Tuesday, July 31, 2018

Poster Session: 9:30 AM - 5:00 PM

Hematology/Coagulation

A-294

Does Vitamin B12 behave as an acute phase reactant

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Backgound: High serum vitamin B12 (vitB12) levels are found in myeloproliferative diseases including chronic myeloid leukemia, polycythemia vera, and also infectious and autoimmun diseases. The purpose of this study to determine whether high serum vit B12 levels may be used as an acute phase reactant. For this aim the inflammatory markers leukocytes (WBC), absolute neutrophil count (ANC), and erythrocyte sedimentation rate were investigated in patients whose serum vitB12 levels were above 1000 pg / mL associated with high or normal serum C-reactive protein (CRP) levels. **Methods:** Children who 0-18 years old were included to the study. The patients were divided into two groups: Group 1 (n=32, 17 F/15 M) containing the patients with high vitB12 (>1000 pg/mL) and high CRP (>0,8 mg/dL) levels, and Group 2 (n=19, 9F/ 10M) containing the patients with high vitB12 (>1000 pg/mL) and normal CRP (0-0,8 mg/dL) levels. Serum VitB12 assay was performed by competitive-binding immunoenzymatic assay on DXI800 (Beckman Coulter, Inc, US) with an analytical sensitivity of <50 pg/mL. Serum CRP was measured by nephelometry (Beckman Coulter, Inc, US). WBC was counted by Beckman DXH800 (Beckman Coulter, Inc, US) Absolute neutrophil count (ANC) was calculated. Erythrocyte sedimentation rate was measured by kinetic method of Test 1-ALIFAX (Italy). **Results:** The mean age of group 1 and 2 were 9,47 ± 5,77 (0,82-18,1) and 6,19 ± 5,3 (0,42-17,5), respectively. The median levels of the parameters are shown in Table 1. Table 1:

Conclusion: Only the ESR was significantly different between the two groups. This is a 50-patient sampling study in patients underlying different disorders and/or infectious. Extended study with different cut offs is planned.

Table 1

A-295

Prevalence of Factor V Leiden and Prothrombin mutation assessed by data from a large clinical laboratory in Sao Paulo

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Background: Deep venous thrombosis (DVT) has multifactorial causes and 5-10% morbidity rate if not treated. Activated Protein C (mainly due to Factor V Leiden) and G20210A Prothrombin mutation are amongst genetic risk factors. Factor V Leiden (FVL) and G20210A Prothrombin mutation are due to punctual mutations that cause amino acid exchanges. Activated FVL looses Activated Protein C proteolytic site and becomes resistant to this enzyme's action. This mutation increases 3-8 times DVT risk in heterozygous patients, and 80 times in homozygous patients. G20120A Prothrombin mutation increases blood stream Prothrombin levels, causing hypercoagulability, yielding 2-3 times DVT risk. Therefore genetic thrombophilia diagnostic tests are important to reveal DVT cause and predict risk-benefit of anticoagulants treatment. The present study was performed to evaluate FVL and G20210A Prothrombin mutation prevalence amongst patients from a Sao Paulo large diagnostic laboratory. **Methods and Casuistic**: Peripheral blood samples collected into EDTA, using Biopur Extra Mini Spin Kit®. FVL and G20210A Prothrombin mutations were verified through Real Time PCR and allelic discrimination performed with TaqMan® probes in a 7500 RT (Applied Biosystems®) thermocycler. The data bank with FVL and G20210A Prothrombin mutation exam results was analyzed between 2006 and 2017. **Results:** From a total of 13286 patients, 10503 (79.05%) were female. The prevalence found for both mutations was similar to previous studies. FV Leiden prevalence was higher between male patients, as found in previous studies as showed in the table 1. **Conclusion**: It is important to diagnose FVL and G20210A Prothrombin mutation to evaluate anticoagulants treatment and use of birth control pills risk-benefits, as well to evaluate recurrent abortion risk in patients with 3 or more losses as a prenatal public health policy, since live births are significantly lower in women with recurrent early and late abortions who have FVL allele and in women who have Prothrombin mutation.

Table 1: FV Leiden and G20210A Prothrombin mutation prevalence.

FV Leiden	G20210A Prothrombin mutation			
	Wild Type	Heterozygous	Homozigous	Total
Wild Type	12033 (95.82%)	511 (4.07%)	14 (0.11%)	12558 (100%)
Heterozygous	675 (94.54%)	38 (5.32%)	1 (0.14%)	714 (100%)
Homozigous	13 (92.86%)	1 (7.14%)	0 (0%)	14 (100%)
Total	12721 (95.7%)	550 (4.1%)	15 (0.1%)	13286 (100%)

A-296

Sysmex BeyondCaresm Quality Monitor: Closing the Loop on Quality Control

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Background: Quality control for a clinical laboratory is designed to detect issues related to the analytical process with the aim to maximize the quality of the results. Most laboratory quality control systems use a Levy-Jennings chart, where data is easily visible and trends and other changes clearly identified. Sysmex uses this method of quality control for hematology and has now added an additional layer on top of the standard Levy-Jennings charts, introducing the BeyondCare℠ Quality Monitor (BCQM) quality control management program. The application takes the guesswork out of quality control, and has the potential to identify quality control failures with minimum false rejection, ensuring analyzer reliability while providing a consistent quality control process. **Methods:** BCQM uses multiple algorithms incorporating Six Sigma methodologies to interpret the quality control data from the analyzer. A dashboard provides at-a-glance analyzer status tabs with real-time quality control information, a benefit for laboratories with varying degrees of skill. An analyzer with a green status indicates that precision and accuracy have passed the necessary criteria. If the status is yellow, more information is needed before patient testing can occur. If laboratory involvement is needed, the application will provide guided troubleshooting prompts to help the technologist resolve any issues and notify Sysmex for service if necessary. A red status indicates there is a precision or accuracy issue and generates a service request followed by a phone call from Sysmex. **Results:** MultiCare recently participated in a pilot study of the BCQM on the Sysmex XN-9000. An analyzer at one of the MultiCare Health System hospitals had a mean corpuscular volume (MCV) low bias that resulted in a standard deviation index (SDI) of -2.3 based on the BCQM criteria. A SDI result greater than ± 2.0 requires follow-up by the technologist reviewing the quality control data. The difficulty is that the daily quality control data was acceptable and within the normal ± 2SD non-BCQM criteria, so no apparent issue seen. This issue would not have been found until a monthly quality control review, possibly weeks later. BCQM caught this issue immediately and had service dispatched. The analyzer was calibrated and values went back to the mean compared to other analyzers. This issue is important because middleware rules dictate that the MCV will not autofile into the patient's chart with an MCV difference of ± 3.0 from the previous sample. The strong negative bias from this analyzer would cause many samples that would normally autofile be held up in the middleware, requiring action from a technologist. This increases turnaround time for patients, including emergency room patients. This unnecessary delay impacts throughput of these patients, which is critical for a high volume area. **Conclusion:** BCQM closes the loop on the quality control process for hematology. The complex algorithms are married to a simplified process that elevates the degree of quality while encompassing ease of use. This superior quality control model is invalu-

able to laboratories because major issues can be found and corrected quickly before any impact is seen in patient results.

A-297

System performance evaluation of the cobas t 711 and cobas t 511 analysers

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Background: The fully automated **cobas t** 711 and **cobas t** 511 coagulation analysers perform qualitative and quantitative in-vitro coagulation analyses to aid the diagnosis of coagulation abnormalities and monitor anti-coagulation therapy. The objective of this validation study was to evaluate the functionality, reliability and analytical performance of the **cobas t** 711/511 analysers in a clinical laboratory setting under routine-like conditions at three European sites. **Methods:** Anonymised human citrated plasma samples were evaluated on the **cobas t** 711/511 analysers using a variety of coagulation tests. Functionality and analytical performance were monitored via daily quality control (QC) runs assessed twice daily. Intermediate precision was assessed over 21 days using control samples according to Clinical and Laboratory Standards Institute EP05-A3 guidelines. Routine Simulation Series (RSS) were used to confirm absence of random errors when running the analysers under routine-like conditions. Routine workloads of the laboratory were replicated and re-processed on the **cobas t** 711/511 analysers with the aim of verifying the performance by re-measuring all left-over specimens from the respective routine run. **Results:** All samples tested showed a stable QC performance over ~12 weeks. Of 4216 results, 19 were outside the ±2 SD (±10%) range due to reagent (or control material) related issues. Out of range results were repeated according to the QC re-run rules; all were resolved. No QC recovery issues due to reagent/instrument malfunction. All assays passed acceptance criteria for repeatability, intermediate and total precision of the respective assay (**Table**). For RSS, good comparability between runs was shown when testing single samples under random mode conditions (**Table**), no system malfunctions observed. **Conclusion:** The performance of the **cobas t** 711/511 analysers was excellent and the analysers are suitable for the accurate and reliable measurement of coagulation in routine clinical practice.

	aPTT	aPTT Lupus	aPTT Screen	D-Dimer[a]	Fibrinogen	PT-derived fibrinogen	PT Owren	PT	Thrombin time[a]
CV% day-to-day									
cobas t 711[b] / cobas t 511[c]	0.0-0.5 / 0.2-0.3	0.1-0.7 / 0.4-0.6	0.0-0.6 / 0.0-0.3	0.0-0.0; 0.0-0.0 / 0.6; 1	0.0-1.5 / 0.0-0.1	0.0 / 0.8	0.0-0.9 / 0.6-1.3	0.0-0.6 / 0.0-0.4	0.6-0.9; 0.0-1.2 / 0.9; 0.7
CV% within run									
cobas t 711[b] / cobas t 511[c]	0.2-0.4 / 0.3-0.4	0.3-0.6 / 0.4-0.5	0.3-0.9 / 0.6-1.0	1.4-1.5; 0.6-1.5 / 1.5-2.7; 1.0-2.0	1.2-3.0 / 1.7-2.6	1.5-2.5 / 2.1	0.4-1.3 / 0.7-0.9	0.3-1.1 / 0.3-0.5	1.0-2.0; 1.1-2.3 /1.1; 1.4
CV% total (within laboratory									
cobas t 711[b] / cobas t 511[c]	0.4-0.7 / 0.4-0.6	0.6-1.4 / 0.8-1.3	0.5-1.3 / 1.0-1.3	1.5-2.7; 1.0-2.0 / 2.4; 2	1.8-3.6 / 2.0-3.2	2.3-2.9 / 2.2	1.0-2.2 / 1.8-2.2	0.5-1.8 / 0.6-1.8	1.5-2.1; 1.9-2.5 / 1.6; 1.8
Correlation (Pearson's r)									
cobas t 711[b] / cobas t 511[c]	0.992-1.000 / 0.98	0.997-1.000 / 0.998	0.998-0.999 / 0.999	1 / 1	0.994-0.999 / -	0.995-0.999 / 0.998	0.999-1.000 / 1.000	1.000-1.000 / 1.000	0.967-0.991 / 0.952
Slope (Passing-Bablok)									
cobas t 711[b] / cobas t 511[c]	0.951-0.987 / 0.937	0.950-1.002 / 0.98	0.985-1.005 / 1	0.992-1.000 / 1.002	1.006-1.032 / -	0.987-1.000 / 0.993	1.000-1.004 / 1.000	0.991-1.000 / 0.988	0.968-1.023 / 1.040
Intercept									
cobas t 711[b] / cobas t 511[c]	0.132-0.337 / 0.444	-0.0431-1.21 / -0.052	-0.100-0.454 / 0	-0.00642-0.00799 / 0.00145	-12.1-0.0774 / -0.00145	-2.00-2.75 / 0.0987	-0.05000-0.0133 / 0.100	0.000-0.0313 / 0.0702	-0.476-0.525 / -1.18

[a]Results for control samples with the same acceptance criteria are pooled, results for control samples with difference acceptance criteria are presented separately (D-Dimer and thrombin time); [b]**cobas t** 711 analyser tested at three sites; [c]**cobas t** 511 analyser tested at one site.
aPTT, activated partial thromboplastin time; PT, prothrombin time

