin concentration was measured, as well as the creatinine concentration using five different methods (Jaffé and enzymatic, both in regular and diluted (4x) mode, and with a blood gas analyser). All values were compared to creatinine concentrations determined with the LC-MS/MS method, a reference method not influenced by bilirubin. In addition, we investigated the influence of the different methods for creatinine measurement on the MELD score (PT-INR was set to 1). The obtained data were analysed using Spearman correlation and Passing-Bablok comparison.

Results:
When compared to LC-MS/MS, the enzymatic creatinine measurement resulted in a concentration that was 16% lower (P<0.0001), which declined to 7% (P<0.0001) using the diluted mode. The correlation between LC-MS/MS and the enzymatic assay was better than with the Jaffé assay (r=0.95 vs 0.91). The blood gas analyser had both highest concordance and correlation (r=0.99).

When calculating the MELD scores using the different creatinine methods and comparing them to the LC-MS/MS creatinine-based MELD score, relevant differences were observed: ≥3 points in 8% of the patients when the undiluted enzymatic assay was used, which was reduced to 4% when sera were diluted. For the Jaffé method these numbers were 14% and 8%, respectively. Using the blood gas analyser, only 1% of the MELD scores differed ≥3 points.

Conclusion:
When interpreting a MELD score one should be aware that icteric interference of creatinine influences the score. From a workflow perspective, the enzymatic creatinine assay using an automated reflex test diluting the icteric interference is a smart and efficient alternative for reporting reliable creatinine concentrations.
DEVELOPMENT OF A MASS SPECTROMETRY BASED METHOD FOR TARGETED QUANTITATION OF CLINICALLY RELEVANT PROTEOFORMS OF ANTITHROMBIN

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Background: Antithrombin (AT) is an anticoagulant protein, and reduced AT activity is associated with increased risk of thrombosis. Several genetic mutations that hamper AT activity have been reported. Moreover, protein glycosylation plays an important role in AT activity: ß-AT, having only three of four glycosylation sites occupied, has slow activity compared to α-AT. Currently, AT activity is analysed using a functional assay that measures overall activity. The compounds that contribute to total activity assays are unknown because the specific contribution of α-AT, ß-AT and genetic variants goes unrecognized. Better assays are required. This could be achieved by quantitative clinical chemistry proteomics using liquid chromatography coupled to mass spectrometry (LC-MS).

Methods: Different proteases were considered to enable the analysis of peptides, peptides with mutations and glycopeptides. AT isolated from plasma was digested using either of the proteases, and the digests were analysed using LC-QQQ-MS. Based on these results peptides and stable isotope labelled peptides were synthesized and used for further optimization of MS measurement, defining MRM transitions with regard to retention time, collision energy, and precursor and product ion. Transitions for glycopeptides were optimized using proteolytic digests of isolated AT.

Results: Multiple peptides were observed in the proteolytic digest of isolated AT and 3 peptides were selected for further optimization. Furthermore, two peptides with potential mutations as well as glycopeptides originating from all four sites were identified. Optimized transitions were used to generate calibration curves, and LOQ values were found within the relevant range. Peptides were also observed in plasma digests, suggesting that AT quantitation is feasible directly from plasma without further protein isolation.

Conclusions: Using LC-MS we were able to identify proteotypic peptides, genetic variants and glycopeptides of AT, which allows us to identify and quantitate clinically relevant AT-proteoforms. Further work is needed to improve quantitation of glycopeptides and to standardize the LC-MS assay to guarantee metrological traceability of test results.
ONE-TUBE SCREENING FOR PRIMARY IMMUNODEFICIENCIES

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Primary immunodeficiencies (PID) are rare diseases with a prevalence of 1 in 1000 for all currently recognized forms of such diseases. Most patients present with susceptibility to recurrent episodes of severe or unusual infection. A steadily increasing number of single gene mutations (to date over 300) have been described that explain defects in cell function and/or maturation. Established routine laboratory tests are generally not sufficient for detecting most of these entities because the numbers of basic cell populations in peripheral blood commonly remain within the wide reference ranges. The aim of our project was to set up a screening tube for most common immunodeficiencies, compatible with various systems, as far as possible standardized, and easy to use as routine test in most laboratories.

We designed an 11-parameter panel including absolute cell count which can be analyzed with common diagnostic flow cytometers with at least 8 color detectors. In a single step, with off-the-shelf reagent, using a simple lyse/no wash procedure, results were generated with previously established protocols in 5 different laboratories across Europe. Our results show that the panel is easy to set up on FACS CANTO II (BD Biosciences) and NAVIOS or CYTOFLEX (both Beckman-Coulter) instruments. Results are comparable with previously used panels. The main immune cell populations can be detected and counted: CD4+ or CD8+ T cells, B and NK cells, monocytes, and PMNs. Furthermore, naïve and mature T cells (CD45RO/CD27), B cells (CD38/CD27/IgD), and functional entities of NK cells (CD16high CD56low) can be quantified as well. Finally, activated CD38+ T and NK cells can be counted. Instrument settings can be standardized based on target fluorescence provided for different systems. A typical template allows for additional drop-in parameters where appropriate lasers and detectors are available in the system.

In conclusion, such a panel can be easily implemented in non-specialized laboratories as a screening tool for detection of potential immunodeficiency and can overcome disadvantages of the common immune status tube in these patients. Following pathological findings, samples have to be further explored with detailed panels, functional tests, and genetic testing. Our novel panel can also be used for secondary immunodeficiencies as observed in chronic inflammatory diseases or sepsis.
Analytical technologies and applications

Cod: M098

STANDARDIZATION OF CARBOHYDRATE DEFICIENT TRANSFERRIN (CDT) MEASUREMENT FOLLOWING IFCC PROTOCOL ON TWO CAPILLARY ELECTROPHORESIS SYSTEMS

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Background: Excessive alcohol detection is based on questionnaires and self-report measures, and on the results of laboratory tests. Carbohydrate-deficient transferrin (CDT) is considered the most reliable biomarker in routine conditions. Because CDT measurement was not standardized, a wide array of method-dependent cut-off values has been used. This prompted the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to initiate a Working Group on Standardization of CDT (WG-CDT). The measurand for CDT standardization was defined as disialotransferrin to total transferrin fraction in serum. An HPLC method was validated as the reference measurement procedure (RMP). Target values for secondary calibrators are assigned by the network of CDT reference laboratories, which allows the manufacturers to produce standardized CDT values, named CDTIFCC. This study checked if the standardization process developed by the manufacturer (Sebia, Lisses, France) for the Capillarys® and Minicap® capillary electrophoresis systems was successful.

Methods: CDT was measured in 126 samples using five analytical procedures: the RMP, the standard CDT procedure on Capillarys® and Minicap®, and the standardized CDTIFCC procedure on Capillarys® and Minicap®. The regression equations and the concordance coefficient (rc) were calculated. The acceptable difference limit (ADL) was found to be 18.4%.

Results: Both systems gave highly correlated results (r=0.9994) for both standard CDT and CDTIFCC procedures. Moreover, the concordance coefficient was 0.9993 between both systems using the CDTIFCC procedure, which is “almost perfect” according McBride classification. Standard CDT results and CDTIFCC results given by both systems were compared to the results obtained with the RMP. For both systems, the concordance coefficient with the RMP was increased to “almost perfect”. The mean bias between the RMP results and the standard procedures were respectively 15.8% (Capillarys®) and 20.3% (Minicap®). After standardization, these values were reduced to 1.6% and -0.5% respectively. Consequently, the number of individual unacceptable differences was 0.8% (Capillarys®) and 4% (Minicap®). These values are lower than the 5% unacceptable results defined by McBride to conclude that methods give identical results.

Conclusions: This study demonstrated that the standardization process of CDT measurement is successfully achieved for the Capillarys® an Minicap® systems.
INTRAOPERATIVE MONITORING OF PARATHORMONE DURING SURGERY FOR PRIMARY HYPERPARATHYROIDISM: A CASE REPORT SHOWING THE IMPORTANCE TO USE A THIRD GENERATION PARATHORMONE ASSAY.

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Background
Rapid intraoperative parathyroid hormone (PTH) assay is used to guide the surgeon in the adequacy of resection during surgery for primary hyperparathyroidism (PHP). Actually, only 2nd generation PTH (PTH2) assays are used, because no rapid 3rd generation PTH (PTH3) assays are available. We report a case of PHP surgery where a 2nd intraoperative PTH test was used and led to a misinformation for the surgeon.

Methods
Intraoperative rapid PTH2 was measured in a 34 years old woman, presenting a normal renal function, undergoing parathyroidectomy for PHP using the Rapid Elecsys PTH assay (Roche, Germany) at 5 time points: incision, removal and subsequently 5, 10 and 15 minutes after curative resection. PTH increased from 293 ng/L at incision to 443 ng/L at +15 minutes. Using a 50% reduction in PTH from incision at 15 minutes as the criterion for adequate resection, we concluded that surgery failed. The next day, calcium level decreased from 2.8 mmol/L to 2.3 mmol/L, which on the contrary supports a successful surgery.

