Tuesday, August 2, 2016

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

Hematology/Coagulation

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The VCS parameters: a potential hematological indicator for antituberculosis drug-induced neutropenia

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Background: The morphological changes in reactive neutrophils associated with antituberculosis drugs can be measured by volume, conductivity, and scatter (VCS) technology on the Coulter LH750 hematology analyzer. We conducted the current study to further validate the clinical usefulness of the neutrophil VCS parameters in predicting drug-induced neutropenia.

Methods: Peripheral blood samples were collected from 52 patients with drug-induced neutropenia, 309 patients without any abnormal complete blood counts, and 237 healthy controls. The mean neutrophil volume (MNV) with its distribution width (NDW) and the mean neutrophil scatter (MNS) were studied.

Results: We observed a significant increase in the MNV with NDW and a significant decrease in patients with neutropenia compared to healthy controls, as well as to case samples after therapy. The ROC curve analyses showed that the MNV,MNV-SD and MNS had larger areas under curves compared to conventional parameters. With a cutoff of 150.15 for the MNV, a sensitivity of 84.4% and specificity of 75.7% were achieved prior to neutropenia.

Conclusion: The neutrophil VCS parameters may be clinically useful as to be a potential hematological indicator for predicting drug-induced neutropenia.

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Performance Evaluation of the Beckman Coulter DxH 500 vs. the COULTER HmX Five-part Differential

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Introduction:

The DxH 500 analyzer from Beckman Coulter is a quantitative, multi-parameter, automated hematology analyzer for in-vitro diagnostic use in clinical laboratories. These clinical laboratories include low-volume hospitals, small reference laboratories, and physician's office laboratories. The DxH 500 is used to identify the normal patient with normal system-generated parameters from patients with abnormal parameters and/or flags that require additional studies.

The DxH 500 analyzers identify and enumerate the complete blood count (CBC) parameters and five-part differential in only 12 microliters (µL) of whole blood (venous or capillary) sample types making it ideal for pediatric and geriatric populations.

Methods:

The purpose of the study is to evaluate the DxH 500 performance in a clinical lab setting and to assess acceptance of the instrument. We utilized our established method, the COULTER HmX, as the comparator system. Simple Deming regression were applied for method-comparison data analysis.

Results:

Using Deming approach to estimate regression parameters on 436 specimens, the correlation coefficient for the five-part differential percentage were as follows: Neutrophils = 0.990; Lymphocytes = 0.993; Monocytes = 0.939; Eosinophils = 0.882; and Basophils could not be estimates since most of the results were near zero. The WBC Differential parameter regression scatterplots are included in Figure 1.

Figure 1. WBC Differential Parameter Regression Scatterplots

DxH 500 vs. COULTER HmX

Conclusion: The DxH 500 is a small footprint five-part differential hematology analyzer designed for low-volume laboratories that provides differential results comparable to larger hematology systems.

Footnotes

[1] K. Linnet. Stat Med. 9,1463-1473.1990

A-281

Reliability Proof of the Beckman Coulter DxH 500 System[1]

E. Lin, G. Scott. *Beckman Coulter, Miami, FL*

Background: Reliability is a key differentiator for Beckman Coulter products and is a vitally important factor for customers when selecting diagnostic instrumentation. To address the needs of the low-volume user for hematology testing, Beckman Coulter developed the DxH 500, a highly reliable automated hematology analyzer and WBC differential counter for use in the clinical laboratory. Reliability represents the probability of components, subsystems and system to perform intended functions for a desired period of time without failure in specified environments with a confidence level. To ensure that the critical components and the system meet or exceed the reliability requirements, the reliability testing of the DxH 500 was comprised of multiple test stages with rigorous test plans and methods.

Methods: To efficiently identify and correct all potential malfunctions and design weakness, reliability testing focused on hardware issues, confirmed software, system, and user interface performance. A reliability growth model was developed using Army Material Systems Analysis Activity (AMSAA) for reliability improvements and predictions after design changes and corrective actions. Prior to the DxH 500 commercial release, external reliability studies were conducted at 21 clinical sites across five continents and multiple countries to ensure the reliability requirement was met or exceeded. **Results:**

To demonstrate an ESC (Emergency Service Call) rate of < 2 per instrument per year with 80% confidence level, up to 1 ESC was allowed, the total accumulative test time of 294 days and 12,558 cycles are required.

The actual accumulative test time from the 21 instruments were 1,342 days and 36,932 cycles with just 1 ESC incident reported. Therefore, the results had surpassed the test criteria. In addition, there were no hardware parts replaced on any of the instruments tested.