A-298

Discriminating Red Cells, White Cells and Platelets in Chordate Blood Samples using a Simple Detection System and a Single Analysis Cycle

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Background: We show that Red Cells (RBC), White Cells (WBC), and Platelets (PLT) can be discriminated in a single analysis cycle by an optical system consisting of only a 405 nm laser, a flow cell, and two detectors; one parallel to the flow cell front face collecting a 23 degree cone of light about incidence and another parallel to a side face collecting 70-110 degrees. No lenses are required for the detectors. The system works for all chordates, regardless of RBC size, shape or nucleation. Discrimination is based on absorption of 405 nm light by hemoglobin, present only in RBC, and WBC and PLT side scattering patterns. More than 95% of light scattered by a blood cell remains within a 23 degree cone. Therefore the front detector signal is dominated by absorption. Cells do scatter <5% of light outside 23 degrees, generating "pseudo-absorption" signals, so that WBC pseudo-absorption signals overlap RBC absorption signals if MCV<30 fL. Therefore a side scatter detector is also used, to distinguish between WBC and small RBC. For samples with MCV>30 fL, only the absorption detector is needed. PLT produce such small pseudo-absorption signals that they do not overlap even very small RBC signals. **Methods:** This method is a significant improvement over current flow cytometric methods that rely on light scattering, in terms of both simplicity and application range. The total scattering intensity relationships: WBC>RBC>PLT should provide discrimination. However, the cell concentration ratio in e.g. humans is typically 450 RBC/25 PLT/1 WBC. To count sufficient WBC in a diluted unlysed whole blood sample quickly enough to be commercially useful, RBC counting frequency would produce 3%-7% signal doublets. These would overlap WBC signals, and WBCs could not be counted. A separate analysis cycle involving RBC lysis is therefore required. Even this approach does not work for bird, fish, and reptile WBC because the lysed RBC release nuclei, generating multiplets that overlap WBC.Our method is not subject to RBC/WBC signal overlap; in fact, RBC doublets are better discriminated from WBCs than single cells, since more HGB is detected. Blood samples can be analyzed in a single cycle even at very high concentration;

e.g. 50:1 dilution, with cycle time <60 seconds. The method applies regardless of RBC shape and to nucleated-RBC species as well as to mammals, because absorption signals are only weakly sensitive to shape and all RBCs contain hemoglobin. **Results, Conclusion:** We have analyzed samples whose MCV range from 16 fL to 325 fL, including human, other mammalian, bird, and reptile samples, and shown discrimination for all samples. RBC- and WBC absorption channel signals are detected, while PLT signals are too small to be detected in this version of the system, even for bird and reptile samples which have large thrombocytes. PLT can be detected in an expanded dynamic range version.

A-299

Development of a liquid fibrinogen calibration verification set to verify the method's linearity and validate the analytical measurement range (AMR).

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Introduction: Fibrinogen, a 340kDa plasma glycoprotein, is a primary component of blood clots. Reduced levels may contribute to bleeding risk. Increased fibrinogen consumption in cases of disseminated intravascular coagulation (DIC) or decreased fibrinogen synthesis by liver disease, may result in decreased fibrinogen levels. Fibrinogen assays have been cleared by the U.S. FDA as non-waived laboratory tests, therefore, calibration verification is required under CLIA '88. Our objective was to develop a liquid-stable, human plasma fibrinogen test kit to meet calibration verification needs of clinical laboratories. The kits are for the Instrumentation Laboratory (IL) ACL TOP® 500, Siemens CS 2500 and the Stago Compact Max. **Methods:** VALIDATE® Fibrinogen, prepared in a human plasma matrix, was formulated according to CLSI EP06-A into five equal-delta concentrations to cover the manufacturer's analytical measuring range (AMR) for fibrinogen. Manufacturing and recovery targets were adjusted to meet the manufacturer's reportable range: IL (30 to 1000 mg/dL), Siemens (80 - 450 mg/dL, CCR 50 - 860 mg/dL) and Stago (150 - 900 mg/dL). The CLSI EP05-A3 guideline for Evaluation of Precision of Quantitative Measurement Procedures was followed. The three different calibration verification formulations were tested on their respective systems at two separate sites for reproducibility and precision assessments. Linearity was assessed, using samples tested in triplicate, per level. Linearity was evaluated using MSDRx® (LGC Maine Standards' proprietary linearity software). **Results:** For the IL ACL TOP® 500, reproducibility results for Levels 1 through 5 (n = 75/level) ranged from 6 to 8 total %CV. Precision results for Levels 1 through 5 (n = 240/level) ranged from 6 to 10 total %CV. For the Siemens CS 2500, reproducibility results for Levels 1 through 5 (n=75/level) ranged from 3 to 10 total %CV. Precision results for Levels 1 through 5 (n=240/level) ranged from 2 to 6 total %CV. For the Stago Compact Max, reproducibility results for Levels 1 through 5 (n = 75/level) ranged from 3 to 4 total %CV. Precision results for Levels 1 through 5 (n =240/level) ranged from 3 to 7 total %CV. All formulations demonstrate a linear response on their respective platforms and have excellent reportable range coverage. **Conclusion:** VALIDATE® Fibrinogen, as a liquid, five-level, ready-to-use test kit, is effective for calibration verification testing and reportable range method validation. Each formulation provides coverage of the respective manufacturer's claimed reportable range for the fibrinogen assay. The IL, Siemens and Stago formulations are currently available and stability studies are on-going. All formulations are listed with the FDA.

A-300

Multicenter Study of the High-volume Sysmex CS-5100 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Background: The objective of this study was to compare the performance of two automated coagulation analyzers, the high-volume Sysmex® CS-5100 System (CS-5100) and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Healthineers. Instrument performance for factor V Leiden (FVL), factor II deficiency (FII), factor VIII deficiency (FVIII), factor IX deficiency (FIX), factor X deficiency (FX), factor XI deficiency (FXI), factor XII deficiency (FXII), and lupus anticoagulant (LA screening [LA1], LA confirmation [LA2], and LA ratio [LAR]) were compared. **Methods:** A measurement comparison (MC) study was performed according to CLSI EP09-A3E (*Measurement Procedure Comparison and Bias Estimation Using Patient Samples*). Four clinical sites or internal measurements were included for testing de-identified leftover samples. The MC of the CS-5100 versus the CA-1500 was based on 165–495 results per parameter (total of 3136 results). A reproducibility study was performed according to the CSLI EP05-A3 (*Evaluation of Precision of Quantitative Measurement Procedures*) guideline in three laboratories. Between five and seven samples were measured covering medical decision points and the clinical reportable ranges for each test. The complete dataset contained 14,968 results. Additional performance data were determined for regulatory clearance. **Results:** Results correlated well between the CS-5100 and the CA-1500. The MC studies showed Passing-Bablok regression slopes ranging from 0.94 to 1.05 and Pearson correlation coefficients ≥0.966 (depending on the application). Reproducibility testing for the new device/test combinations showed low CV values. The mean reproducibility (total CV combined labs) of all samples and parameters was 3.7%, ranging from 0.9 to 7.3% (depending on application and sample). **Conclusion:** The CS-5100 compares well to the CA-1500 and offers the benefits of state-of-the-art functionality and ease of use in high-volume coagulation laboratories. *Product availability varies by country. Sysmex is a trademark of Sysmex Corporation.

A-301

Acute promyelocytic leukemia with atypical presentation and fatal outcome. Case description and literature review.

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Background: Acute promyelocytic leukemia (APL) represents about 25% of all acute myeloid leukemia in Latin America. There are two morphological variants, hypergranular (M3) and microgranular (M3v); the latter is rare and it is often confused with myelomonocytic leukemia (M4). Phenotypically more than 94% of the cases are positive for the CD117 antigen and above 95% and 75% are negative for HLA-DR and CD34 respectively. Just over 95% of the cases present translocation (15;17), which confers susceptibility to conventional pharmacological treatment, while about 3% of patients express the chimeric protein ZBTB16 / RARA, resulting from the t(11;17) and are resistant to conventional treatment. **Objective:** Description of a M3v-APL, CD117 negative / HLA-DR positive, t (11; 17) positive with fatal outcome. **Case description:** A 40 year old male with a persistent headache, ecchymosis in the right arm and infiltrative lesion on the palate, whose blood test results showed anemia, thrombocytopenia and leukocytosis (89,000 leukocytes per µL) along with 71% blasts of myelomonocytic appearance in the peripheral blood. The bone marrow cells showed similar characteristics to those found in peripheral blood. In order to classify the leukemia and establish the prognosis flow cytometry and molecular analysis were performed. The immunophenotype showed 96% of myeloid blasts and a treatment with all-trans retinoic acid (ATRA) was initiated. In a computerized tomography scan a hyperdense image in the right frontal region suggestive of leukemic infiltration was observed. It was not possible to perform a lumbar puncture. The patient suffered right parenchymal hematoma and uncal herniation, dying on the next day.

Method: Immunological phenotype based on the identification and quantification of cellular antigens by means of flow cytometry. Genetic abnormalities assessed by reverse transcription polymerase chain reaction (RT-PCR) for simultaneous detection of six myeloid leukemia translocations. **Results:** Blasts cells were negative for CD117 and CD34, with heterogeneous expression of CD36, CD64 and HLA-DR. RT-PCR revealed thet (11;17) confirming the diagnosis of APL. **Discussion:** Different reports describe atypical APL as M3v leukemia with an unusual antigenic expression pattern or a rare translocation. In a series of 20 cases with APL M3v all were CD117 positive and HLA-DR negative. In another series with 16 cases of M3v APL, 12 were positive for CD117 and only 1 for HLA-DR. The expression of the CD36 antigen has not been described to our knowledge in any APL report. The combination of CD36 and CD64 is used for the identification of leukemia with monocytic differentiation. Despite its low frequency, t (11; 17) has only been described in APL with habitual phenotypic expression. **Conclusion**: This case confirms that t (11; 17) can occur in an APL with morphological and phenotypic characteristics similar to myelomonocytic leukemia. Fatality was associated with the genetic alteration responsible for clonality and resistance to conventional treatment with ATRA.

A-302

Reliability of automated platelet counts in moderate to severe thrombocytopenia. comparison of optical with impedance methods

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BACKGROUND: Patients with thrombocytopenia (platelet<150x10^3/uL) require accurate and reliable platelet counts for effective clinical assessment and management. Current methodologies for automated platelet counting include impedance and optical light scatter. Limitations exist with the impedance method as microcytes and fragments maybe ascribed as platelets. **OBJECTIVE:** We aim to investigate the impact of different platelet counting methods in moderate and severe thrombocytopenia. **METHODS:** Full blood counts were performed on the Sysmex XN-9000 hematology analyzer, with our primary platelet count method being optical light scatter. We compared the platelet counts of 105 patients (64 male, 41 female) with moderate (50-100x10^3/uL) and severe (0-50x10^3/uL) thrombocytopenia by replicate analyses using the impedance and optical counting modes on the XN-9000. Statistical analyses were performed using MedCalc v16.0 (MedCalc Software, Ostende, Belgium). **RESULTS:** In subjects with normal platelet counts, there was close correlation between platelet methods with impedance giving higher values than optical (R=0.964). This discrepant platelet counts were more pronounced in moderate (R=0.831) and severe (R=0.905) thrombocytopenia. Optical platelet counts agree closely with the optical fluorescence platelet method (R=0.983). **CONCLUSION:** The optical platelet modality on the XN9000 showed good agreement with the fluorescent method. Platelet counts on the impedance mode shows a positive bias of 6.3 to 11.5% with respect to the optical mode. The platelet methodology employed in practice may impact optimal clinical management especially in states of thrombocytopenia.