Results
In view of these contrary results, all samples were tested for PTH2 on Liaison XL (DiaSorin) and Elecsys Cobas (Roche). PTH3 assays were performed on Liaison XL and Lumipulse (Fujirebo).

As with the Rapid Elecsys assay, PTH2 increased from incision to +15 minutes when tested on Liaison and Cobas. Interestingly, a 50% reduction in PTH3 was observed on Liaison and Fujirebo.

As the calcium results, the decrease of PTH3 confirms the success of the surgery and the recovery of the patient.

Conclusion
We reported a case of intraoperative PTH assay where interferences from undefined origin, only observed on 2nd generation PTH assays (a HAMA interference was ruled out), leaded to misinformation given to the surgeon. This case shows the clear relevance of using 3rd generation PTH assays.
Analytical technologies and applications

Cod: M100

PRECISION OF 19 ASSAYS TESTED ON COBAS E 801 AT 4 EUROPEAN STUDY SITES

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BACKGROUND
The new high throughput immunochemistry module from Roche Diagnostics, cobas e 801 is designed to cover almost double the testing capacity of the predecessor cobas e modules. During this study at 4 European sites, multiple assays routinely used in our laboratories were applied and intensively tested on the new analyzer. Here we report on the precision of the results observed for 19 assays within each lab and across labs.

METHODS
At all sites, the used study system included a cobas e 801 module connected to one or more cobas 8000 clinical chemistry and or immunochemistry modules. At one site two cobas e 801 modules were integrated.

Assays routinely used by at least 3 of the participating sites were selected for this randomized precision testing covering the indication areas Thyroid (TSH, FT4, FT3), Oncology (AFP, CEA, CA 125, CA 15-3, CA 19-9, HCG + beta, free PSA, total PSA), Cardiac markers (hsTnT, NT-proBNP), Fertility (Progestosterone, Testosterone, FSH, LH) and Anemia ( Vit B12 and Folate).

The applied protocol for testing was based on guidance from CLSI EP5-A3. Per assay, 2 quality control materials with analyte concentrations in the normal and in the pathological range respectively were used. Testing was done in 2-fold, twice per day, over 21 days. From the results, repeatability (within-run) and intermediate (within-lab/total) precision CVs were calculated as well as the reproducibility over all labs.

RESULTS
Repeatability and Intermediate precision CVs: all cardiac marker CVs were <2%, for the thyroid assays, all CVs were < 2.5%, all oncology and fertility assay CVs were <3%; all Vit B12 CVs were <3.6% and <6% for Folate. Between – lab CVs were only marginally higher than repeatability and intermediate precision CVs.

CONCLUSIONS
The precision of the data generated on the study systems for the 19 assays within each site and across sites over about 5 weeks demonstrates the consistency of cobas 8000 < e 801> results.
Analytical technologies and applications

Cod: M101

USEFULNESS OF THE REACTION DATA MONITORING SYSTEM IN THE AUTOMATED BIOCHEMICAL ANALYZER

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BACKGROUND
In laboratory tests using an automated biochemical analyzer, unexpected problems lead to erroneous test values. Recently, to detect abnormal reactions and failures of the device, a reaction data monitoring system has been provided for analyzers. We previously reported that this system is able to detect the abnormal reaction in clinical chemistry measurement (Clin Chim Acta 2015). In this study, we aimed to investigate the usefulness of this system to prevent errors in routine tests.

METHODS
Written informed consent was obtained from all participants prior to blood sampling in Chiba University Hospital. The absorbance of various test items measured during a 5-day period from July 13, 2010 was summed, and the following items (cut-off values) were calculated: 1) variances of operated absorbance at photometric time-points to calculate the measurement result, 2) variances of differences in absorbance after mixing the sample and reagent between adjacent photometric points. When an abnormality was detected by the system in a routine test, the person in charge confirmed the reaction data in the reaction monitor of the analyzer.

RESULTS
We identified cross contamination with other test reagents, abnormal test values due to clouding caused by mixing a sample and reagent, and variation in absorbance due to deterioration of the halogen lamp. These were detected by the reaction data monitoring system, and the reporting of erroneous test results could be prevented. When samples in which clouding was caused were subjected to protein electrophoresis or immunofixation electrophoresis, 22 cases of monoclonal gammopathy were detected in 20 months.

CONCLUSIONS
The reaction data monitoring system of the automated biochemical analyzers was useful to prevent false reports due to unexpected problems. It was also suggested that, when a false reaction with a reagent is detected, a new pathology, such as monoclonal gammopathy, may be identified by close and careful examination of the sample.
Analytical technologies and applications

Cod: M102

EVALUATION OF THE ATELlica CH ALBumIN, ENZYMATIC CREATININE_2, AND GLUCOSE HEXOKINASE_3 ASSAYS*

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Background: The purpose of the investigation was to evaluate the analytical performance of representative clinical chemistry assays including the Atellica™ CH Albumin (Alb), Enzymatic Creatinine_2 (Ecre_2), and Glucose Hexokinase_3 (GluH_3) Assays on the Atellica™ CH Analyzer**.

Methods: Performance testing included precision and method comparison. Assay precision was evaluated using Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with patient sample results compared with results from the ADVIA® 1800 Clinical Chemistry System.

Results: For Alb, within-lab precision ranged from 1.85 to 2.42% CV in serum/plasma samples. For Ecre_2, within-lab precision ranged from 1.9 to 2.7% CV in serum/plasma samples and from 2.3 to 3.2% CV in urine samples. For GluH_3, within-lab precision ranged from 1.0 to 2.0% CV in serum/plasma samples, from 1.3 to 3.7% CV in urine samples, and from 1.2 to 2.3% CV in cerebrospinal fluid (CSF) samples.

The Alb serum method comparison study yielded a regression equation of y = 0.99x + 0.1 g/dL, with r of 0.995, versus the ADVIA 1800 ALB assay. The Ecre_2 serum method comparison study yielded a regression equation of y = 1.00x - 0.04 mg/dL, with r of 1.00, versus the ADVIA 1800 Ecre_2 assay. The Ecre_2 urine method comparison study yielded a regression equation of y = 1.03x + 2 mg/dL, with r of 1.00, versus the ADVIA 1800 Ecre_2 assay. The GluH_3 serum method comparison study yielded a regression equation of y = 1.03x - 1 mg/dL, with r of 1.00, versus the ADVIA 1800 GluH_3 assay.

Conclusions: The Atellica CH Alb, Ecre_2, and GluH_3 Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

*Under development. Not available for sale.

**The products/features (here mentioned) are not CE marked and are not commercially available. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.
Analytical technologies and applications

Cod: M103

PERFORMANCE EVALUATION OF THE ATELlica CH CREATINE KINASE, ALANINE AMINOTRANSFERASE (WITH AND WITHOUT P5P), ASPARtATE AMINOTRANSFERASE (WITH AND WITHOUT P5P), AND LIPASE ASSAYS*

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Background: The purpose of the investigation was to evaluate the analytical performance of representative enzyme assays including the Atellica™ CH Creatine Kinase (CK_L), Alanine Aminotransferase (ALT), Alanine Aminotransferase with P5P (ALTPLc), Aspartate Aminotransferase (AST), Aspartate Aminotransferase with P5P (ASTPLc) and Lipase (Lip) Assays on the Atellica™ CH Analyzer**.

Methods: Performance testing included precision and method comparison. Assay precision was evaluated using Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with Deming regression of patient sample results compared with the ADVIA® 1800 Clinical Chemistry System.

Results: For CK_L, within-lab precision ranged from 0.7 to 2.4% CV. For ALT, within-lab precision ranged from 1.0 to 2.8% CV. For ALTPLc, within-lab precision ranged from 0.7 to 2.7% CV. For AST, within-lab precision ranged from 0.9 to 3.4% CV. For ASTPLc, within-lab precision ranged from 1.1 to 1.8% CV. For Lip, within-lab precision ranged from 2.5 to 4.1% CV.

The CK_L method comparison study yielded a regression equation of y = 0.96x – 3 U/L, with r of 1.000, versus the ADVIA 1800 CK_L assay. The ALT method comparison study yielded a regression equation of y = 1.00x – 1 U/L, with r of 1.000, versus the ADVIA 1800 ALT assay. The ALTPLc method comparison study yielded a regression equation of y = 1.04x – 5 U/L, with r of 1.000, versus the ADVIA 1800 ALTPLc assay. The AST method comparison study yielded a regression equation of y = 1.02x – 0 U/L, with r of 0.999, versus the ADVIA 1800 AST assay. The ASTPLc method comparison study yielded a regression equation of y = 1.00x – 4 U/L, with r of 0.999, versus the ADVIA 1800 ASTPLc assay. The Lip method comparison study yielded a regression equation of y = 0.97x – 1 U/L, with r of 1.000, versus the ADVIA 1800 Lip assay.