The system interruption rate due to some error events was predicted to be < 1 per 1,000 operational cycles after the corrective actions have been implemented after the external reliability testing, with the majority of the errors recoverable via the user interface diagnostic tool.

Conclusion:

The reliability of the DxH 500 was validated using in-house metrics that ensured critical components and the system met or exceeded key reliability requirements. External site testing further verified the high level of confidence in the reliability performance of the DxH 500 expected in the clinical laboratory.

[1] Not available for sale in the US

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Effect of −158 Gγ (C→T) Xmn1 Polymorphism on HbF level in a goup of sickle cell disease patients from Siwa oasis Egypt

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Background: Sickle cell disease (SCD),an autosomal recessive disorder is caused by a single point mutation in position 6 of the β globin gene. The World Health Organization (WHO) estimated that each year about 300,000 infants are born with major haemoglobin disorders - including more than 200,000 cases of sickle-cell anaemia .It is the most common genetic disease in Africa, the Caribbean, the Americas, the Middle East, and India.In Oasis Siwa Egypt ,the prevalence rate of SCD (trait and anemia) is approaching 20%.Clinical severity of sickle cell disease is extremely variable. higher expression of fetal haemoglobin (HbF) in adulthood ameliorates morbidity and mortality in sickle cell disease. The −158 Gγ (C→T) Xmn1 Polymorphism -a common sequence variant in all population groups is known to influence the γG gene expression. It predisposes carriers to increased Hb F concentrations in particular when they are under conditions of erythropoietic stress ameliorating the clinical phenotype. The present study aims to investigate the

frequency of −158 Gγ (C→T) Xmn1 Polymorphism and its association with high HbF level in sickle cell disease patients from Siwa oasis in Egypt .

Methods: This study was performed on 65 SCD cases and 65 age and sex matched healthy controls. Both cases and controls were selected out of a screening program conducted on primary school children in Siwa oasis by Alexandria Faculty of Medicine during years 2011- 2012. All patients and controls were subjected to complete blood count,capillary electrophoresis for the detection of HbS and HbF levels and PCR-RFLP for detection of −158 Gγ (C→T) Xmn1 Polymorphism using the XmnI restriction enzyme. The mean age of SCD patients was11.3 ± 2.4 years and controls was10.1 ± 2.5 years. Because of the influence of age on the HbF level, patients younger than five years were excluded from the study. Genomic DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). A 650-bp fragment 5' to the γG gene was amplified using the primer 5'- AAC TGT TGC TTT ATA GGA TTT T-3' and 5'- AGG AGC TTA TTG ATA ACC TCA GAC-3'.

Results: Genotype frequencies of the −158 Gγ (C→T) Xmn1 Polymorphism among SCD cases revealed that 84.6% of cases were homozygous for the wild-type allele (CC) and 15.4% were heterozygous (CT), The genotype frequencies among controls were 83.1% homozygous for the wild-type allele (CC) and 16.9% heterozygous (CT). In cases and controls there was no significant difference between the wild-type and heterozygous genotypes as regard HbF level.

Conclusion: From the current study we conclude that the −158 Gγ (C→T) Xmn1 Polymorphism has no effect on HbF level in sickle cell disease patients from Oasis Siwa Egypt. A wide range of HbF was obtained both in the presence and absence of this site. Further studies with a larger sample size are needed for a better understanding of the association between the −158 Gγ (C→T) Xmn1 Polymorphism and Hbf level in sickle cell disease patients from Siwa Oasis .

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Point-of-care PT and aPTT in Patients with Suspected Deficiencies of Coagulation Factors