A-303

Early urinary biomarkers of renal damage in sickle cell disease patients

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Background: Sickle cell disease is a genetic monogenic disorder with variable clinical manifestations and is characterized by the presence of hemoglobin S. Renal damage is common in patients with this disease which begins in childhood, progressing with age, a fact that makes nephropathy one of the possible complications. This can compromise patients' quality of life, and result in decreasing survival. Classical biomarkerssuch as creatinine, are not able to detect early renal lesions which makes kidney injury molecule-1 (Kim-1) and N-acetyl-β-D-glucosaminidase (NAG) possible biomarker candidates in the prediction of renal disease in sickle cell anemia patients. The aim of this study was determine KIM-1 and NAG urine levels in sickle cell anemia patients and in a control group. **Methods:** It was a cross-sectional study with a total of 56 individuals up to 17 years of age, 32 of whom had sickle cell disease and 24 healthy individuals. All were treated at the Laboratorio de Analises Clinicas da Faculdade de Farmácia da UFBA. The study was approved by the local ethical committee and blood samples were collected after signing informed consent forms. Hematological analyses were performed with automated protocols. KIM-1 and NAG urine levels were investigated by immunoenzymatic assay (ELISA) as per manufacturer protocols (USCN, USA). All data were analyzed in SPSS version 24, considering p <0.05. **Results:** The results showed that KIM-1 and NAG levels were significantly higher (p<0.0001 for both) in the patients group, compared with the control group (6.95 ± 7.13pg / mL vs 1.72 ± 1.87 pg / mL for KIM-1 and 0.43 ± 042ng / mL vs 0, 07 ± 0.11 ng / mL for NAG). **Conclusion:** KIM-1 and NAG levels were higher in the patients group, which suggests it may be incorporated in a follow up of sickle cell anemia patients in focus identify early renal damage.

A-304

Installation of Two TEG® Instruments in a Clinical Laboratory. Detection and Corrective Actions when Alpha Angle Level I Control Showed an Unnatural Behavior.

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Background. The thromboelastogram offers to the trauma surgeon both diagnostic information on blood clot formation deficiencies and it monitors the effect of therapeutic interactions. We report the detection of unnatural behavior of one quality control (QC) parameter, namely alpha angle, while installing two TEG® 5000 analyzers and the subsequent corrective actions. **Materials.** Two TEG® 5000 instruments (Haemonetics®), QC material (Level I, lot #1101-1201, HMO #3137; Level II, lot #1022-1202, HMO #3132, Haemonetics). The short-term precision was evaluated by assaying both levels of QC material with each instrument five times throughout the day for five consecutive days. This was performed by the manufacturer's representative (SS) and one of us (KB). The long-term precision was evaluated by assaying both levels of control material with both instruments for thirty days. The assays were performed by the technologists assigned to each of the three daily shifts. The data were transferred manually from the instruments printout to Minitab® (version 17 Minitab Inc.) statistical software. **Results.** We present the results for alpha angle control material. The short term precision showed for both levels of controls quasi-normal distribution (normal probability plots), independence (P>0.05), equality of standard deviations (Multiple comparisons P>0.05, Levene's P>0.05), and of daily means (ANOVA by day P>0.05, Instrument P>0.05). The long-term precision showed that while for angle level II the data showed a natural pattern as observed for the short-term precision study, for angle level I the data showed a negatively skewed distribution (skewness = -3.44), the histogram and the normal probability plot showed a shift to the left. The parallel box plot by technologist showed differences for mean performance and variability (length of the whiskers). ANOVA showed statistically significant differences (P<0.05) by technologist and multiple comparisons (P<0.05) and Levene's (P<0.05) tests with the Bonferroni's 95 % C.I. showed statistically significant differences between technologist's standard deviations. Upon obtaining new, calibrated pipettes (Pipet-Lite XLS®, Raining), an anti-vibrational table, and intense technologists training (performed by two members of the team (SS and KB) the unnatural distribution of the QC data was corrected as demonstrated by the quasi-normal distribution, independence, equality of the technologists means (ANOVA, P>0.05) and standard deviations by technologists (multiple comparisons (P<0.05) and Levene's (P<0.05) tests) for the data collected during 30 days of operation. **Conclusions.** The TEG technology requires good manual dexterity and a vibration free surface. The assistance of the manufacturer's representative (SS) was critical in training the younger technologists who are more adapted to hands-free methodologies. The availability of QC statistical software such as Minitab, was critical for analysis and interpretation of the data.

A-305

Acute Myeloid Leukemia with Erythrophagocytosis in Peripheral blood smear and marrow bone: case report

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Background: Patient 28 year-old woman was admitted to our Institute emergency service with the diagnosis of Acute Myeloid Leukemia (AML) with monocytic component, the patient presented loss of alertness, radiological suspicion of infiltration to the central nervous system and magnetic resonance with subarachnoid hemorrhage, at diagnosis with 43% of blasts in hemodiluted bone marrow aspirate. During admission she developed febrile peaks, a thorax tomography was performed in which a bilateral basal infiltrate was evident, suggestive of a multiple foci infectious process, a treatment with piperacillin-tazobactam and vancomycin was initiated. Since admission she presented headache, infectious complications with pulmonary Aspergillosis, herpetic

gingivostomatitis, transvaginal bleeding, sphenoidal and frontal sinusitis. At admission she had anemia, neutropenia and thrombocytopenia, she was on chemotherapy treatment and multiple transfusions of blood components. Since she was diagnosed with AML she had three relapses; June 2014, April 2016 and March 2017. She was in protocol for bone marrow transplantation, but due to the poor evolution and lack of a donor, transplant was not performed. Finally the patient died, her diagnostic end was hypoxemia refractory. **Results:** Hemostasis: Prothrombin time 11.4 s (normal) aPTT 22.4 s (low). Hemogram: Leukocytes $4.7x10^3$, Hemoglobin 7.2 g / dL, Platelets $4.0x10^9$ / L, 48% blasts with isolated images of erythrophagocytosis, presence of Auer rods and hypogranular neutrophils. Most recent bone marrow aspirate: 48% blasts (IF> 20% myeloid blasts). Immunophenotype: CD45dim/ CD34 positive / CD13 positive / CD117 positive / HLADR positive / CD33 positive, MPO positive, CD7 positive. Cytogenetic Study: 11 metaphases 45, X, -X, der(3) t(X;3) (q29;p11.2), t(8,21) (q22; q22). **Conclusion:** Erythrophagocytosis is a rare finding in acute leukemias described in less than 1% of cases. In these, erythrophagocytosis has been associated with some subtypes of AML, especially with the M4 and M5 subtypes (according to the FAB classification). These subtypes have been associated with alterations involving the C-MOZ gene, located in chromosome (8) (p11), the most frequent alteration being t (8; 16) (p11;p13); in this translocation the C-MOZ gene in (8)(p11) is rearranged with the CBP gene in 16p13 giving rise to the C-MOZ / CBP7 fusion gene. The second less frequent translocation is t (16; 21). The exact mechanism by which leukemic blasts phagocytose erythrocytes is not well known. It has been postulated that it could be related to the aberrant premature expression of complement receptors CR1 and CR3 and of the FcR receptors of IgG and gp150. Another hypothesis is that all these alterations could be stimulated by the coexistence of disseminated intravascular coagulation. This case is very interesting because it is the first case described in Mexico. Leaves context for being an AML with erytrophagocytosis t(8;21), that is not described in the clinical cases reported in the literature.

A-306

Comparison of Advia 120 CHr with the Abbott Alinity H MCHr as a potential analyte in patients with Chronic Kidney Disease

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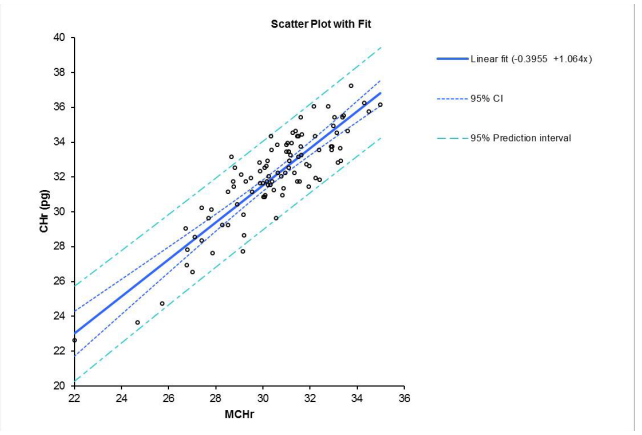
Functional Iron Deficiency (FID) exists in chronic kidney disease where there are adequate iron stores but a failure to incorporate the iron into the developing erythron. Mean cell haemoglobin (MCH) and mean cell volume (MCV) vary over a period of weeks and may not be useful in monitoring FID or its treatment. Reticulocyte haemoglobin (CHr or Ret-He) gives a direct estimate of the availability of functional iron over days, rather than weeks, and is useful for monitoring acute changes in renal patients receiving Erythropoiesis stimulating agents. A CHr value <29 pg predicts for iron response in patients with chronic kidney disease (CKD) receiving ESA therapy. In this study the CHr and MCHr were measured and compared on the ADVIA 120 (CHr), and the Abbott Alinity H (MCHr) respectively. The Alinity H analyser is a new high throughput analyser from Abbott. 99 whole blood EDTA samples with an MCH of ≤ 34pg in were chosen. Samples were aliquoted in two allowing simultaneous testing on both analysers. The results showed excellent linear correlation between the two datasets with a consistent positive bias of the CHr over the MCHr. These results fit the equation for Linear fit and the Altman Bland test gave a p value of <0.0001. Using the linear fit equation of MCHr = (Chr+0.3995)/1.064 a CHr of 29pg is equivalent to 27.6pg. Given this and clinical utility of the CHr, using an MCHr cut-off of 27.6pg may be used to predict iron responsiveness in patients with CKD in laboratories using the Alinity H analyser. References: NICE: Chronic kidney disease: managing anaemia. June 2015 Update. **National Institude for Health and Care Excellence,** NG8, 2015. THOMAS, D. W. et al. Guideline for the laboratory diagnosis of functional iron deficiency. **British Journal of Haematology,** 161, (5), p. 639-648, 2013.



A-307

IgD Multiple Myeloma: clinical features and prognosis

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Background: IgD Multiple Myeloma (MM) is a rare variant (2% of all MM) associated with a poor prognosis. The aim of our study is to describe the clinical and analytical characteristics of patients with MM IgD in our geographical area and to highlight the fundamental role played by the clinical laboratory in the study of these patients. **Methods**: Five patients diagnosed of IgD-MM between 2010 and 2015 have been studied. Data were analyzed from the medical record of the patients. **Results**: The main presenting features of the patients were: median age at disease presentation of 63 years, male predominance (80%), bone pain (80%), lytic bone lesions (100%), renal function impairment (100%), creatinine of 3,37 mg/dl, predominance of lambda light chain (80%), M-protein undetected by serum protein electrophoresis (40%), serum free light chains ratio abnormal at baseline (100%), hypercalcemia (40%), presence of plasmacytomas (20%), associated amyloidosis (20%) and aggressive clinical course (ISS-3, 80%). The median value of plasma cell in bone marrow was 26%. In the patient with IgD Kappa MM; the value of free kappa at diagnosis was 24769 mg/L with free lambda of 15.78 mg/L and a ratio of 1570. In IgD Lambda MM patients; the median value of free lambda was 2859 mg/l with median free kappa of 7.57 mg/L and a median ratio of 0.002. There were four disease-related deaths with a short median survival of 21 months.

Patient	**1**	**2**	**3**	**4**	**5**
Age (years)	83	50	63	54	77
Plasma Cells (%)	28	4	15	60	26
Serum Protein Electroforesis	Positive Large peak	Negative	Negative	Positive Small peak	Positive Small peak
Serum Immunofixation	IgD-L	IgD-K	Negative	IgD-L	IgD-L
Bence Jones Protein	No sample	Kappa	Negative	Lambda	Lambda
Free Kappa (mg/L)	11.20	24769	1.57	3.15	8.62
Free Lambda (mg/L)	1410	15.78	3290	4025	2427.5
Ratio K/L	0.008	1570	0.0005	0.001	0.0036
Beta-2-Microglobulin (mg/L)	23.06	21.82	3.0	6.2	18.7
Creatinine (mg/dL)	8.3	14	1.20	4.24	3.37
Diagnosis	IgD-L MM ISS 3	IgD-L MM ISS 3	IgD-L NSMM ISS 1	IgD-L MM ISS 3	IgD-L MM ISS 3
Survival (months)	1½ (Exitus)	21 (Exitus)	48 (Exitus)	10 (Exitus)	5 (Alive)

Conclusions: IgD MM presents clinical and laboratory findings that defines a distinct entity. In our population, IgD MM is characterized to have poor prognosis with a median survival of 21 months after diagnosis confirming previous studies. The recognition of IgD monoclonal component can be sometimes difficult to detect by SPE and the quantification of serum free light chains are essential for the diagnosis of these patients and improve the management of patients these patients.