Conclusions: The Atellica CH CK_L, ALT, ALTPLc, AST, ASTPLc and Lip Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

*Under development. Not available for sale.

**The products/features (here mentioned) are not CE marked and are not commercially available. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.
FUNCTIONAL IN-SOLUTION STABILITY OF ANTIBODIES CONJUGATED TO A NOVEL INHERENTLY FLUORESCENT EUROPium CHELATE

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BACKGROUND The latest inherently fluorescent WN europium chelate (WN-Eu3+) synthetized at the University of Turku has unique characteristic, improved stability in aqueous solutions being one of them. A stable label molecule–antibody conjugate obviates the need for dry reagent preparation, which can be advantageous in various applications. In this work functional stability of WN-Eu3+-conjugated antibodies was assessed when stored as ready-to-use solutions.

METHODS Optimal conjugation reaction pH with the WN-Eu3+ was studied with several monoclonal antibodies. Two different monoclonal antibodies (Mab1 and Mab2) were conjugated to WN-Eu3+ or reference chelates and stored as ready-to-use liquid solutions. Antibody conjugates were stored at 4 °C, 22 °C, and 37 °C and used as tracer antibodies in assays that were performed from 0 to 41 weeks. Assay with fresh tracer solution was used to monitor inter-assay variation. Assays were sandwich immunoassays using PSA as the model analyte. Stability was assessed by comparing maximum specific signal levels to the medians of control assays.

RESULTS Conjugation of WN-Eu3+ to studied Mabs was optimal in pH 7. Maximum specific signals with WN-Mab1 conjugate were stable for up to 41 weeks (89-97 % of control median) at 4 °C, 20 weeks (89-96 %) at 22 °C and 5 weeks at 37 °C (89-102 %) compared to the reference chelate conjugate that had stability for up to 16 weeks (92-98 %) at 4 °C and only 1 week at 22 °C (78-99 %). With Mab2, differences in stability between the two different conjugates were similar to Mab1, but otherwise stability with the WN-Mab2 conjugate was slightly reduced to 30 weeks (99-104 %) at 4 °C, 4 weeks at 22 °C (88-93 %) and 1 week at 37 °C (90-92 %). Gradual increase in background was seen with both WN-Mabs.

CONCLUSIONS The maximum specific signals obtained with WN-Eu3+-conjugated tracer antibodies exhibited excellent stability compared to the reference chelate. Functional stability is the combination of the label molecule’s and antibody’s stability. Mab1 showed better stability than Mab2, highlighting the difference in stability between antibodies. Since the stability of the chelate is not the limiting factor, liquid storage of tracer solutions can be optimized to enhance the stability of the antibody itself.
QUANTITATIVE ANALYSIS OF STEROIDS IN SERUM USING TRIPLE QUADRUPOLE LC-MS/MS

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BACKGROUND
Serum steroid assays are major tools in the clinical evaluation of adrenal disorders. The main adrenal steroids are routinely measured with immunoassays. However, immunoassays are among the most sensitive and precise analytical methods. The antibodies used in many commercially available immunoassays lack specificity owing to cross-reactivity. Chromatographic methods are known to offer better specificity. We report a triple quadrupole LC-MS/MS assay for simultaneous quantification of 13 adrenal steroids targeting the mineralo- and gluco-corticosteroid pathways.

METHODS
For analysis was used Agilent 6470 Triple Quadrupole LC/MS system. Column: Zorbax Eclipse Plus C18 (3 × 50 mm; 1.8 mm). Mobile Phase: A: Water + 1mM NH4F, B = Methanol + 1mM NH4F Chromsystem standards: MassChrom® Steroids in Serum/Plasma - LC-MS/MS and MassChrom® Internal Standard Mix by deuterium labeled internal standards.

RESULTS
This LC-MS/MS method provides highly sensitive and specific assessments of thirteen major steroids: Androstenedione, Aldosterone, Progesterone, 17-OH-Progesterone, Cortisol, Cortisone, Corticosterone, Deoxycortisol, Testosterone, 11-Dihydrotestosterone (DHT), Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone sulfate (DHEA-S), Estradiol. Mobile phase with ammonium fluoride (NH4F) is significantly better for the ionization of steroid hormones in both positive (ESI+) and negative (ESI−) mode. Compared with ammonium formate, sensitivity of estradiol was about 300× better and also for all other steroids was achieved about 1.5 - 4 times higher responses in NH4F.

CONCLUSIONS
LC-MS/MS has become an essential clinical research tool for analysis of endogenous steroids because of its ability to simultaneously analyze multiple analytes with high sensitivity, excellent specificity and reproducibility. Selection of the mobile phase can significantly affect the sensitivity of analysis and NH4F in very low concentrations (1 mM) is ideal for the analysis of steroid hormones in both polarities.

This work was supported by a Research Project of the General University Hospital Prague RVO-VFN 64165 and Research Project of the Grant Agency of Ministry of Health of the Czech Republic MZ0/NT 11277
In this study we obtained the quantitative profiles of biochemical composition in urine samples from healthy individuals living in Romania and we evaluated the gender-related and age-related urinalysis differences by proton nuclear magnetic resonance spectroscopy (1H-NMR). The metabolic profiles were compared with 1H-NMR urine profiles from Italian, Greek, British and Swedish healthy subjects.

167 normal subjects living in Romania (89 females and 78 males, mean age 38.3±12.1 years, ranging between 25-67 years old) were recruited with the following characteristics: no metabolic diseases such as diabetes, no hypertension, no urinary infections, no renal impairment and no alcohol consumption for 24 hours before sampling.

The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer operating at field strength of 9.4 Tesla. To 0.9 ml urine, 0.1 ml of stock solution of 5 mM sodium 3-(trimethylsilyl)-[2,2,3,3-d4]-1-propionate (TSP) in D2O has been added. The 1H-NMR spectra have been recorded with water presaturation. Metabolite concentrations were expressed in mmol/mol of creatinine.

There is a gender-related difference in the excretion of citrate between males and females in healthy Romanians. The healthy subjects above 35 years old tended to have higher urinary concentrations of trimethylamine-N-oxide, dimethylamine and 3-hydroxyisovaleric acid compared to subjects below 35 years old. There are significant differences in the urinary excretion of pyruvate, 3-hydroxyisovaleric acid and gamma-aminobutyrate between Romanian volunteers below and above 45 years old. There are significant decreased differences between the excretions of citrate, hippurate and trimethylamine-N-oxide, while the values of alanine were increased in Romanians healthy subjects vs. Italian normal group. In the Romanian volunteers, the two genders excreted similar concentrations of hippurate and men excreted lower concentrations of trimethylamine-N-oxide than women, whereas the excretion of this metabolite in Greek men was higher. The qualitative metabolic picture showed differences between the excretion of dimethylamine and similar profiles for citrate, hippurate and glycine in Romanians vs. British and Swedish healthy groups.

There is a strong need for large inter-laboratory and inter-country trials for establishing normal ranges and geographical variations of metabolite concentrations in urine.
Analytical technologies and applications

Cod: M107

QUANTITATIVE ANALYSIS OF LOW MOLECULAR WEIGHT METABOLITES FROM CEREBROSPINAL FLUIDS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY METHOD (1H-NMR)

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Nuclear magnetic resonance spectroscopy method (1H-NMR) is a powerful tool to analyze the chemical composition of biological fluids and show that it is more effective to measure small molecules than conventional techniques. The aim of this study was to identify and to measure low molecular weight metabolites from CSF samples by 1H-NMR, to compare the NMR metabolite concentrations with data obtained by conventional methods (published in the literature) and to develop our NMR database for further CSF studies.

87 cerebrospinal fluids (CSF) samples were examined by 1H-NMR method. CSF samples were collected by lumbar puncture by specialized staff from patients admitted to Craiova Emergency Clinical Hospital. 1H-NMR spectra were recorded with a Bruker Avance DRX 400 MHz spectrometer, which generates a magnetic field of 9.4 Tesla. To quantify metabolites we used standard procedure of nuclear magnetic resonance (NMR) signal processing. After applying the Fourier transform we calculated the concentrations of molecules identified in CSF samples by calculating the total height of the NMR signal and normalizing to the number of hydrogen atoms of molecule. MestRe-C 2.3a and GraphPad Prism 5.0 software were used for processing and visualization of spectra, respectively statistical analysis.

Metabolite concentrations were expressed in absolute concentrations (mmol/L) and relative concentrations (mmol/mol of lactate). Standard laboratory analysis of CSF samples included the examination of physical characteristics (transparency, color, fluidity, presence of heterogeneous formations), chemical examination (Pandy reaction, determination of albumin, glucose and chlorides) and bacteriological exams. In this study we not included the CSF samples that showed positive results in bacteriological and/or changing in biochemical parameters.