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Objective: The objective of this study was to evaluate the performance of the CoaguChek PT Test (POC PT) and CoaguChek aPTT Test (POC aPTT) on the CoaguCheck Pro II meter in hospital point-of-care (POC) settings. **Relevance:** Global coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) are screening tests that are routinely used to assess blood coagulation status represented by factors of the coagulation cascade. In many clinical settings and patient conditions, coagulation status of a patient must be known immediately, including evaluation of hemorrhagic patients, prior to invasive procedures and before starting antithrombotic therapy. POC systems are beneficial in these cases. **Methods:** This non-interventional, multicenter study with prospectively collected whole blood samples was performed in an intensive care unit, one emergency department, one operating theatre and one outpatient coagulation clinic. Diagnostic accuracy of the POC PT and POC aPTT tests with fresh, non-anticoagulated venous blood were compared to laboratory reference tests Innovin (lab PT) and Actin FS (lab aPTT) on Sysmex CA 1500 using venous citrate plasma samples from the same venipuncture in patients with suspected deficiencies of coagulation factors or patients undergoing surgery. **Results:** 390 subjects were tested. Analysis of the comparison of POC PT versus laboratory PT yielded a slope of 0.99 in Passing-Bablok regression and a mean relative difference of 0.2 INR. One-hundred percent of all single POC PT values expressed as INR were within a range of ±20 % compared to laboratory PT, 96.1 % within a range of ±15 %. A measuring range of 0.8 to 6.2 INR was covered with 269 patient samples including statistical outliers. Positive and negative percent agreement of POC aPTT with laboratory aPTT was derived from a concordance table using the upper limits of normal ranges (ULNR) as cutoffs. The ULNR for POC aPTT and laboratory aPTT were determined as the 95th percentile of 120 subjects of a coagulation healthy population. The positive percent agreement was 85 % and the negative percent agreement was 91 %. Moreover three lots of POC PT and POC aPTT were evaluated in the study and each patient blood sample was measured with two different test strip lots per evaluation site. Lot-to-lot variation was below 2 % both for POC PT and POC aPTT. Comparison between venous and capillary blood samples taken from the same patient yielded a mean relative difference of 0.2 % INR (66 patient sample pairs with POC PT) and 8.4 % (63 patient sample pairs with POC aPTT) respectively. The coefficients of variation for repeatability of POC PT using patient blood samples were found to be 2 to 3.6 %. **Conclusion:** In summary both POC PT and POC aPTT showed a good concordance to the laboratory PT and aPTT as the reference methods. Consequently, this POC system allows to obtain reliable quantitative results to support on-site decision making for patients with suspected coagulation factor deficiencies in acute and intensive care. **Disclaimer:** This product is not cleared or approved for use in the USA.

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IKZF-1 and BCR-ABL gene expression signatures in acute lymphoblastic leukemia

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BCR-ABL1 gene fusion [t(9;22)] is rare in B-cell-progenitor acute lymphoblastic leukemia (pB-ALL) children but frequent in adults and it is associated with poor outcome. Microarray expression data showed that a group of high-risk patients, negative for BCR-ABL1, presents similar expression profiles to the BCR-ABL1 carriers, therefore becoming known as BCR-ABL1-like. In the past decade, studies have tried to define genetic hallmarks of BCR-ABL1-like, and one of them is the presence of IKZF1 deletions. The IKZF1 gene is an essential transcription factor in hematopoiesis involved primarily in lymphoid differentiation. IKZF1 deletions are highly incident among BCR-ABL1-positives and BCR-ABL1-like cases. Some studies have shown that IKZF1 deletions are significantly associated with increased relapse rate and adverse events in both pediatric and adult patients. Aims: To gain further comprehension of the biology of these genetic aberrations, we compared the gene expression signatures of BCR-ABL gene fusion and IKZF1 deletions inpB-ALL. Patients and Methods: Thirty-four Brazilian pB-ALL patients (25 adults and 9 children) were retrospectively and non-consecutively included in this analysis. Ten of them carried a BCR-ABL gene fusion, six of whom also carried an IKZF1 deletion, and four carried IKZF1 deletions only. RNA was extracted from bone marrow samples obtained at diagnosis using PAX gene Bone Marrow RNA (QIAGEN/BD). RNA integrity was determined with Bioanalyzer2100 (Agilent Technologies). Gene expression was carried out using the Low Input Quick Amp Labeling kit One Color and the Sure Print G3 Human GE 8 x 60K array (Agilent Technologies). Data were extracted with the Feature Extraction Software v7.5 and normalized using the Gene Spring software v12.5 (Agilent Technologies). Machine-learning supervised analysis was performed in order to build a classifier based on the differential gene expression signatures from carriers and non-carriers of each alteration. The K-nearest neighbors prediction algorithm was selected to identify the 40 informative probes to build the classifiers. Performance of each prediction model was assessed by leave-one-out cross-validation assay through the Gene Pattern platform (Broad Institute).DAVID v6.7 software (NIH) was used for functional annotation analysis. Results: The prediction model based on the gene signature established for IKZF1 deletions had accuracy and precision rates of 93% and 89%, respectively. For BCR-ABL presence, accuracy and precision were 93% and 100%. Hierarchical clustering analysis using probes selected for IKZF1 grouped patients carrying deletions in two neighbor clusters, one of them also included in a sub-cluster patients who were positive for BCR-ABL but negative for IKZF1 deletions. When using probes selected for BCR-ABL, all fusion carriers were grouped in a separate single cluster. Five genes (12.5%) were common to both IKZF1 and BCR-ABL gene signatures: SNORA26, C8orf38, AGFG2, FAM78A and SLC9A9. Both IKZF1 and BCR-ABL signatures contained genes involved in GTPase activity regulation, the function annotation of these clusters, though, were not statistically significant when p-values were corrected. Conclusion: Although IKZF1 deletions are a common finding in BCR-ABL-like patients, we were able to identify distinguished gene signatures for IKZF1 deletion carriers and BCR-ABL carriers. The role of these genes in pB-ALL need to be further investigated.