A-308

Distinct Biomarkers Reflect Pathophysiological Differences of Sickle Cell Disease (SCD) Sub-phenotypes: Viscosity-Vaso-Occlusion vs. Hemolysis-Endothelial Dysfunction

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Background: Patients with SCD have variable phenotypes, with different severity of pain and other symptoms, including lung injury, stroke, leg ulcers, renal injury, osteonecrosis, and systemic pulmonary hypertension. Recently, two distinct sub-phenotypes have been defined: a) Patients with the Viscosity-Vaso-Occlusion sub-phenotype (VVO) suffer mainly from vaso-occlusive pain crises with a relatively high hemoglobin concentration and b) patients classified as the Hemolysis-Endothelial-Dysfunction sub-phenotype (HED) suffer from stroke and pulmonary hypertension with an elevated concentration of LDH. We aimed to explore the correlation of key biomarkers with the two sub-phenotypes of the disease, namely: Placental Growth Factor (PlGF) a member of the Vascular-Endothelial-Growth-Factor superfamily, which plays an important role in both inflammation and neo-angiogenesis; vWF:antigen, a multimeric plasma glycoprotein secreted by the endothelium; Growth-Differentiation-Factor-15 (GDF-15), a member of the TGF-β superfamily, which expression is strongly upregulated in response to oxidative stress, inflammation, tissue injury and in conditions related to ineffective erythropoiesis. We tested these biomarkers in patients with compound heterozygous SCD and beta-thalassemia (HbS/βthal). **-Methods**: Ninety adult Caucasian patients with HbS/βthal were included in the study, while 20-apparently healthy individuals, served as controls. Patients with HbS/βthal were divided in two groups according to their LDH levels: High-LDH (LDH>270U/L) (HED-phenotype) group (42-patients) and the Normal-LDH (LDH<270U/L) (VVO-phenotype) group (48-patients). Along with hematologic and blood chemistry parameters determination, measurements of circulating levels of PlGF, vWF:antigen, GDF-15, hs-CRP, Cystatin C, hs-TnT and D-Dimers were performed in both groups of patients and controls. **Results**: We found that patients with the HED-phenotype compared to the VVO-phenotype of the disease had lower Hb levels ($p<0.001$), higher Reticulocyte-Production-Index and higher bilirubin ($p<0.001$ and $p=0.004$, respectively), while there were no differences regarding HbF levels between the two groups. PlGF levels were significantly elevated only in patients with the HED-phenotype (22.6±7.1pg/mL) compared to the controls (15.2±2.4pg/mL) ($p<0.001$) and patients with the VVO-phenotype (18.2±7.6pg/mL) ($p=0.005$). vWF:antigen concentrations were markedly elevated in both groups of patients compared to controls (186.4±81.7 and 157.8±73.4 vs 85.3±22.1IU/dL, $p<0.001$), with the increase of vWF:antigen levels to be more pronounced in patients with the HED-phenotype ($p=0.008$). Similarly, GDF-15 levels were also markedly elevated in both groups of patients compared to controls (2,346.2±1295.6 and 1,693.5±1398.3 vs 665.4±221.9pg/mL; $p<0.001$), with the increase of GDF-15 levels to be more pronounced in patients with the HED-phenotype ($p=0.006$). We found also significant higher levels of D-Dimers in patients with the HED-phenotype ($p<0.001$) compared to patients with the VVO-phenotype, while no differences were found in parameters of inflammation and renal function. **Conclusions**: These findings demonstrate for the first time the correlation and involvement of PlGF, vWF:antigen and GDF-15 in the pathophysiological mechanisms of the HED sub-phenotype in patients with the HbS/βthal. Although, there is a degree of overlapping between the two sub-phenotypes of SCD, the differences in the specific biomarkers were significant. Thus, these markers along with the clinical profile could better identify the two subtypes of SCD patients and drive an innovative approach with the use of direct personalized therapies for each specific sub-phenotype by targeting the predominant mechanism in this multifactorial disorder.

A-309

Simultaneous Validation and Verification of Multiple Instrumentation Laboratory ACL TOP Coagulation Analyzers in a Clinical Core Laboratory

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Background: Due to a Mayo Clinic Clinical Laboratory coagulation testing standardization process, multiple Instrumentation Laboratory (IL) ACL TOP Family 50 Series LED optical systems were chosen to replace greater than 10 years-old electromagnetic mechanical end-point clot detection system analyzers (Stago) in the core laboratory. The performance of ACL TOP 750 and 550 analyzers was compared to existing Stago analyzers during validation of prothrombin time (PT), International Normalized Ratio (INR), activated partial thromboplastin time (APTT), thrombin time (TT), D-Dimer, fibrinogen (Fib) and heparin anti-Xa (anti-Xa) testing. Here, we describe the processes used to verify the IL ACL TOP manufacturer performance characteristics. **Methods:** A comprehensive validation was performed to verify manufacturer performance characteristics of the ACL TOP Family 50 Series instruments for PT, INR, activated APTT, TT, D-Dimer, Fib, and anti-Xa testing. The focus of the validation was to verify the accuracy (ACL TOP versus Stago method comparison and ACL TOP versus ACL TOP instrument comparison), precision, and reportable range of these tests. A minimum of 30 samples were used for each test method comparison. Slope (0.90-1.10), correlation coefficient (>0.90), and percent and absolute differences (manufacturer stated) were used as criteria for acceptability. Precision testing was performed by testing each control five times within a run for five days, and percent coefficient of variation was calculated (acceptable ranges were <5% CV for PT and TT, and <10% CV for APTT, D-Dimer, Fib, anti-Xa). The IL stated reportable range for each test was verified. Linearity testing was performed for Fib, D-Dimer, and anti-Xa assays. Slope (0.90-1.10), R^2 (>0.95) and percent recovery (90-110%) were used as acceptance criteria for linearity testing. Results were organized by instrument and test and entered into spreadsheets in real time to allow for faster data evaluation and troubleshooting. **Results:** Other than TT and APTT, method comparison results for all R^2 values were within limits. Anti-Xa, APTT, and INR slope results were slightly outside acceptable limits, but the remainder of the results were within acceptable limits. With the exception of TT, the average percent difference results were within acceptable limits. TT testing showed larger differences (IL ACL TOP versus Stago TT tests) due to the different sensitivities of the reagents in each system to heparin and fibrinogen abnormalities. Observed differences in slope and R^2 for APTT testing were also likely due to the different methodologies in the two systems. ACL TOP to ACL TOP instrument comparisons showed good correlation, with all results within acceptance limits. Precision results were all acceptable. Linearity results for fibrinogen and anti-Xa were within acceptable regression and recovery limits. D-dimer results were slightly outside of recovery limits for two samples, but these differences were not clinically significant. **Conclusions:** Although verification of new analyzers is a common process, unique challenges were encountered during the validation process of multiple ACL TOP Family 50 Series analyzers undertaken to replace current outdated coagulation analyzers. However, our clinical laboratory was able to overcome these obstacles through identification of organizational steps that helped to streamline the validation processes and the transition to the new coagulation analyzers.

A-310

Screening for Light Chain Glycosylation: A Potential Route to Earlier Diagnosis of AL Amyloidosis

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Background:
Immunoglobulin light chain (AL) amyloidosis is a rare plasma cell disorder affecting ~10 people/million people/year. AL amyloidosis is characterized by abnormal secretion of immunoglobulin light chains (LCs) from clonal bone marrow plasma cells. Secreted clonal LC misfolds into aggregates, forming fibrils in organs like kidney, heart, liver, etc. Serum free light chain (FLC) ratios are the most sensitive method to detect LCs in AL patients, but given that 45 percent of patients with monoclonal gammopathy of unknown significance (MGUS) also have abnormal FLC ratios, abnormal FLC ratios have low specificity for AL. Hence, there is a critical need for a serum-based diagnostic assay that can stratify AL risk with high specificity, which enables accurate and earlier diagnosis of AL with potential to improve patient outcomes.
Methods:

Serum samples were collected from 189 AL patients (128 AL-lambda, 61 AL-kappa) and 122 non-AL patients (distributed among 54 multiple myeloma, 57 MGUS, 8 Waldenstrom's macroglobulinemia and 3 other plasma cell disorders, with a 2:1 ratio of kappa:lambda M-proteins). Serum LC was purified using nanobody-enrichment and analyzed by MALDI-TOF-MS[1]. LC mass spectra were visually analyzed for abnormally broad complex patterns that are generated by N-glycosylation. Kappa and lambda LCs with potential N-glycosylation were deglycoslylated with PNGase F and N-glycosylation was confirmed by looking for a shift in LC mass (due to deglycosylation) via MALDI-TOF-MS and LC-ESI-Orbitrap MS.
Results:
33% of AL-kappa patients had a suspected LC glycosylation pattern when compared to only 10.2% of AL-lambda patients. The rate of LC glycosylation among non-AL patients was only 4.1%; rate among kappa non-AL patients was 3.7% and among the lambda non-AL patients was 4.9%. In order to confirm that the broad peak pattern observed for native LCs is due to N-glycosylation, 21 kappa M-protein (18 AL and 3 non-AL) and 9 lambda M-Protein (7 AL and 2 non-AL) LCs were deglycosylated with PNGase F and LC mass spectrum were re-examined by MALDI-TOF-MS and LC-ESI-Orbitrap. In all samples, the abnormally broad complex peak patterns observed in native LC spectra were shifted into a monoclonal LC peak, confirming the presence of N-glycosylation in native LCs. The odds of glycosylated kappa LC patient being AL was 12.68 ($p<0.0001$), glycosylated lambda LC patient being AL was 2.20 ($p=0.31$), and LC glycosylation being AL was 4.95 ($p=0.0012$).
Conclusion:
This work confirms the over representation of LC glycosylation in AL patients when compared to other plasma cell disorders. The frequency of kappa LC glycosylation was 5 times higher than lambda in AL patients. The odds of a glycosylated LC being AL was very high and clinically actionable. In addition, the MALDI-TOF-MS method used to detect LC glycosylation is currently being validated for clinical use; allowing for rapid assessment of AL risk in MGUS patients. This potentially could lead to earlier clinical suspicion of AL in MGUS patients with glycosylated LCs.
Reference:
1. Milani P, Murray DL, Barnidge DR et. al. The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic. Am J Hematol. 2017: 92: 772-779.

A-311

Precision and method comparison data for three new coagulation assays measuring activated partial thromboplastin time (aPTT) on the new cobas t 711 and t 511 analysers

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Background: Assessment of aPTT is a good indicator of intrinsic coagulation pathway activity and is widely used to diagnose coagulopathies and monitor anticoagulant therapy. The objective of this multicentre study was to evaluate the precision of three coagulation assays (aPTT, aPTT Lupus and aPTT Screen) with different sensitivities to heparin, lupus anticoagulant and factor deficiencies, and compare their performance against commercially-available assays/platforms.
Methods: Anonymised human 3.2% sodium citrate plasma samples were used (commercially sourced or residual clinical). For each assay, within-run precision was evaluated in a single run comprising three controls and five pool plasma samples covering the relevant measuring ranges (21 replicates per sample); reproducibility was evaluated over five days. Method comparisons were performed on the **cobas t** 711 versus reference reagents on the Siemens Sysmex CS or Stago STA-R Evolution systems (≥120 samples per assay, per site); Pearson's r correlation coefficients were estimated. Results were compared against prespecified acceptance criteria.
Results: Across all three assays, coefficients of variance (CV) for within-run precision and total reproducibility were within the acceptance range **(Table)**. CVs for within-run precision were 0.2-1.5% (**cobas t** 711) and 0.2-1.8% (**cobas t** 511); CVs for total reproducibility were 0.4-3.4% (**cobas t** 711) and 0.3-6.3% (**cobas t** 511). Method comparison experiments for all assays (**cobas t** 711) demonstrated excellent correlation versus their respective reference methods, with Pearson's correlation coefficients within the acceptance range **(Table)**.
Conclusion: Each aPTT assay showed good within-run precision, reproducibility, and excellent correlation with commercially-available assays/platforms, thereby demonstrating their suitability for use in core laboratories.