Examination of CSF samples by 1H-NMR method show a remarkable consistency in aliphatic region of the spectra. Molecules identified and quantified were alanine, lactic acid, acetone, acetic acid, acetoacetate, pyruvate and citric acid. The averaged concentrations for lactate, citrate, pyruvate, acetate and glucose determined by 1H-NMR spectroscopy where in resionable agreement with those obtained by conventional techniques published in literature.

The results presented in this study demonstrate the ability 1H-NMR method to investigate the CSF samples, showing particular interest in the diagnosis of neurological diseases.
Analytical technologies and applications

Cod: M108

INFLUENCE OF LIPEMIA TO BIOCHEMICAL ANALYTES RESULTS

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BACKGROUND

Lipemia is one of the most frequent analytical factors that can interfere with various laboratory methods and can cause falsely elevated or decreased results. There are several methods to remove lipoproteins in lipemic serum samples. The aim of our study was to investigate the influence of lipemia to biochemical analytes results using lipids extraction method.

METHODS

We tested 127 lipemic serum samples, containing triglycerides in concentrations ranging from 2.08 to 93.35 mmol/L, for alanine aminotransferase (ALT), amylase, creatinine, potassium, sodium, IgG and C-reactive protein (CRP) analytes on the Synchron Unicell® DxC 800 (Beckman Coulter, USA) analyzer. Each analyte was determined before and after treatment with LipoClear (StatSpin, Iris, USA). The significance of differences was calculated using the Wilcoxon matched pairs test for non-parametric data (median (interquartile range)). Differences comparing the groups were considered statistically significant when P value was less than 0.05. Test effect size (r) was evaluated according to Cohen criteria: 0.1 = small effect size; 0.3 = medium effect size; 0.5 = large effect size.

RESULTS

ALT, creatinine and sodium results before and after treatment with LipoClear statistically significant increased median (interquartile range), respectively 31 (26) and 29 (30) U/L, 87 (52) and 96 (66) µmol/L, 137 (4) and 142 (5) mmol/L; p<0.001. IgG and CRP results before and after treatment with LipoClear significant decreased median (interquartile range), respectively 10.77 (4.36) and 8.54 (3.05) g/L; 1.72 (4.26) and 0.5 (0) mg/L; p<0.001. Only amylase results before and after lipid extraction were the same.

We found that lipid extraction has large effect size for reducing IgG (r=0.61), CRP (r=0.5), and increasing creatinine (r=0.54), sodium (r=0.56) results.

CONCLUSIONS

We have found significant differences before and after lipid extraction in lipemic serum samples in all analytes except for amylase. Our study demonstrated that lipemia can cause inaccurate results. Unlike for other interferences, lipemia can be removed. Each laboratory should estimate the influence of lipemia and care to avoid these analytical errors.
Analytical technologies and applications

Cod: M109

TWO AUTOMATED METHODS FOR 25(OH) ASSESSMENT – COMPARISON WITH THE USE OF PEDIATRIC SERUM SAMPLES.

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Introduction: The best biomarker of estimation of vitamin D status is measurement of total 25(OH)D. Nowadays, there is a wide spectrum of various methods used for vitamin D level evaluation. Most commercially available automated assays are based on immunochemiluminescence technology, but recently enzymatic method adapted for general chemistry analyzers has been launched.

Aim: We aimed to compare two automated 25(OH)D assays: enzymatic (Pentra 400, Horiba ABX) and immunochemiluminescence (IDS-iSYS Immunodiagnosticsystems) using pediatric blood samples.

Material and methods: 25(OH)D total was measured in the serum of 100 schoolchildren aged 9 - 11 yrs (45 boys and 55 girls) on Pentra 400 and IDS-iSYS platforms. The anthropometric measurements were conducted and body mass index percentiles were calculated with an online BMI calculator (based on the “OLAF” project).

Results: Both methods demonstrated “poor correlation” (pc = 0.26). The Spearman’s rank correlation has shown a positive “moderate correlation” (rho = 0.47). Mean biases differed when comparing two subgroups of children. The one with the optimal body mass achieved the mean bias 35.3% (LoA: 109.8% to -39.1%), whereas in children categorized as overweight/obese the mean bias was only 2.0% (LoA: 80% to -75.9%).

Conclusions: Assays for 25(OH)D evaluation performed on Pentra 400 and IDS-iSYS showed uneven scores. Both analyzed method are not comparable, partly due to high cross-reactivity of the antibodies used in enzymatic method with C3-epimers of 25(OH)D, and cannot be used interchangeably, especially for evaluation of children vitamin D status. Further comparison study with “gold standard” methods and more age-diverse group is recommended.
INVESTIGATION OF NEGATIVE BIAS OBSERVED FOR EQA COBAS JAFFE CREATININE ASSAY STAT APPLICATION

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Introduction:
Participation of EQA programme is part of laboratory’s quality assurance process to ensure consistently good and accurate assay performance. SGH Clinical Biochemistry enrolled to both College of American Pathologist (CAP) programme (method code 1155) and Royal College of Pathologists Australasia (RCPA) (method code G 21Q 069 S) programme for COBAS JAFFE Creatinine assay on STAT application. Despite acceptable performance, consistent negative bias has been observed for the recent few submissions of RCPA EQA results. This paper describes the collaborative effort by lab and vendor in conducting systematic troubleshooting in investigating the probable cause for negative bias observed.

Methods:
The method code classification for COBAS JAFFE Creatinine Assay STAT application was verified to be correct. All RCPA EQA sample materials were repeated on the Roche COBAS c702 module to establish the reproducibility of negative bias observed. The same materials were also analyzed on the same day with COBAS JAFFE Reagent Kit on different module and application as follows; 1) COBAS c702 module on NON STAT application, 2) COBAS c502 module on STAT application and 3) COBAS c502 module on NON STAT application. Some CAP EQA materials were also selected and analyzed on the above platforms. A total of 120 patient samples were later compared on STAT and Non STAT application on JAFFE Reagent kit COBAS c702 module.

Results:
SGH Clinical Biochemistry Lab is the only participant using the method code while majority of Roche participant are using Alkaline Picrate Rate-Blanked Compensated (Non STAT Application) or Enzymatic method. Consistent negative bias was observed on STAT application, with calculated % recoveries using RCPA target values as; 90% (c702 STAT), 107% (c702 Non STAT), 89% (c502 STAT) and 105% (c502 Non STAT). Method correlation of patient samples produce the passing bablok of c702 STAT = 1.05 (c702 Non STAT) – 7.57; n=120 and different plot of 2.1% (-8.9 to 13.0%).

Conclusion:
The negative bias observed in RCPA sample possibly attributed by method specific sample matrices effect. Variation in application setting and instrumentation for COBAS JAFFE Creatinine is not likely the cause for the negative bias observed.
EVALUATION OF THE AUTOMATED URINE ANALYZER COBAS 6500 IN A ROUTINE LABORATORY WORK FOR MONITORING LIVER AND RENAL TRANSPLANT PATIENTS

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BACKGROUND: The aim of our study was to evaluate the analytical and clinical performance of automated urine analyzer Cobas 6500 for monitoring liver and renal transplant patients in routine laboratory work.

METHODS: Urine samples (n=308 for comparison of reagent test strips; n=133 for urine sediment) were collected in Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital accredited according to ISO 15189. Test strips were compared with Siemens Multistix 10G reagent strips read on Siemens Clinitek 500 which served as a referent analyzer as its results were regularly included in the program of external quality control. Urine sediments were compared with manual microscopy analysis.

RESULTS: Repeatability and precision of test strips for hemoglobin, leukocyte esterase, nitrites, ketones, glucose, urobilinogen, bilirubin and pH were 100%, while for proteins, color and relative volume mass were lower but stayed within recommended limits. Agreement rates for all parameters on test strips within one concentration range were 85-100%. Bland-Altman analysis revealed deviations between results for the sediment parameters: red blood cells (RBCs) -1.3% (95% CI: -2.4 to -0.09%), leukocytes -5.1% (95% CI: -6.8 to -3.9%), squamous epithelial cells 0.8% (95% CI: 2.9 to 1.2%) and small epithelial cells 0.1% (95% CI: -0.04 to 0.25%). Total agreement rates for other urine sediment parameters: crystals, bacteria, mucus, hyaline cylinders and yeast were between 76 and 90%.