Within-run precision, reproducibility, and method comparison of the aPTT, aPTT Lupus and aPTT Screen

Assay	Within-run precision* (range of % CV)		Total reproducibility† (range of % CV)		Method comparison (cobas t 711)				
	cobas t 711‡	cobas t 511§	cobas t 711‡	cobas t 511§	Reagent	Instrument	n	Acceptance criteria	Pearson's r
aPTT	0.2-1.5	0.2-0.8	0.4-2.9	0.4-3.8	Actin FS	Siemens Sysmex CS	594	≥0.85	0.980-0.986‡
					STA Cephascreen	Stago STA-R Evolution	175	NA	0.819¶
aPTT Lupus aPTT Screen	0.3-1.2	0.2-1.4	0.4-2.9	0.3-2.2	Actin FSL	Siemens Sysmex CS	620	≥0.85	0.967-0.987‡
					STA Cephascreen	Stago STA-R Evolution	99	NA	0.943¶
					STA-LA	Stago STA-R Evolution	128	NA	0.9575¶
aPTT Screen	0.3-1.2	0.2-1.0	0.8-3.1	0.8-6.3	Pathromtin SL	Siemens Sysmex CS	579	≥0.85	0.964-0.985‡
					aPTT	Stago STA-R Evolution	153	NA	0.833¶

*acceptance criteria CV ≤4.0%; †acceptance criteria CV ≤25.0%; ‡range across four sites; §range across two sites; ¶performed at one site only
aPTT, activated partial thromboplastin time; CV, coefficient of variation; NA, not applicable

A-312

Method comparison of the PT Rec coagulation assay with existing reference methodology for measuring prothrombin time (PT)

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Background: Coagulation screening tests are used to reliably and quickly detect inherited or acquired factor deficiencies and monitor patients receiving anticoagulant therapies. New commercially available assays may offer improvements over current assays; however, it is important that new assays are evaluated against existing assays prior to their adoption in the healthcare setting. The PT Rec assay contains thromboplastin (recombinant human thromboplastin reagent containing a heparin-neutralizing substance) and calcium, which initiates activation of the extrinsic coagulation cascade when added to citrated human plasma. The objective of this study was to compare the new PT Rec assay (measure of prothrombin time) with existing reference methodology.
Methods:
Method comparisons, according to Clinical and Laboratory Standards Institute EP09-A3 (CLSI EP09-A3) guidelines, were performed across four sites for the PT Rec assay (**cobas t** 711 analyser) versus Innovin (Siemens Sysmex CS-5100 or CS-2000i). A minimum of 120 residual anonymised human plasma samples were used per assay, per site to represent the appropriate measuring range. Equivalency of the **cobas t** 711 and **cobas t** 511 platforms was also evaluated by method comparison.
Results:
According to prespecified criteria based on Deming regression analyses, method comparison experiments showed good agreement between PT Rec and the reference method (**Table 1**). Equivalency was also demonstrated between the **cobas t** 711 and **cobas t** 511 analysers, according to prespecified acceptance criteria, based on Passing-Bablok regression analyses (**Table 1**).
Conclusion:
Based on good agreement between PT Rec and the commercially available reference, and equivalency observed between the **cobas t** 711 and **cobas t** 511 analysers, the PT Rec assay has demonstrated suitability for use in core laboratories.

Table 1. Summary of method comparison data.

Comparison	Evaluation	Acceptance criteria	Freiburg	Sheffield	Debrecen
			Lot 1	Lot 2	Lot 3
PT Rec versus Innovin[a]	n	-	131	135	130
	Slope (Deming)	1.00 ± 0.10	1.008	1.036	0.9
	Intercept	NA	0.090	0.045	0.238
	Pearson's r	≥0.900	0.9985	0.9966	0.9883
	Bias at 1.0 INR	≤0.15	0.097	0.081	0.138
PT Rec[b]	n	-	129	135	-
	Slope (Passing-Bablok)	1.00 ± 0.10	1.006	0.984	-
	Intercept	NA	0.006	0.033	-
	Pearson's r	≥0.900	0.9999	0.9996	-

[a]Analyses based on INR, method comparison between **cobas t** 711 and reference method; [b]Analyses based on INR, method comparison between **cobas t** 711 and **cobas t** 511 analysers; INR, International Normalised Ratio; NA, not applicable; PT, prothrombin time

A-313

Sensitivity of the aPTT, aPTT Lupus and aPTT Screen assays towards heparin and lupus anticoagulant

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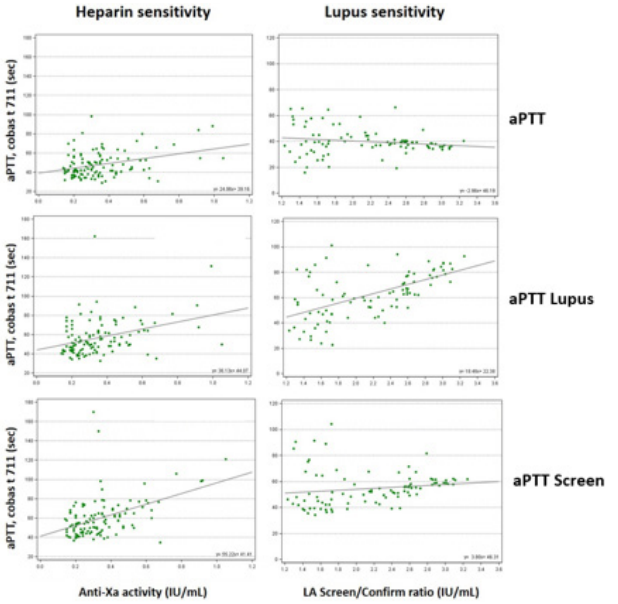
Background: Activated partial thromboplastin time (aPTT) tests are used to monitor anticoagulation therapy and for the assessment of coagulopathies. To assist accurate diagnosis, a range of aPTT assays that give different responses to anticoagulants (heparin) and have varied lupus anticoagulant sensitivity, is required. The objective of this study was to evaluate the sensitivity of three new aPTT assays (aPTT, aPTT Lupus and aPTT Screen) towards unfractionated heparin (UFH) and lupus anticoagulant. The aPTT assay has reduced lupus antibody sensitivity, whilst the aPTT Lupus assay has increased lupus antibody sensitivity. **Methods:** For heparin sensitivity experiments, platelet-poor plasma samples from patients receiving UFH (n=117) were analysed. For lupus sensitivity experiments, lupus anticoagulant-positive commercially available plasma samples (n=96) were analysed. Therapeutic/reference ranges were determined (**cobas t** 711) or data from the package insert were used (Siemens BCS XP); prolongation of clotting times measured using aPTT, aPTT Lupus and aPTT Screen (**cobas t** 711) were then compared with the reference assay (reagents used: Actin FS, Actin FSL, Pathromtin SL). **Results:** Sensitivity to UFH (aPTT Screen > aPTT Lupus > aPTT) and lupus anticoagulant (aPTT Lupus > aPTT Screen > aPTT) varied between assays (**Figure**). Using aPTT Lupus or aPTT Screen, ≥80% of samples from UFH-treated patients within the therapeutic range (anti-Xa activity, 0.3-0.7 IU/mL) resulted in a measurable clotting time. For each assay, prolongation of clotting times in the presence of UFH and lupus anticoagulant were within 0.75-1.25 times that of the reference assay; clotting times for aPTT, aPTT Lupus and aPTT Screen were 0.97, 0.95 and 0.94 with UFH, respectively, and 1.00, 1.04 and 0.96 with lupus anticoagulant, respectively. **Conclusion:** aPTT, aPTT Lupus and aPTT Screen met the pre-specified acceptance criteria for sensitivity to UFH and lupus anticoagulant.



A-314

Derivation of a new reference interval for Reticulocytes counted on an automated platform using the Sysmex XN-9000

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BACKGROUND: The reticulocyte count in peripheral blood reflects bone marrow erythropoiesis and is used in the differential diagnosis of anemia and monitoring bone marrow response to therapy. The current reticulocyte reference intervals for a normal adult population employed in most books and laboratories are 0.2-2.0%. This reference range is historically based on a manual reticulocyte count derived from small samples (usually <100) of "normal" subjects.Manual quantitation of reticulocytes involve staining the ribonucleic acid (RNA) content with supravital stains and counting the number of cells that appear polychromatic or have basophilic precipitate of granules or filaments. **OBJECTIVE:** With a contemporary and more specific automated method of reticulocyte counting, we aim to derive an updated reticulocyte reference interval. **METHODS:** Our current hematology analyzer (Sysmex XN-9000) performs the reticulocyte count using fluorescence flow cytometry. Apart from estimating the reticulocyte count, it also provides information on reticulocyte-hemoglobin and minimizes interference from leucocytes, erythrocyte inclusions and parasites. Reticulocyte counts were performed on the Sysmex from ambulatory subjects with normal hemoglobin (male: 13.0-17.0g/dL; female: 11.5-15.0g/dL) and MCV (76-96fL). Children and pregnant women were excluded.After excluding 31 outliers (Tukey), the distribution of the reticulocyte data was tested for normality (Kolmogorov-Smirnov). Statistical analyses were performed using MedCalc v16.0 (MedCalc Software, Ostende, Belgium). **RESULTS:** The distribution of the reticulocyte counts from our reference population (n=3940, male=2096, female=1844) was non-Gaussian. The derived reticulocyte reference interval (2.5-97.5 percentile) for adults was 0.7% to 2.7%. Gender comparison, tested by non-parametric means (Mann-Whitney U test), showed no significant difference. **CONCLUSION:** Our findings suggest that our reticulocyte reference interval upwards needs to be revised upwards.

A-315

Automated Determination of Leukocyte Cell Population Data in Detecting Neonatal Sepsis

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Background: Neonatal sepsis (NS) is one of the leading causes of morbidity and mortality despite advances in neonatology. Diagnosis which is made by clinical and laboratory findings is challenging because the signs and symptoms are highly variable. Blood culture, considered as a gold standard for diagnosis, has the disadvantages of a low positivity rate and the time delay of 48 to 72 h. Acute-phase reactants such as C-reactive protein (CRP) and procalcitonin (PCT) along with hematological parameters strengthen the diagnosis of NS. Unfortunately none of these tests have been particularly useful in identifying many of the cases. The objective of the study was to evaluate the value of volume conductivity scatter (VCS) parameters of leukocytes in detecting NS and to estimate their optimal cutoff levels using receiver operating characteristic (ROC) curves.**Methods:** The study was conducted at Marmara University Pendik E&R Hospital. A total of 66 babies from neonatal intensive care unit were included, of whom 19 had proven sepsis with blood culture positivity and 22 had clinical sepsis (clinical course consistent with sepsis, culture-negative) with 25 control cases. Complete blood count (CBC) and cell population data including VCS parameters were retrospectively analyzed. VCS parameters had been analyzed by UniCel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). CRP levels and PCT had been determined nephelometrically (Dade Behring BN II, Siemens Laboratory Diagnostics, Germany) and by electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics, Germany), respectively. Statistical analysis was performed using the SPSS software, version 17.0. Results were expressed as the mean±standard deviation. All parameters were compared using analysis of variance (ANOVA). P value<0.05 was considered statistically significant. Cut-off values were established based on ROC curves.**Results:** While CRP and PCT values were not significantly different among all groups, mean neutrophil volume, lymphocyte conductivity SD, mean monocyte volume, and mean monocyte volume SD differentiated sepsis and control cases (P= 0.009; P=0.020; P=0.017, P<0.001, respectively), but these parameters were not different between sepsis and clinical sepsis groups. Lymphocyte median-angle light scatter and upper median-angle light scatter of neutrophils and monocytes were significantly decreased in the clinical sepsis group when compared to the controls (P=0.036; P=0.017; P=0.023, respectively), but these parameters were not different between sepsis vs. control and sepsis vs. clinical sepsis groups (P>0.05). However, upper median-angle light scatter of lymphocytes (MN-UMALS-LY) was significantly decreased in the clinical sepsis group compared to both control and sepsis group. ROC curve analysis revealed 73.2% sensitivity and 81.2% specificity at 56.5 arbitrary units for MN-UMALS-LY in the discrimination of clinical sepsis.**Conclusion:** Early diagnosis in NS may not only prevent delay in treatment and improve the outcome of the infants, but also avoid unnecessary and prolonged usage of antibiotics. This study shows that VCS parameters may provide significant value for the detection of NS, especially in clinical sepsis. When validated by further studies, incorporation of VCS parameters during initial CBC count analysis could be used to provide a timely and convenient sepsis diagnostic tool and lead to early identification of NS.

A-316

Method comparison of cobas m 511 Integrated Hematology Analyzer and Sysmex® XN-10 Automated Hematology Analyzer using samples with targeted medical conditions

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Background: The **cobas m** 511 integrated hematology analyzer (**cobas m** 511 system) is a novel slide-based system that performs a complete blood count, white blood cell differential, reticulocyte count, and nucleated red blood cell count using digital analysis. This single-center study investigated whether the **cobas m** 511 system delivered comparable results to the Sysmex® XN-10 Automated Hematology Analyzer using samples from patients with medical conditions. **Methods:** Laboratory hematology results were reviewed to identify subjects with 23 targeted medical conditions (including hematological malignancies and disorders of cell numbers and function). Residual whole blood samples (n=130) were processed on both systems within 8 hours of venipuncture. Consistent with CLSI EP09-A3, a method comparison was used to assess the correlation and bias between the systems for all parameters. Individual patient parameter results that were valid on both instruments were included. **Results:** All 26 reportable parameters evaluated showed good-to-excellent correlation between the automated results of the **cobas m** 511 system and Sysmex Analyzer, with no significant bias (Table 1; *Data for MCHC, RDW, RDW-SD, %NRBC, #NRBC, %NEUT, %LYMPH, %MONO, %EO, %BASO, %RET, and HGB-RET not shown*). **Conclusions:** The **cobas m** 511 system and Sysmex Analyzer produce comparable results for samples with targeted medical conditions. This demonstrates the robustness of the **cobas m** 511 system when abnormal samples are encountered.

Table 1: cobas m 511 vs. Sysmex Analyzer results

Parameter [units]	Sample range	Pearson's *R*	Intercept	Slope
WBC [10^3/µL]	(0.11–95.41)	0.999	-0.02	0.995
RBC [10^6/µL]	(1.79–7.68)	0.996	-0.01	0.992
HGB [g/dL]	(6.28–17.24)	0.995	-0.31	1.064
HCT [%]	(18.60–56.00)	0.982	-0.53	1.034
MCV [fL]	(69.50–107.80)	0.879	5.35	0.975
MCH [pg]	(20.22–36.48)	0.977	2.87	0.946
PLT [10^3/µL]	(9.00–1379.00)	0.994	-2.02	0.943
MPV [fL]	(8.40–13.00)	0.843	0.82	0.915
#NEUT [10^3/µL]	(0.46–36.82)	0.999	0.03	1.008
#LYMPH [10^3/µL]	(0.16–4.89)	0.985	0.01	0.976
#MONO [10^3/µL]	(0.14–7.65)	0.991	-0.03	1.013
#EO [10^3/µL]	(0.00–1.23)	0.978	0.00	1.031
#BASO [10^3/µL]	(0.00–2.51)	0.962	-0.08	1.829
#RET [10^6/µL]	(0.00–0.34)	0.971	-0.01	0.982

A-317

Accuracy evaluation of low platelet counts on the cobas m 511 Integrated Hematology Analyzer

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Background: The **cobas m** 511 integrated hematology analyzer is a novel slide-based system that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using digital analysis of a microscope slide. This single-center study assessed the accuracy of low platelet (PLT) counts on the **cobas m** 511 compared with the Sysmex® XN-10 Automated Hematology Analyzer and BD FACSCanto™ Flow Cytometer. **Methods:** Residual whole blood samples (n=115) in the PLT range 1-505 x10^3/µL (based on flow cytometer results using CD61-FITC, CD41a-PerCP-Cy5.5, and CD42b-APC antibodies) were randomly processed on the three systems. Sysmex samples were set to automatically reflex to PLT-F function, which is marketed as more reliable for thrombocytic or problematic samples than the standard impedance PLT-I function. Three analyses were performed: **cobas m** 511 and Sysmex Analyzer versus flow cytometer (reference), and **cobas m** 511 versus Sysmex Analyzer (reference). Data were compared using three different thresholds and results included if a valid PLT result was obtained on both systems. **Results:** The **cobas m** 511 demonstrated excellent accuracy compared with flow cytometry, with ≥93.6% sensitivity, specificity, and agreement, and good accuracy compared with Sysmex Analyzer (Table 1). Attempts were made to also study flags for the Sysmex Analyzer PLT-I function however excessive flagging (100% for PLT counts ≤10, 80% for PLT 11-20, and 28% for PLT 21-50 x10^3/µL) indicated these values were unreliable and prevented any further comparison. **Conclusions:** These data demonstrate the robustness and accuracy of platelet counts reported by the **cobas m** 511 system. Many samples were flagged using the Sysmex PLT-I function that, in routine use, would have reflexed to the PLT-F function. All samples on the **cobas m** 511 system had valid PLT results.

Table 1: Accuracy analyses				
	PLT threshold (10^3/μL)	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**
cobas m 511 vs. flow cytometer (N = 115)	10	95.5	96.8	96.5
	20	93.6	94.1	93.9
	50	98.6	97.6	98.3
Sysmex Analyzer (PLT-F) vs. flow cytometer (N = 113)	10	100.0	96.7	97.3
	20	100.0	92.5	95.6
	50	100.0	97.6	99.1
cobas m 511 vs. Sysmex Analyzer (PLT-F) (N = 113)	10	88.0	97.7	95.6
	20	94.1	100.0	97.3
	50	100.0	100.0	100.0

A-318

The research of pak1 function in different BCR-ABL subtype in leukemiagenesis and treatment through STAT5 pathway

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Background: BCR-ABL fusion gene has different subtypes such as P210, P190 and is the main aetiological agent for Chronic myelogenous leukeamia (CML) and can also be found in Acute Lymphocytic Leukemia (ALL). Although p21-activated kinase (PAK1) gene is proved to paly an important role in leukemia caused by different BCR-ABL susptype by NimbleGen expression profile microarray analysis and verified by RT-PCR, the mechanism remain unclear. Our research aims to clarify the difference in regulation of PAK1-STAT5 biological axis between different BCR-ABL subtype and its influence for proliferation and apoptosis in leukemia cell and to discuss if there is a synergy or antagonism effect between PAK1 expression and TKI treatment in different BCR-ABL subtype in hope of finding new biomarker and therapeutic targets. **Methods:** PAK1 interference and overexpression lentivirus was constructed and transfered into P210(+) positive CML cellline K562 and P190(+) positive ALL cell line SUP-B15. Western-blot was used to analysis phosphorylation of STAT5,MAPK in cell line treated with pak1 inference/overexpression lentivirus and TKI. BrdU was used to detect cell proliferation and MTT was used to detect cell apoptosis. Flow cytometry was used to detect cell appotosis and proliferation for cell lines treated with PAK1 interference /overexpression lentivirus and TKI. **Results:** WB testing indicated that in K562 cell line, after PAK1 interference, phosphorylation level in STAT5,ERK, JUK have decreased and cells treated with both PAK1 interference and TKI have the lowest phosphorylation level. In SUP-B15 cell line, after PAK1 overexpression, phosphorylation level in STAT5,ERK, JUK have decreased and cells treated with both PAK1 overexpression and TKI have the lowest phosphorylation level. BrdU and MTT test showed that, in K562 cell line, Cell proliferation rate decreased in 24, 48, and 72h after PAK1 interference, and cells treated with both PAK1 interference and TKI have the lowest proliferation rate. In SUP-B15 cell line, Cell proliferation rate decreased in 24, 48, and 72h after PAK1 overexpression, and cells treated with both PAK1 overexpression and TKI have the lowest proliferation rate. Flow cytometry test showed that, in K562 cell line, Cell apoptotsis rate increased and Cell cycle proliferation index(PI) decreased after PAK1 interference, and cells treated with both PAK1 interference and TKI have the highest Cell apoptotsis rate and lowest PI. In SUP-B15 cell line, Cell apoptotsis rate increased and Cell PI decreased after PAK1 overexpression, and cells treated with both PAK1 overexpression and TKI have the highest Cell apoptotsis rate and lowest PI. **Conclusion:** PAK1 is an important differential expression gene between different BCR-ABL subtype. In P210(+) CML, down-regulated PAK1 gene expressions may lead to suppression in cell proliferation and promotion in apoptosis through phosphorylation of STAT5, with a reverse effect in P190(+)ALL, which showed PAK1 might be an an important molecular mechanism of pathogenic difference between different BCR-ABL subtype.In P210 (+) CML, down-regulated PAK1 expression may enhance the effect of TKI, while the reverse in P190(+)ALL, which showed PAK1 might be an an important molecular mechanism of prognosis difference between different BCR-ABL subtype.

A-319

Evaluation of Volume, Conductivity and Scatter Properties of Leukocytes (VCS Technology) as Screening for Infection in Patients Undergoing Therapeutic Hypothermia after Cardiac Arrest

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Background: Induced hypothermia is increasingly applied in patients with cardiac arrest as a therapeutic intervention in intensive care unit (ICU) to prevent ischemia reperfusion injury, resulting in a better neurologic outcome and reduced mortality. One of the underlying mechanisms of the beneficial effects of hypothermia is proposed to be reduction of the inflammatory response. However, a pitfall of reducing the inflammatory response is an increased infection risk. Therefore, we sought to investigate value of VCS parameters of leukocytes and compare it to C-reactive protein (CRP) and procalcitonin (PCT), in the early detection of infection in these patients. **Methods:** A total of 27 adult patients admitted at the ICU after surviving cardiac arrest were included. Twelve patients received standard post-resuscitation care according to the current best practice as control group while 15 patients received the post-resuscitation protocol of the target temperature management (TTM) trial. Blood samples were drawn before initiation of hypothermia, at the 24th hour and at the time the cultures were taken. Presence of an infection was confirmed either by a positive culture (blood, urine, deep tracheal aspirate) result or by the presence of pulmonary infiltrate on chest radiography. VCS parameters were analyzed by UniCel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). CRP levels were determined nephelometrically (Dade Behring BN II, Siemens Laboratory Diagnostics, Germany) and PCT were measured by electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics, Germany). Statistical analysis was performed using the SPSS software, version 17.0. Results were expressed as the mean±standard deviation (SD). All parameters were compared among groups using analysis of variance (ANOVA) and subsequent post hoc range tests. P value <0.05 was considered statistically significant. **Results:** While PCT values didn't differ from the baseline, CRP values were significantly increased at the time cultures were taken, compared to the baseline values in both control and hypothermia group (P=0.18; P<0.001,respectively). There were significant differences for mean axial light loss of neutrophil (MNAL2-NE; P=0.016), SD of volume of lymphocyte (SD-V-LYM; P=0.021), SD of axial light loss of monocyte (SDAL2-MO; P=0.036), monocyte mean volume (MN-V-MO; P=0.009) and SD of volume (SD-V-MO; P=0.015) values at the baseline and at the time cultures were taken in hypothermia group. However, there was no statistical difference between control subgroups for all VCS parameters (P>0.05). **Conclusion:** Infectious complications after cardiac arrest may be more frequent after TTM. Diagnosis of infectious events is complicated in patients after cardiac arrest not only by the physiological effects of TTM but also by the consequences of reperfusion injury and development of postresuscitation disease associated with systemic inflammatory response syndrome. Furthermore, the significance of the usual symptoms of infections is reduced, as well as the value of laboratory markers such as PCT and CRP. VCS parameters which can be obtained easily using an automated blood analyzer, may be promising for helping clinicians in the prediction and early management of infection especially in cardiac arrest patients undergoing TTM. Nonetheless, further research is needed to identify a biomarker with high diagnostic accuracy and validity.