CONCLUSION: Variation of agreement rates within one concentration range on test strips can be explained with lower detection limits on Cobas 6500 analyzer. In relation to manual microscopy, results of the urine sediment analysis are satisfactory. However, pathological urines (especially samples with present cylinders and crystals considering that Cobas 6500 doesn't differentiate between different crystal and cylinders types) should be checked microscopically as it is very important to differentiate for monitoring transplant patients. Nevertheless, automated urine analyzer Cobas 6500 shortens the turn-around-time and by standardization of preanalytical and analytical process reduces the possibility of error which is necessary for laboratories with large amounts of samples per day.
COMPARISON OF TWO METHODS FOR DETERMINATION OF HUMAN GROWTH HORMONE LEVELS: ELISA AND ECLIA

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BACKGROUND: Physiologically, Human Growth Hormone (HGH) has general anabolic effects and its major function is to stimulate the elongation of bones in childhood. It is difficult to diagnose HGH deficiency, because the pituitary gland produces growth hormone in bursts. One way of testing HGH deficiency is to give the child a substance (e.g. insulin, arginine, glucagon, L-dopa, clonidine), that causes the release of a HGH burst in normal children and measure the levels of HGH in several blood samples obtained over a period of time. The aim of the study was to compare two analytical methods for measurement of serum HGH levels: enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA).

METHODS: Two assays were used to measure serum HGH levels: a) ELISA kit (DRG International Inc.) and b) the analyzer COBAS e411 (Roche Diagnostics Laboratories). The study material was the serum obtained from 10 healthy children after HGH stimulation with glucagon (mean age 12.67 ± 3.65).

RESULTS: After the calculation of the results, we divided them into two groups, the first group was consisted of the lower values <1.0 ng/ml and the second group was consisted of the higher values >1.0 ng/ml. The mean values and standard deviation of serum HGH levels in the first group were 0.59 ± 0.22 ng/ml measured by ELISA and 0.54 ± 0.19 ng/ml by ECLIA (p=0.55). For the second group the mean values and standard deviation of serum HGH levels were 5.93 ± 2.26 ng/ml and 5.79 ± 2.39 ng/ml, respectively (p=0.9).

CONCLUSION: The comparison of data obtained by ELISA and ECLIA showed no significant difference between the two methods for both groups. However, from laboratory’s point of view ECLIA method allows rapid determination of serum HGH concentrations and is less expensive than ELISA method.
Analytical interference due to the presence of heterophilic antibodies is a significant problem for immunoassays. Latex-enhanced turbidimetric immunoassays are prone to such interference being single step immunoassays lacking any washing step.

In the past, our Ferritin reagent based on a rabbit-derived polyclonal anti-ferritin antibody showed a prevalence of 0.1% in producing results in serum samples where heterophilic interference was present.

In order to solve the above problem two different Ferritin reagents were developed following two alternative approaches. The first approach consisted in using only the F(ab)_2 fragment of the polyclonal antibody (FERA), while the second in substituting the polyclonal antibody with two mouse-derived monoclonals (FERB).

Over a period of six months, and after screening a large number of samples, 12 samples have identified where our Ferritin reagent reported statistically significant higher values when compared to Siemens Advia chemiluminescence immunoassay for Ferritin on the Centaur Analyzer. This positive bias was confirmed to be due to a heterophilic interference since it was completely or partially neutralized after treatment of the patient samples with heterophilic blocking tubes. Thus, mean Ferritin concentration was reduced from 167.5 ng/ml observed initially, to 114.8 ng/ml after treatment.

Contrary to previous Ferritin reagent, the two assays showed no positive bias in the same samples when compared against the reference immunoassay. The mean Ferritin concentration observed was 103.6 ng/ml, 91.3 ng/ml and 102.9 ng/ml for FERA, FERB and reference immunoassay, respectively.

These results suggest that, epitopes responsible for heterophilic interference probably reside to the Fc region or very near to the hinge region of the rabbit polyclonal antibody. In FERA reagent these epitopes are either removed (Fc) or hindered (hinge region) after immobilization of F(ab)_2 fragment on the polystyrene bead while, in FERB assay are absent due to the substitution of rabbit polyclonal by the two mouse monoclonals.
Background: Urine chemistry is one of the most commonly used tests for screening, diagnosis, and monitoring of renal, urinary, and metabolic disorders. Results are semi-quantitative with limited analytical sensitivity. Aims of study were: a) to assess the comparability of glucose, bilirubin, blood, leukocytes and protein using Combur10Test®M test strips on Cobasu411 (AI) (Roche, Mannheim, Germany) and iChem Velocity strips on iChem VELOCITY (AII) (Iris Diagnostics, Chatsworth, USA) and b) to assess accuracy of glucose, bilirubin, protein, and leukocytes of each analyzer. Methods: Comparability was evaluated on 229 patient’s samples for bilirubin and blood, 227 for glucose, 43 for protein and 40 for leukocytes on AI and AII. Accuracy was tested by comparing the results of patients samples obtained by test strips and using reference methods on Architect c8000 (Abbott, IL, USA) and XN-1000 (Sysmex, Kobe, Japan). Kappa coefficients with 95% confidence interval were calculated as a measure of results comparability. Kappa $\geq 0.6$ was considered acceptable. In order to evaluate accuracy, diagnostic specificities and sensitivities were calculated. MedCalc (v12.7.2.0, Ostend, Belgium) was used for statistical analysis. Results: Results have shown substantial to almost perfect agreement for all tested parameters between two analyzers. Accuracy was not acceptable for glucose on both analyzers (kappa (AII)=0.595 (0.508-0.683); kappa (AI)=0.488 (0.388-0.588)) and for leukocytes on AII (kappa=0.409 (0.206-0.612)). Diagnostic sensitivity was the lowest for glucose on both analyzers (AI-57%; AII-58%). All other parameters have shown diagnostic sensitivity from 72-100%. Diagnostic specificity was the lowest for leukocytes on both analyzers (AI-57%; AII-58%). All other parameters have shown diagnostic specificity from 75-100%. Conclusion: Although different manufacturers offer test strips with different specifications we have concluded that for the tested parameters tested strips can be used interchangeably. However, we have to be careful when purpose of urine chemistry testing is considered. Glucose testing should not be used in screening program due to the low sensitivity and positive leukocytes should be confirmed (e.g. urine sediment microscopy).
S302

Analytical technologies and applications

Cod: M115

EVALUATION OF ADAMS™ A1C MENARINI HA-8180 THALASSEMA MODE HPLC ANALYZER FOR HbA1c DETERMINATION

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Background: ADAMS A1c HA-8180 Thalassemia mode is a system for the measurement of HbA1c. The analysis time is 3.5 minutes per sample, HbA2 and Hb variants can be detected. The analytical performance was evaluated to verify quality of results.

Methods: Trueness is studied with controls. Carry over and within run imprecision studies were performed according to ICLH guidelines and ICLS guides (N5-A2, EP6, EP9) for reproducibility, recovery and method comparison.

Effect of the concentration total Hb: a sample was centrifuged 10 minutes to 300 rpm. Red blood cells and plasma obtained are mixed in proportions 9:1, 8:2...and samples analyzed.

Effect of interfering substances was studied adding increasing concentrations of Glucose, Sodium Cyanide or Acetaldehyde solutions; the samples were incubated to 370 C for 2 hours and analyzed.

Comparison with ADAMS™ HbA1c 8180 variant mode: 110 samples were analyzed with both modes of operation; Passing - Bablok regression applied to the couples of results.

Results: trueness 40 mmol/mol bias 0.6%; 95 mmol/mol bias 0.13%.
Carry over 0 % for HbA1c 104 and 30 mmol / mol.
Within run imprecision (blood samples): mean 40 and 102 mmol/mol CV 0%, mean 53 mmol/mol CV 0.4%.
Reproducibility: mean 40 mmol/mol, within run CV 0.71 %; between run CV 0.77 %; between-day CV 0.36 %; Total CV 0.93 %.
Mean 95 mmol/mol within run CV 0.43 %; between run CV 0.29 %; between-day CV 0 %; total CV 0.48%. Total analytical error 1.74 %.
Recovery: range 31-113 mmol/mol Y = 0.93 x + 0.51, R2 = 0.994
average recovered 98.7%, 95% CI slope (.824-1.043) intercept (-.31-1.54).
HbA1c 37 mmol / mol is not affected by a Hb in the range 214-40 g / L.
HbA1c 31 mmol/mol is not affected by the presence of labile, carbamylated or acetylated fractions of 27 mmol/mol.