A-320

Multicenter Study of the Mid-volume Sysmex CS-2500 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Background: The objective of this study was to compare the performance of two automated coagulation analyzers, the mid-volume Sysmex® CS-2500 System (CS-2500) and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Health-

ineers. Instrument performance for factor V Leiden (FVL), factor II deficiency (FII), factor VIII deficiency (FVIII), factor IX deficiency (FIX), factor X deficiency (FX), factor XI deficiency (FXI), factor XII deficiency (FXII), and lupus anticoagulant (LA screening [LA1], LA confirmation [LA2], and LA ratio [LAR]) were compared. **Methods:** A measurement comparison (MC) study was performed according to CLSI EP09-A3E (*Measurement Procedure Comparison and Bias Estimation Using Patient Samples*). Four clinical sites or internal measurements were included for testing de-identified leftover samples. The MC of the CS-2500 versus the CA-1500 was based on 157–494 results per parameter (total of 3189 results). A reproducibility study was performed according to the CSLI EP05-A3 (*Evaluation of Precision of Quantitative Measurement Procedures*) guideline in three laboratories. Between five and seven samples were measured covering medical decision points and the clinical reportable ranges for each test. The complete dataset contained 14,948 results. Additional performance data were determined for regulatory clearance. **Results:** Results correlated well between the CS-2500 and the CA-1500. The MC studies showed Passing-Bablok regression slopes ranging from 0.92 to 1.04 and Pearson correlation coefficients ≥0.958 (depending on the application). Reproducibility testing for the new device/test combinations showed low CV values. The mean reproducibility (total CV combined labs) of all samples and parameters was 4.1%, ranging from 0.7 to 10.5% (depending on application and sample). **Conclusion:** The CS-2500 compares well to the CA-1500 and offers the benefits of state-of-the-art functionality and ease of use in mid-volume coagulation laboratories.
*Product availability varies by country.
Sysmex is a trademark of Sysmex Corporation.

A-321

QIP-MS: A specific, sensitive, accurate, and quantitative alternative to electrophoresis for the identification of intact monoclonal immunoglobulins

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Background: The development of the serum free light chain assay at the turn of the century heralded a significant improvement in the identification and quantification of these important biomarkers. In contrast, for the last 3 decades identification of monoclonal intact immunoglobulins has had little innovation. Here for the first time we present performance data for Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS), a polyclonal antibody-based technology to identify and quantify intact immunoglobulins. **Methods:** Modified sheep polyclonal antibodies (anti-IgG, -IgA, -IgM, -κ and -λ) were covalently attached to blocked magnetic microparticles. The microparticles were incubated with serum, washed, and then treated with 20mM TCEP in 5% (v/v) acetic acid to reduce patient immunoglobulin heavy and light chain disulphide bonds. Mass spectra were generated on a Microflex LT smart matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) system. Specificity was assessed using normal polyclonal serum (IgG = 8.25g/L, IgA = 1.67g/L, IgM = 0.7g/L, total κ = 7.7g/L, total λ = 3.67g/L) and patient sera containing monoclonal immunoglobulins (IgGκ = 33.2g/L, IgGλ = 28.3g/L, IgAκ = 21.1g/L, IgAλ = 20.4g/L, IgMκ = 23.1g/L, IgMλ = 19.1g/L). Accelerated stability was assessed at 22°C over 12 weeks using normal human serum. LoB and LoD were determined by serial titrations of polyclonal human serum diluted in sheep serum and linearity was assessed by serial titrations of a known monoclonal Ig diluted in normal polyclonal serum. Sensitivity was compared to CZE and IFE. **Results:** Mass spectra were acquired in positive ion mode covering the m/z range of 10,000 to 30,000 which includes the singly charged (+1, m/z 23330 to 24650) and doubly charged (+2, m/z 11168 to 12401) ions. Polyclonal molecular mass distributions for the light chains from: IgG (median IgGκ/IgGλ ratio 2.3:1 (CV=4.4%)), IgA (IgAκ/IgAλ = 1:1 (CV=3.5%)) and IgM (IgMκ/IgMλ=1.5:1 (CV3.7%)), total kappa, and total lambda were observed. No other peaks were observed in the polyclonal light chain molecular mass distributions confirming the high specificity of the antibodies. Normal human sera assessed at 22°C/12 weeks gave reproducible intensities for the polyclonal molecular mass distributions without loss of activity (48 week 4°C equivalent stability). The LoD for monoclonal proteins diluted into sheep serum were: 0.7mg/L for IgG, 1.4mg/L for IgA, IgM and total kappa, and 0.17mg/L for total lambda. Serial dilution of known monoclonal immunoglobulins into normal polyclonal serum gave acceptable linearity (IgGκ = y = 1.1x + 0.23, IgGλ = y = 1.02x + 0.06, LoD 24mg/L; IgAκ = y = 1.01x + 0.2, IgAλ = y = 0.95x + 0.44, LoD 8mg/L; IgMκ = y = 1.21x + 0.42, IgMλ = y = 1.15x + 0.17, LoD 8mg/L). In a blind study QIP-MS had a greater sensitivity for the detection of monoclonal immunoglobulins than either serum (100x) or immunofixation (10x) electrophoresis. **Conclusion:** QIP-MS provides a highly reproducible, linear, and sensitive alternative to conventional electrophoresis. The ability to measure a unique molecular mass for any myeloma paraprotein offers an innovative addition for the identification and quantification of monoclonal immunoglobulins.

A-322

Pediatric reference ranges for the cobas m 511 Integrated Hematology Analyzer

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Background: The **cobas m** 511 system is a novel slide-based automated hematology analyzer that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using automated digital microscopy. This single-center study determined pediatric reference ranges for the 26 parameters measured by the system. **Methods:** Residual EDTA whole blood samples were obtained from 245 healthy subjects covering four age ranges (6-≤24 months, 2-≤6 years, 6-≤12 years, and, separately for males and females, 12-≤18 years). A hematologist reviewed the subjects' medical records to rule out the presence of pathologies or therapies known to affect blood cell counts. Samples were analyzed on the **cobas m** 511 system and reference ranges were calculated as the central 95% of values obtained for each parameter according to CLSI guideline EP28-A3c. **Results:** Reference ranges for each pediatric cohort for the 26 blood count parameters measured by the system are presented in **Table 1** (*Data for RDW, RDW-SD, MPV, %NRBC, %NEUT, %LYMPH, %MONO, %EO, %BASO, %RET, #RET, and HGB-RET not shown)*. **Conclusions:** The observed reference range for the 26 parameters analyzed on the **cobas m** 511 Hematology Analyzer, and the age and sex differences, are consistent with reference ranges determined for other automated hematology analyzers.

Table 1: Pediatric reference ranges

Parameter [Units]	6-≤24 months	2-≤6 years	6-≤12 years	12-≤18 years (females/males)
WBC [10^3/µL]	5.06-14.94	4.46-13.42	4.28-12.68	3.86-11.03/3.87-12.53
RBC [10^6/µL]	3.97-4.98	4.01-5.02	3.99-4.99	3.88-5.18/4.09-5.34
HGB [g/dL]	10.8-13.3	11.0-13.6	11.6-14.1	11.1-15.0/12.3-15.9
HCT [%]	31.7-38.3	32.7-40.1	33.5-42.1	33.4-44.0/36.6-47.7
MCV [fL]	72.4-86.0	72.5-87.5	71.6-94.6	71.9-95.4/79.7-96.5
MCH [pg]	23.9-29.3	23.2-29.9	24.0-32.0	24.8-31.9/26.5-31.7
MCHC [g/dL]	32.4-35.1	32.4-34.9	32.4-35.4	32.2-35.1/32.5-34.5
PLT [10^3/µL]	222-551	213-579	199-420	184-409/138-409
#NRBC [10^3/µL]	0.00-0.01	0.00-0.01	0.00-0.02	0.00-0.01/0.00-0.03
#NEUT [10^3/µL]	1.45-5.64	1.18-6.51	1.63-6.90	1.69-6.70/1.50-9.55
#LYMPH [10^3/µL]	2.68-9.85	2.27-6.86	1.20-5.82	1.40-3.40/1.27-3.24
#MONO [10^3/µL]	0.38-1.51	0.34-1.07	0.29-0.91	0.22-1.09/0.35-1.30
#EO [10^3/µL]	0.02-0.91	0.07-1.82	0.01-1.37	0.01-0.51/0.02-0.64
#BASO [10^3/µL]	0.00-0.16	0.00-0.12	0.00-0.13	0.00-0.12/0.01-0.12

A-323

Performance Evaluation of Automated Urine Analyzers to enhance laboratory efficiencies for Urinary Tract Infection Diagnosis

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Background: Rapid and accurate identification of urinary tract infection (UTI) is one of the most important issues in both health-care facilities and community settings. Urine culture is "gold standard" until now, but time-consuming, labor-intensive, and costly. Newly developed automated urine analyzers are expected to help reduce unnecessary culture and provide prompt data to clinicians for determination of the treatment plan. **Methods:** Compared to urine culture, a total of 528 samples were analyzed using five automated urine analyzers: UF-5000 (Sysmex Corporation), UAS800 (Siemens Healthineers), Cobas® u701 (Roche Dignostics), Iris iQ®200SPRINT (Beckman Coulter) and URiSCAN® PlusScope (YD diagnostics). The criteria for UTI was defined if the inoculated plate yields more than 10^4 CFU/mL from all specimens, or more than 10^3 CFU/mL from specimens that were collected from patients with foley catheter or urinary symptoms. Performances of indicators - leukocyte esterase (LE), nitrite, white blood cell (WBC) and bac-

teria - were analyzed by single indicator alone or in different combinations. **Results:** By considering the indicators alone, diagnostic performances of bacteria were various according to each of urine analyzers compared with urine culture. The specificity and negative predictive value (NPV) were 97.7%, 56.0%, 68.8%, 95.7%, 97.7% and 92.3%, 90.1%, 90.4%, 90.3%, 89.4% for UF-5000, UAS800, Cobas® u701, Iris iQ®200SPRINT and URiSCAN® PlusScope, respectively. For LE, the specificity ranged from 65.1 to 72.0% and NPV ranged from 87.1 to 88.9%. And after considering the combinations, diagnostic performances were improved, but not satisfactory because negative predictive values (NPVs) were less than 95%. The combination of LE and nitrite showed similar NPV, ranging from 88.9 to 90.3%. And the combination of bacteria and WBC showed NPV from 91.2 to 94.0%. **Conclusion:** In this study, we evaluated diagnostic performances of recently introduced automated urine analyzers compared with conventional urine culture. There are still limitations for automated urine analyzer to replace conventional urine culture in some cases. But, this study is a statistical approach regarding the feasibility as a screening test for UTI. As sensitive and rapid diagnostic tools, urine sediment analyzers can be one of the important tools in the near future, and reduce unnecessary culture and give a guidance for clinicians to determine treatment plan.

A-324

A simple and rapid LC-MS method for identification of hemoglobin variants

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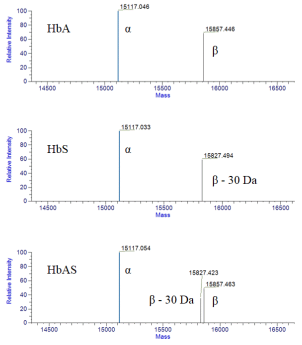
Background:
Hemoglobin (Hb), as the most abundant protein in the red blood cells, carries the important function of transporting oxygen from the lungs to tissues. Hb consists of four globin subunits (two α and two non-α units, e.g., β, γ and δ). Hemoglobinopathies are disorders resulted from gene mutations of the globin subunits which often cause structural change. Traditional methods of analyses are gel electrophoresis and chromatography; however, these methods are limited by low resolution and have difficulty in identifying less-commonly encountered variants. The goal of this work was to develop an LC-MS method to identify Hb variants that are challenging for the traditional methods. **Methods:** EDTA whole blood (10 µl) was mixed with 1 mL of sample buffer (3 % acetonitrile, 0.5 % formic acid and 1 % trifluoroacetic acid in water) followed by centrifugation at 13K rpm for 10 min. Ten microliter of the supernatant was then taken and diluted with sample buffer for another 100 folds. Samples were injected directly to a TLX-II LC system coupled with a Q-Exactive high resolution MS (Thermo Scientific). The intact Hb proteins were separated on a C18 column with total LC time of 5.5 min. Mass deconvolution was achieved using BioPharma Finder software (Thermo Scientific). **Results:** This LC-MS method was proven to be able to identify normal Hb and variants that were tested in both homozygous and heterozygous specimens (Figure 1). **Conclusion:** The developed LC-MS method is able to accurately identify Hb variants based on accurate mass of the intact protein subunits.
Figure 1. Deconvoluted mass of samples with HbA, HbS, and HbAS



A-325

Significance of High Serum Ferritin in Discrimination of Various Diseases

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Background: Serum ferritin is an acute phase reactant and increases in various infectious and inflammatory conditions. Besides, serum ferritin is high in patients with transfusional iron overload. Severity of the rise in serum ferritin may be used as an indicative for much severe conditions, such as primary hemophagocytic syndrome. The objective of this study was to determine whether serum ferritin levels could be used to make a differential diagnosis between different diseases. **Materials and methods:** The patients below 18 years of age were included. Out of 11809 patients, who were tested for serum ferritin levels between January 2015 and February 2018, 260 (2.2%) were found to be above 1000 ng/ml. Those patients who were found to have serum ferritin above 1000 ng/ml were analyzed retrospectively in terms of the underlying diagnosis. **Results:** Mean age of the patients in study group were 90±71 months (0-215), 56% were males. Out of 260 patients, 70 (27%) had an underlying malignancy. Of the patients with malignancies, 32 (46%) had acute leukemia and 17 (24%) had adrenal neoplasm. Sixty-four patients (24.6%) had underlying infection, 21 (8%) had immune deficiency and infection, 17 (6.5%) had thalassemia, 17 (6.5%) had secondary hemophagocytic lymphohistiocytosis (HLH), 16 (6.1%) rheumatological diseases, 15 (5.7%) had chronic renal failure, 9 (3.4%) had secondary HLH, 9 (3.4%) had other types of hemolytic anemias, 6 (2.3%) had DBA, 6 (2.3%) had aplastic anemia, 2 osteopetrosis, 2 metabolc disease, 1 hydrops fetalis, 1 acute renal failure, 1 Celiac disease, 1 sickle cell disease, 1 cirrhosis, 1 had burn injury. The cut-off serum ferritin level that differantiates primary HLH from secondary HLH was 3282 ng/ml (100% sensitivity, 53%specificity). **Discussion:** High serum ferritin levels can be seen at diagnosis or during follow-up of various conditions. Levels above 3000 ng/ml might be indicative for primary HLH.

A-326

Significance of Leukocyte VCS (Volume, Conductivity and Scatter) Parameters in the Diagnosis of Acute Exacerbations of Cystic Fibrosis

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Background: Cystic fibrosis (CF) is the most common inherited disease and is characterized by chronic sinopulmonary infection besides gastrointestinal, nutritional, and other abnormalities. The main clinical features of CF lung disease are chronic airway infection and repeated acute exacerbations, with a distinctive bacterial flora facilitating irreversible lung damage. Early diagnosis of acute exacerbations in CF patients is very important for the initiation of treatment. Our aim was to evaluate diagnostic significance of volume, conductivity, and scatter (VCS) parameters in cystic fibrosis with or without acute lung exacerbation, and healthy controls. **Methods:** CF patients (n=43) that are followed by the Department of Pediatric Respiratory Disease at Marmara University Pendik E&R Hospital and who were younger than 25 years of age were enrolled in the study. Acute exacerbations of CF cases were determined as episodes of acute worsening of respiratory symptoms and decline in lung function. A further age and gender matched 39 children without CF were also included as the control group. We extracted complete blood count data with VCS parameters of CF patients at the time of acute exacerbations which was determined clinically and during the follow-up visits without acute exacerbation together with those of the control cases from laboratory database. All parameters had been measured by Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). Data analysis was performed using SPSS Statistics 17 software. Results were expressed as the mean±standard deviation (SD). Comparisons among mean values of the groups were performed by ANOVA and $P<0.05$ was determined as significant.**Results:** The mean age was 7.4±7.6 years in CF group, while control group mean age was 5.8±4.5 years. WBC counts were significantly different in all groups. Mean neutrophil volume (MN-V-NE) (148±10, 148±8.6 respectively) and SD of neutrophil low-angle light scatter (SD-LALS-NE) (37.5±9.7, 35±9.5 respectively) were similar in CF with or without acute exacerbation ($P>0.05$). However, these parameters were significantly different compared to the control group (141±5.4; 30±6.5, respectively) ($P=0.002$; $P=0.022$, respectively). Lymphocyte median-angle light scatter (MN-MALS-LY) and upper median-angle light scatter (MN-UMALS-LY), and MN-MALS-MO of monocytes

were significantly increased in the CF group with acute exacerbation when compared with CF cases without acute exacerbation (P=0.006; P=0.002; P<0.001, respectively) but both groups of CF did not differ from control group (P>0.05). Mean conductivity (MN-C) of monocytes were significantly different among all groups (P<0.001, P<0.044, P<0.023). ROC curve analysis revealed 72.1% sensitivity and 69.8% specificity at 11 arbitrary units for SD of low-angle light scatter of lymphocyte (SD-LALS-LY) in the discrimination of acute exacerbation of CF patients. **Conclusion:** Volume, conductivity and scatter parameters like MN-MALS-LY, MN-UMALS-LY, SD-LALS-LY and MN-MALS-MO from automated analysers have emerged as promising parameters for the follow-up and management of acute exacerbation of cystic fibrosis. However, further studies are required to demonstrate their diagnostic value.

A-327

Comparison of Five Automated Urine Sediment Analyzers to Manual Microscopy for Accurate Detection of Urine Particles

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Background: Urinalysis is one of the most commonly performed diagnostic test in clinical laboratory, and gives information regarding the wide range of disorders including kidney, metabolic, and systemic disease. For urine sediment analysis, microscopic examination is still "gold standard", but is time-consuming, labor intensive, and has large interobserver variation. Automation in urinalysis has been developed over time. And in this study, we evaluated analytical and diagnostic performances of recently introduced automated urine sediment analyzers. **Methods:** A total of 1016 samples were analyzed using five automated urine sediment analyzers: UF-5000 (Sysmex Corporation), UAS800 (Siemens Healthineers), Cobas® u701 (Roche Dignostics), Iris iQ®200SPRINT (Beckman Coulter) and URiSCAN® PlusScope (YD diagnostics). UF-5000 is based on flow-cytometry based system, and others are based on digital image-based system. Manual microscopy using KOVA chamber (KOVA International Inc.) was performed as reference method. **Results:** All of the five urine sediment analyzers showed acceptable performances in precision, linearity, and carry-over study, according to manufacturer's instruction. For semi-quantitative parameters, these five urine sediment analyzers showed good concordance rates within 1 grade difference for semi-quantitative parameters compared with manual microscopy: 92.2-94.7% for red blood cell (RBC), 92.2-93.8% for white blood cell (WBC), and 96.9-99.0% for squamous epithelial cell (SQEP). And diagnostic performances for qualitative parameters were also evaluated. The sensitivity and specificity for crystal were 44.8%, 49.1%, 61.2%, 68.1%, 18.1%, and 99.8%, 97.9%, 95.1%, 90.4%, 96.7%, for UF-5000, UAS800, Cobas® u701, Iris iQ®200SPRINT and URiSCAN® PlusScope, respectively. And the sensitivity and specificity for pathologic cast were 23.6%, 81.8%, 74.5% and 95.9%, 84.8%, 90.2%, for UF-5000, UAS800 and Cobas® u701, respectively. **Conclusion:** In this study, we compared five recently introduced automated urine analyzers with manual microscopy, for accurate detection of urine sediments. Compared to manual microscopy, there are still limitations in terms of detection of particles, particle recognition software, and interpretation system, and more technical advances are needed. But, automated urine analyzers are expected to reduce the burden of manual processing, with reliable results. And with image and microscopic review, routine urinalysis laboratories can provide more accurate results.

A-328

Using QIP-MS to distinguish a therapeutic mAb from an endogenous M-protein in patients being treated for multiple myeloma

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Background: Therapeutic monoclonal antibodies (t-mAbs) daratumumab and elotuzumab have revolutionized the treatment of patients with multiple myeloma (MM). These t-mAbs present a challenge to laboratories using electrophoresis to monitor MM patients due to peak serum concentrations which can interfere with serum protein electrophoresis and immunofixation electrophoresis (IFE). Since the majority of monoclonal therapeutics have an IgG heavy chain isotype and a kappa light chain isotype, it is likely that newly developed t-mAbs will interfere with electrophoretic methods. An alternative solution is to utilize molecular mass to distinguish between an M-protein and a t-mAb. In this study we present the use of quantitative immunoprecipitation mass spectrometry (QIP-MS) as a method to distinguish patients Mprotein from daratumumab and elotuzumab. **Methods:** Briefly, modified sheep polyclonal antibodies (anti-IgG) were covalently attached to blocked magnetic microparticles. An IgGk patient monoclonal protein was diluted to 2.0g/L in normal human sera (IgG = 8.25g/L, IgA = 1.67g/L, IgM = 0.7g/L, total κ = 7.7g/L, total λ = 3.67g/L) and either daratumumab or elotuzumab was added at between 0-5-5g/L. Separately, 2xGK and 2xGL MM patient sera were diluted to 1.0 g/L in pooled normal human serum containing either daratumumab or elotuzumab at a concentration of 0.2g/L. Microparticles were incubated for 15mins, before being washed and treated with 20mM TCEP in 5%(v/v) acetic acid to reduce immunoglobulin heavy and light chains. Mass spectra were generated on a microflex LT "smart" MALDI-TOF-MS system. IFE was performed in a certified clinical laboratory in accordance with the manufacturer's instructions. **Results:** QIP-MS mass spectra identified the monoclonal GK patient at 2g/L in the same mass spectrum with daratumumab and elotuzumab present between 0.5-5g/L. By contrast, IFE was only able to identify the t-mAb when present at >1g/L. In an expanded study, QIP-MS was able to distinguish the monoclonal light chains originating from the patient's M-protein and the t-mAb at therapeutically relevant concentrations (0.2g/L) in all samples analyzed. The +2 charge states were monitored for the monoclonal light chains from daratumumab (11,689.0 *m/z)* and elotuzumab (11,710.5 *m/z*) and were clearly distinct from the +2 charge states of the monoclonal light chains from the patients (GK1 = 11,752.9 *m/z*, GK2 = 11,730.8 *m/z*, GL = 11,378.8 *m/z*, GL2 = 11,271.5 *m/z*). **Conclusion:** Our findings show that by combining the specificity of polyclonal anti-IgG antibodies bound to magnetic particles with the mass resolution and mass measurement accuracy of a MALDI-TOF mass spectrometer, daratumumab and elotuzumab were easily distinguishable from the patient M-protein, even in the presence of a high polyclonal background and at levels below the detection limit of IFE. Furthermore, this approach is agnostic to the therapeutic antibody and therefore can be used to monitor patients irrespective of their treatment modality, a distinct advantage over idiotypic gel shift assays.