Method comparison: range 15-130 mmol/mol.
\[ y = 1.0 x + 0.10, r = 0.999 \] 95% CI slope (1.000-1.000) intercept (0.0-0.1)

Conclusions: Total error is lower than the recommended total analytical error 2.0 %.
The results are independent of the concentration total of Hb and of the presence of modified Hb, precise and linear in the clinically significant analytical range.
It is a reliable system for the control of diabetic patients and the detection of Hemoglobinopathies, both maladies of increasing prevalence in our area.
SEBIA FLC: A NEW SERUM FLC ELISA-BASED ASSAY THAT BRINGS COHERENCE WITH SERUM PROTEIN ELECTROPHORESIS RESULTS

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BACKGROUND Since the availability of the serum Free Light Chain (sFLC) assay, diagnosis, monitoring and prognosis has greatly improved for plasma cell dyscrasias. In parallel, many publications appeared on the analytical limitations, pitfalls and technical difficulties of nephelometric techniques. Strong discrepancies between Freelite (The Binding Site, Birmingham) sFLC concentrations and the FLC monoclonal band on Serum Protein Electrophoresis (SPE) have been reported. Freelite overestimation can be greater than 10-fold and has been attributed to sFLC polymerization leading to larger immune complexes and greater scatter by nephelometry. We have previously published sFLC concentrations within close range of the values obtained with SPE using our home-made ELISA sFLC assay. Here we present data of a large scale validation of this observation using the industrialized version of our ELISA assay: the Sebia FLC assay (Sebia, Lisses, France).

METHODS Sera obtained from 53 patients with measurable FLC peaks on SPE (Capillarys 2, Sebia) were analyzed using both the Freelite and Sebia FLC assays. The SPE FLC peak concentration was compared to Freelite and Sebia FLC concentrations.

RESULTS SPE concentrations of the FLC in the 53 serum samples ranged from 50 to 8400 mg/L. Freelite FLC concentrations were consistently higher in all 53 tested sera, with a mean 12-fold overestimation compared to SPE. The observed differences between Freelite and SPE were patient dependent and the absolute Freelite overestimation of $\kappa$-FLC ranged from 0.5 to 31 g/L. The Freelite overestimation of $\lambda$-FLC ranged from 0.2 to 21 g/L. Sebia FLC concentrations fluctuated around the SPE FLC peak concentrations, with a mean 0.8-fold underestimation compared to SPE. For Sebia $\kappa$-FLC the absolute deviation ranged from -1.6 to 2.5 g/L. The Sebia $\lambda$-FLC absolute deviation ranged from -4.7 to 1.7 g/L.

CONCLUSION We underline here a well known limitation of the nephelometric sFLC technique: the overestimation of sFLC concentrations. The nephelometric units are not “real mg/L” but are more “arbitrary units” in sera with relatively high sFLC concentrations. Even though laboratories are now used to this unit shift, the possibility to use sFLC assays that give coherent results with SPE is an important added value for the laboratory. We show here that the new Sebia FLC ELISA allows this coherence with electrophoresis bringing consistency to FLC testing and leading to easier assay interpretation.
EVALUATION OF 3 NEW AUTOMATED MICROSCOPY URINE SEDIMENT ANALYZERS

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Introduction
Urinary analysis remains important in the diagnosis and monitoring of urinary tract infections and renal diseases. This study comprises an evaluation of 3 recently launched automated microscopy urine sediment analysers: sediMAX 2, sediMAX Lite, sediMAX conTRUST (A. Menarini Diagnostics Benelux S.A.).

Methods
Freshly collected random urine samples sent to laboratory for urine sediment analysis (n=336) were prospectively included in the study. The samples were routinely analysed on sediMAX FL in combination with biochemistry screening on an automatic strip reader AutionMAX AX-4030 (A. Menarini Diagnostics Benelux S.A.). Parallel microscopic analysis was randomly performed on sediMAX 2, sediMAX Lite and sediMAX conTRUST. For 87 urine samples, positive for red blood cells (RBC) and/or white blood cells (WBC), manual urine sediment analysis was also conducted by phase contrast microscopy. Analytical performance (imprecision, carry-over, correlation and categorical agreement) and diagnostic performance (sensitivity, specificity) were evaluated for all urine sediment analysers.

Results
All analyzers showed acceptable imprecision (3 levels RBC/WBC). No carry-over of RBC/WBC was detected. Regarding accuracy, a positive bias was revealed for sediMAX 2, sediMAX Lite, sediMAX conTRUST, attributable to the correction factor used (RBC factor 2x, WBC factor 1.5x). Using correction factor modified semi-quantitative interpretation categories, very good correlations (weighted kappa > 0.8 for RBC and WBC), were obtained for all analysers with sediMAX FL and manual microscopy. The implementation of the correction factors did not significantly improve the quantitative correlation of RBC/WBC counts with manual microscopy. The diagnostic performance of the review flagging (discordance with biochemistry screening, pathological casts, sperm cells, yeasts) was not significantly better for the new urine sediment analysers than for sediMAX FL.

Conclusion
In combination with an automatic strip reader and using modified semi-quantitative interpretation categories, sediMAX 2, sediMAX Lite and sediMAX conTRUST are reliable alternatives for manual microscopic analysis of urine sediment.
FOUR CALPROTECTIN METHODS EVALUATED, COMPARING TWO AUTOMATED ASSAYS, ONE SEMI-AUTOMATED ASSAY AND ONE ELISA ASSAY

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Background
Fecal calprotectin is excreted by neutrophilic granulocytes during gastrointestinal inflammation. Calprotectin (calp) is an important biomarker to distinguish between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) and also predicts relapses in IBD. Measuring calp is a cost-effective approach to prevent unnecessary colonoscopies. As a consequence, calp determinations increased rapidly over the years. Several suppliers have introduced fully automated assays, with obvious advantages as better imprecision, minimal analytical errors, and reduction in turn-around time. Here, we compared four quantitative calp assays: the Bühlmann ELISA, the semi-automated Calpro ELISA, the Bühlmann automated fCal turbo (on a Cobas 6000 platform, Roche Diagnostics), and the automated Phadia EliA2 (on an ImmunoCap250 platform, Thermofischer).

Material and Methods
In total, 88 fecal samples were collected in a stool specimen collector. Fecal extracts were prepared with extraction devices (Roche Diagnostics), using 0.1 gram feces and the appropriated volume of buffer (according to the manufacturer’s recommendations). Extracts were stored at -20°C until analysis. All calp assays were performed in the same week, according to the manufacturer’s instructions.

Results
Assay comparison was performed by non-parametric Passing Bablok regression analysis. Only results within the manufacturer’s range were included. Bühlmann ELISA versus fcal turbo showed a proportional bias of 0.88 and a constant bias of -6 (n=61); Calpro ELISA versus Bühlmann ELISA showed 1.90 and 1 (n=60); Calpro ELISA versus fcal turbo showed 1.83 and -15 (n=65); Phadia EliA2 versus Bühlmann showed 1.08 and 10 (n=82); Phadia EliA2 versus fcal turbo showed 0.85 and 27 (n=67) and Phadia EliA2 versus Calpro ELISA showed 0.41 and 31 (n=66) respectively.

Conclusion
Overall both Bühlmann (ELISA, fCal turbo) and Phadia EliA2 calp assays showed good concordance. In contrast, the Calpro ELISA showed poor concordance to Bühlmann and Phadia assays. Substantial lower concentrations were detected using this Calpro ELISA, especially in the higher concentration range. Due to the proven clinical value of calp measurement, the workflow in the laboratories increases. Both automated calp assays are suitable candidates for processing this increased calprotectin workload.
Background. Thyroid-Stimulating Hormone (TSH) has been suggested to be the most sensitive indicator of hypo- or hyperthyroidism. Third generation TSH sensitivity tests provide a more precise and sensitive detection to aid in the diagnosis of abnormal thyroid function. The aim of this study was to develop a third generation biochip based TSH assay applied to the first high throughput, random access with STAT capability, fully automated biochip analyser, Evidence Evolution. Based on biochip array technology, this application represents a new analytical tool for the quantitative detection of TSH in the investigation of thyroid function.

Methods. A sandwich chemiluminescent immunoassay, applied to the biochip analyser Evidence Evolution, was used. Functional sensitivity and intra-assay precision were evaluated using serum based precision material. Serum patient samples (n=60) were assessed and the results compared with a commercially available method.

Results. The biochip assay showed a functional sensitivity value of 0.01 µIU/mL. Intra-assay precision, expressed as CV (%), showed values of 3 %, 3 % and 8 % for low, medium and high levels of TSH in serum based precision material. An r value of 0.99 was obtained following regression analysis of the results after the assessment of the 60 serum samples with the biochip assay and another commercially available method.

Conclusion. This new biochip based third generation TSH assay on Evidence Evolution allows the high sensitive quantitative determination of TSH in serum samples. This study demonstrates the applicability of this new system to the reliable measurement of one of the tests included in the Thyroid Function Array. As biochip array technology offers flexibility to incorporate other assays on the same biochip i.e. free T3 and free T4. The measurement of these three analytes simultaneously will aid in the efficient diagnosis of patients with thyroid disorder, as previous studies on the application of this technology on other Evidence platforms have demonstrated. The new Evidence Evolution platform also incorporates STAT sample and random access capabilities.
EVALUATION OF ROCHE COBAS C513 HbA1c DEDICATED HIGH THROUGHPUT ANALYZER IN A HIGH VOLUME LABORATORY

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Background: Changes to the recommended use of HbA1c to include diagnosis and screening for diabetes have led to increased demand for HbA1c testing. HbA1c testing in high volume laboratories requires streamlined pre-analytical processes, and analytically robust high throughput analyzers. This study evaluated the suitability of Roche Cobas c513 HbA1c dedicated high throughput analyzer for a laboratory testing 8000 HbA1c samples/24h.

Methods: Analytical evaluation included imprecision (commercial quality control (QC) material and patient sample), accuracy (National Glycohemoglobin Standardization Program reference samples), linearity (commercial linearity material), sample carry-over (high total hemoglobin (Hb) patient sample and saline), and method comparison studies (50 patient samples and 11 patient samples with low total Hb). Robustness was evaluated by simulating the daily workflow for 4 days. The effect of erythrocyte sedimentation (20 patient samples) was evaluated for specimens typically arriving in the laboratory up to 48h after collection.

Results: The within-run (n=20) and between-day (n=2/day, 15 days for QC material, 7 days for patient sample) imprecision was 0.5% coefficient of variation (CV) and 1.2% CV, respectively for the low level control material, 0.6% CV and 1.2% CV, respectively for the high level control material, and 0.7% CV and 0.9% CV, respectively for a patient sample at 49mmol/mol HbA1c (6.6%). Accuracy was acceptable with a bias of 0.0-4.4mmol/mol (0.0-0.4%). Linearity range from 27-162mmol/mol (4.6-17.0%) was established. No sample carry-over was detected. The c513 method compared well with the Roche Integra 800 method (range 26-124mmol/mol [4.5-13.5%], correlation coefficient 0.99, slope 0.98, intercept 4.4mmol/mol [0.4%], Deming linear regression). Low total Hb samples (8/11 containing Hb variants) showed comparable “out of range” error flagging between the two methods. An uptime of 92-100% and an average throughput of 344 tests per hour (range 335-362) were demonstrated. Erythrocyte sedimentation produced clinically significantly different results after 24hr compared to baseline results (slope 0.91, intercept 5.5mmol/mol [0.5%], Deming linear regression).

Conclusions: Roche c513 HbA1c shows comparable analytical performance to the Integra 800 method. In addition, c513 shows adequate robustness and throughput for a high volume laboratory. Samples received in the laboratory ≥ 24h after collection require mixing prior to HbA1c analysis.
Analytical technologies and applications

Cod: M121

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AS ANALYTICAL METHOD FOR STUDY OF URINARY CALCULI

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BACKGROUND

A good qualitative analysis of urinary calculi is essential for the treatment and lifestyle that the patient should follow to prevent future repetition of calculi. This study aims to compare the results obtained using Fourier transform infrared spectroscopy (FTIR) with previously used techniques (macroscopic, microscopic and chemical analyzes), taking as an example a cystine stone.

METHODS

Firstly a macroscopic analysis of the fresh sample was performed. Secondly 20 mg cystine samples were dissolved by pulverization and addition of 5 drops of H2SO4 (95%) followed by addition of 50 mL of distilled water under stirring until complete dissolution was reached. The dissolved sample was photometrically analyzed on a Beckman Coulter AU5800 autoanalyzer. The pulverized cystine sample was analyzed with FTIR, and the IR spectra were compared with a microscopic study of cystine calculi and a semi-quantitative chemical method from DiaSys® (“urinary calculi analysis”). In this method specific reagents, cations (calcium, ammonium and magnesium), anions (oxalate, phosphate and carbonate) or whole molecules (uric-urate, cystine) are used.

We also perform a microscopic analysis of the urinary calculi.

RESULTS

Macroscopic analysis showed a dark brown colored smooth surface which after pulverization turned into a light brown powder. The cystine powder did not release any CO2 gas after the addition of H2SO4, suggesting that the powder does not contain carbonates. The solution of the powder in H2SO4 did not show any chemical reaction after the addition of water, which indicates that uric acid is not present in the powder. No significant values were obtained from the spectrophotometric routine analysis of uric acid, phosphorus, ammonium, calcium and magnesium. IR spectrum was compared with spectra from the German library Nierstenen Golden Gate provided by Shimadzu, and comparison with the most similar spectrum indicated a composition of 100% of cystine. Chemical analysis by DiaSys® detected the highest level of cystine according to their colorimetric scale. Dilution of the powder with distilled water and subsequent evaporation showed under the microscope showed hexagonal crystals, which are typical of cystine crystals.

CONCLUSIONS

When the gold standard method (electron microscopy) is not available, another methods of analysis can be used, such as FTIR, which proved useful tool on the determination of the composition of cystine crystals. Determination of singled composition crystals possess high precision, and while more infrequent calculi with complex compositions can be determined, addition of more spectra to the reference library will improve the determination of these calculi.
LOCKING UP 24,25-DIHYDROXYVITAMIN D: A NEW ASSAY FOR 25-HYDROXYVITAMIN D WITH IMPROVED SPECIFICITY

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With the availability of automated assays and the increase in demand, 25-hydroxyvitamin D (25(OH)D) has become a routine parameter in most countries worldwide. Although the Standardization and Certification Program greatly improved assay comparability for healthy subjects, variations still exist for certain clinical cohorts due to the presence of vitamin D metabolites which are co-detected by some methods. A prominent example is 24,25-dihydroxyvitamin D (24,25(OH)2D) that cross-reacts in most immunoassays. Roche developed a new Elecsys Vitamin D total II assay with negligible cross-reactivity to 24,25(OH)2D by using a specific blocking antibody.

The Elecsys Vitamin D total II assay is traceable to the ID-LC-MS/MS at the University of Ghent and was validated on cobas e 411, e 601, e 602 and e 801. The standardization was verified using a set of reference samples provided by the CDC. The Deming regression was y=0.954*x-0.707 (r=0.982). The good alignment to the reference measurement procedure (RMP) was confirmed by CAP and DEQAS samples with a mean recovery of 99% (83-111%, ABVD-01 to -12) and 100% (84-110%, #476-490), respectively. LoQ was determined as ≤4.96 (with CV 17.3%) and CVs for repeatability and intermediate precision were ≤7.4% (10.5 ng/mL) and ≤9.2% (12.5 ng/mL) in a 21-day precision model for all analyzer platforms. The efficacy of the specific blocking antibody was assessed in two ways: i) Based on four DEQAS samples with high amounts (3.28-4.32 ng/mL) of 24,25(OH)2D. The mean recovery was 103% (96-110%) compared to 114% (100-132%) for another immunoassay with cross-reactivity to 24,25(OH)2D. ii) Based on dialysis patients which are known to have a lower ratio of 24,25(OH)2D / 25(OH)D as compared to healthy subjects. This usually causes under-recovery if an assay cross-reacts with 24,25(OH)2D. A method comparison with samples from dialysis patients gave a Deming regression of y=0.970*x+0.601 (r=0.971) proving correct recovery.

In summary, the new Elecsys Vitamin D total II assay is very well aligned to all listed RMPs and demonstrated a good overall performance. The negligible cross-reactivity to 24,25(OH)2D improves its specificity to the relevant vitamin D metabolites and ensures better comparability to LC-MS/MS and between different cohorts.
Analytical technologies and applications

Cod: M123

FATTY ACID PROFILING OF DAIRY PRODUCTS IN THE CAMBRIDGESHIRE AREA OF UK

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Recently studies suggested that increased intake of fermented dairy products (yogurts and cheese), measured through dietary assessment, and are associated with reduced risk for type 2 diabetes (Sluijs, Forouhi et al. 2012). The measurement of the fatty acids of plasma phospholipids showed that increased levels of odd chain saturated fatty acids associated with reduced risk of type 2 diabetes (Forouhi, Koulman et al. 2014). The only dietary source of odd chain fatty acids are from dairy products. Therefore, the development of microwave- accelerated sample extraction for fatty acid profiling of dairy products was urgently requested.

A simple method of microwave- accelerated sample extraction used for sample preparation, followed by gas chromatography with flame ionization detector to profile the 40 fatty acids of total lipid from various dairy samples has been developed. The precision and the accuracy of this analytical method were validated by using a certified reference material BCR-163 (beef-pork fat blend). More than 100 dairy and dairy related products purchased in the Cambridgeshire area were analyzed for 40 fatty acid profile by using the above method. The dairy products were divided into sub-whole milk, semi-skimmed milk, skimmed milk, cheese, yogurt and non-dairy product groups, and means of fifteen saturated fatty acids, nine cis-monounsaturated fatty acids, thirteen cis- polyunsaturated fatty acids (PUFA), five cis-n-3 PUFA, seven cis-n-6 PUFA and three trans- unsaturated fatty acids in different types of dairy product groups were reported. Results from our study showed that fatty acid profile of dairy products is remarkably constant across the different products; the fatty acid profiles of milks with different fat levels or cheeses or yogurts is very similar. There are not statistic differences between the fatty acid profiles of these products for a robust classification. The only main difference is the increase in the fatty acid 17:1, which is higher in fermented dairy products. Our study also showed that this method used to profile the 40 fatty acids of total lipid in food was simple, less time consuming, inexpensive and suitable to facilitate a large scale study.
Towards a reference material for the standardization of calprotectin (S100A8/A9; MRP8/14) immunoassays

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Background: Calprotectin (Cp) is a multifunctional protein that plays important roles in the diagnosis, prognosis and follow-up of a series of inflammatory diseases. Elevated levels of Cp can be found in stool, blood, synovial fluid, saliva and many other inflamed tissues. Cp is a heterodimeric or - in the presence of calcium/zinc – tetrameric protein. Its complex oligomerization process and protein conformation is hypothesized as the reason behind the different standardizations of commercial Cp immunoassays. The aim of this study was to prepare Cp reference material and to assess it in an in-house research (Muenster) and a commercial IVD/CE (BÜHLMANN) immunoassay.

Methods: Cp was prepared from human granulocytes by two independent laboratories (T.V. in Muenster; T.N. in Moss). The Cp homogeneity, purity and concentration was checked by SDS-PAGE, 3 different protein determination methods and UV/VIS spectroscopy (ε=0.75 mL/g*cm at 280nm). The Cp reference materials were spiked into different buffer systems, human sera and fecal extracts and the accuracy of the respective ELISA standardizations in regard to the biological matrix was assessed.

Results: Six (2 in Moss, 4 in Muenster) reference preparations with a purity of >95% (by SDS-PAGE) have been produced in mg amounts. The protein concentrations varied between 1.5 and 2.9 mg/mL whereby the UV/VIS results were very close to the Biuret method while Bradford and Coomassie gave very discrepant results. One reference preparation from each laboratory was diluted in saline or BÜHLMANN assay buffer. In the Muenster universal research ELISA, standardized with PBS/BSA-based calibrators, the Cp in saline showed an excellent recovery of 97%. The BÜHLMANN ELISA, standardized with serum-based calibrators, yielded a mean recovery of 102% when the Cp reference materials were spiked into BÜHLMANN assay buffer. Consequently, serum samples (n=4) and fecal extracts (n=3) spiked with highly purified Cp reference material from both laboratories yielded recoveries of 82-109% over the entire standard range of the BÜHLMANN ELISA.

Conclusions: The consortium was able to prepare highly purified human Cp reference material in reproducible quality and quantity. The effective Cp concentration could be consistently determined by UV/VIS spectroscopy and confirmed by Biuret protein assay and two independent ELISA methods. Possible interferences of the biological matrix need further investigation in the respective immunoassays.
Validation study and sigmametric performance of DCA Vantage point-of-care analyzer for HbA1c measurement

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Background

The glycosylated hemoglobin assay provides the most objective and reliable information about long term glucose control in diabetic patients. In this study, we aimed to perform validation study for DCA Vantage point-of-care HbA1c device (Siemens Healthcare Diagnostics, USA) and evaluate the laboratory performance of HbA1c using Sigma-metrics.

Methods

The reagents and reference materials are provided from Siemens Healthcare Diagnostics, Turkey for the study. HbA1c levels were measured at two different concentrations (level 1 and level 2). CLSI EP-5 protocol was used for the assessment of the analytical reproducibility. Method comparison was performed according to the CLSI EP9-A protocol for determining bias in HbA1c between DCA Vantage and reference HPLC analyzer (ADAMS A1c, HA-8180, Arkray, Japan). For sigma-metric analysis, CAP, Clia, Ricos and Rilibak guidelines were accepted for the total allowable error values.

Results

For level 1 were; mean HbA1c: 5.34 % (target: 5.5±1.1 %), standard deviation (SD): 0.1, % bias: 2.8, total % CV: 1.8, Sigma-metric values were (according to CAP, Clia, Ricos and Rilibak guidelines) respectively: 2.3, 0.1, 0.8, 8.4. For level 2, results were; mean HbA1c: 10.82 % (target: 10.8±2.2 %), standard deviation (SD): 0.28, % bias: 0.2, total % CV: 2.58, Sigma-metric values were (according to CAP, Clia, Ricos and Rilibak guidelines) respectively: 2.6, 1.1, 1.6, 6.9. According to the HbA1c results from DCA Vantage analyzer compared to the reference HPLC method; the slope was 0.941 (95% CI 0.896-1.002), the intercept was -0.232 (95% CI -0.575-0.002) and the correlation coefficient was 0.997.

Conclusions

Current clinical practice guidelines advise that the HbA1c test can be used to diagnose diabetes and identify at risk patient. High performance liquid chromatography still considered the reference method. We found that this new analyzer has a similar performance and can work compatible with the reference HPLC method. Sigma-metric values were also quite satisfactory for HbA1c.
EVALUATION OF THE ABBOTT ALINITY NEXT GENERATION CLINICAL CHEMISTRY ANALYZER

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Objective/Background: To evaluate the analytical performance and system reliability of Alinity, Abbott’s next-generation clinical chemistry instrument, in an external clinical laboratory setting. Toronto General Hospital performed the testing, using third party quality control materials and sequestered, de-identified, clinical specimens. Acceptability criteria for assay precision were determined while instrument reliability was recorded during continuous testing over four weeks.

Methods: Precision profiles for select assays, CO2, Magnesium, and ICT assays (Sodium, Potassium, and Chloride) were evaluated using 3 levels each of Bio-Rad and Technopath control materials. Testing was performed on each control sample in duplicate, two runs per day for 5 days to calculate and verify expected % CV characteristics (N=20). Precision was also tested using clinical specimens, tested in triplicate, for Glucose, Magnesium, Creatinine, Calcium, and ICT assays each day for a period of 4 weeks comprising 2,052 clinical specimens tested in triplicate or 43,078 total generated results during the duration of the study.

Results: All assays met and exceeded expected precision performance during the 5-day precision study using third party control materials and during the 4 week clinical specimen study. The observed ranges of imprecision for the third party control materials were as follows: CO2 (2.4 – 4.1%), Mg (1.5 – 2.0%), (Sodium (0.2 – 0.4%), Potassium (0.0 – 0.3%) and Chloride (0.2 – 0.4%). In total, approximately 44,000 replicates were tested during the study period, with no service calls for instrument failures reported. Overall, 99.98 % of the evaluated replicates met or exceeded assay design goals as follows: Glucose (5.0%), Calcium (3.0%), Creatinine (6.0%), Magnesium (3.8%), Sodium (1.5%), Potassium (2.7%) and Chloride (2.0%).

Conclusion: Representative assay precision performance and system reliability results on Alinity demonstrated excellent reproducibility with both control materials and clinical specimens. Continuous testing over 4 weeks support the instrument’s ability to reliably generate results for today’s clinical laboratory demands.
Analytical technologies and applications
Cod: M127

DEVELOPMENT OF APPLICATION PROTOCOLS FOR 27 CLINICAL CHEMISTRY PARAMETERS ON DIATRON P500 CHEMISTRY ANALYZER

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The objective of this study was to develop the application protocols for 27 clinical parameters for Medicon reagents on Diatron P500, with the objective to use results in filling our FDA 510k premarket notification submission. The development of application protocols and subsequent evaluation was performed in serum, plasma (heparin or EDTA) and urine samples, where appropriate.

Total precision, Linearity, Interferences, LoB, LoD, LoQ and Method comparison were measured following procedures according to the CLSI protocols EP05-A3, EP06-A, EP17-A2 and EP-09-A2, respectively. Moreover, within-run precision in three concentration levels, on-board stability of the reagents, carry-over and analytical drift were assessed using previously approved procedures.

Within-run CV% was for all parameters between 0.21 % and 4.99% and total precision CV% was between 1.52% and 9.17%. When a deviation higher than 5% from theoretical values was considered as an indication of non-linearity, all assays were within expected linearity claim. The estimated lower limit of analytical range, as determined from the LoB, LoD, LoQ protocol was also within the expected limit for any parameter. The levels of each interferent (Bilirubin, Hemoglobin, Lipemia, Ascorbic acid) causing interference lower than 10% were determined for two levels of analyte concentration. On board stability was determined for every reagent to be at least 1 month with the exception of Creatinine Jaffe (15 days) and Magnesium (7 days). No systematic analytical drift was observed within 8 hours. No relevant sample and reagent carry-over could be detected.

Method comparison was performed against the Beckman Coulter Reagent System on a AU2700 chemistry analyzer. All methods recovered within expected ranges (slope 0.95 - 1.05 and relative intercept 90 – 110%).

In conclusion, Diatron P500 system, in combination with Medicon reagents demonstrated excellent reproducibility and accuracy in reporting results which were highly comparable with those attained with well established analytical systems.