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Impact of serum free light chains in the screening of acute kidney injury of unknown origin

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Background: The "International Kidney and Monoclonal Gammopathy Research Group (IKMG)" have defined an algorithm that included the quantification of serum free light chains (FLC) for the screening of monoclonal gammopathies in the study of acute kidney injury (AKI) of unknown origin. This algorithm allows us a rapid identification of a monoclonal FLC as the possible cause of a tubular interstitial process:

1.Clonal FLC>500 mg/L: probable FLC tubular interstitial pathology. Requires hematology work-up and initiation of disease-specific treatment to reduce serum FLC levels.

2.Clonal FLC<500 mg/L: alternative monoclonal FLC pathology (amyloidosis, light chains deposition disease, cryoglobulinemia) or incidental MGUS. Requires renal biopsy.

3.Absence of clonal FLC: AKI of another cause.

Our aim is to show the utility of this algorithm in the study of AKI of unknown origin.

Methods: Descriptive study of eight patients with AKI of unknown origin where this algorithm was applied. Serum FLC were quantified by the assay Freelite (The Binding Site).

Results: The results are shown in the table.

Case	Patient findings	Serum FLC	IKMG Algorithm	Diagnosis
Female, 74 years	AKI, proteinuria, edema	kappa=17.7 mg/L, lambda=180 mg/L, ratio=0.09	Positive Clonal FLC<500 mg/L	Primary Amyloidosis
Female, 75 years	AKI, anemia, back pain	kappa=22.4 mg/L, lambda=3510 mg/L, ratio=0.01	Positive Clonal FLC>500 mg/L	Lambda Light Chain Multiple Myeloma
Male, 58 years	AKI, proteinuria, dyspnoea	kappa=81.5 mg/L, lambda=82.3 mg/L, ratio=0.99	Negative Absence of clonal FLC	IgA Nephropathy
Female, 82 years	AKI, proteinuria, edema	kappa=477 mg/L, lambda=23 mg/L, ratio=20.73	Positive Clonal FLC<500 mg/L	Light Chain Deposition Disease
Male, 51 years	AKI, proteinuria, bone pain	kappa=3600 mg/L, lambda=6.74 mg/L, ratio=534.12	Positive Clonal FLC>500 mg/L	IgA Kappa Multiple Myeloma
Male, 53 years	AKI, proteinuria, hematuria	kappa=12.1 mg/L, lambda=13.9 mg/L, ratio=0.87	Negative Absence of Clonal FLC	IgA Nephropathy
Male, 81 years	AKI, back pain	kappa=332.5 mg/L, lambda=29.4 mg/L, ratio=11.31	Positive Clonal FLC<500 mg/L	Monoclonal Gammopathy of Renal Significance
Female, 67 years	AKI, proteinuria	kappa=19810 mg/L, lambda=5.9 mg/L, ratio=3357.67	Positive Clonal FLC>500 mg/L	Kappa Light Chain Multiple Myeloma

Conclusions: Nephrotoxic serum FLC can cause a progressive and irreversible kidney damage in patients with AKI of unknown origin. The IKMG algorithm is easy and quick that can help us to guide the study of a patient with AKI.

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Comparative Study of the Sysmex CS-2100*i* and CS-2500 Coagulation Analyzers

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Background: The objective of this study was to compare the performance of the Sysmex® CS-2100*i* System and the new Sysmex CS-2500 System, automated coagulation analyzers from Siemens Healthcare, using different Siemens reagents and applications. Performance characteristics of the two systems for prothrombin time (PT sec and INR), activated partial thromboplastin time (APTT), fibrinogen, antithrombin (AT), D-dimer, and coagulation factor VIII (FVIII) were evaluated.

Methods: Result comparability of the two devices was investigated using previously frozen clinical samples purchased from commercial vendors. Method comparison studies were carried out at Sysmex Corporation, Kobe, Japan, according to CLSI guideline EP09-A3. The method comparison was based on a total of 2184 results (PT_{sec}: n = 302, PT_{INR}: n = 300, APTT: n = 304, fibrinogen: n = 300, AT: n = 301, D-dimer: n = 302, and FVIII: n = 375) distributed over the clinical reportable range (CRR).

Precision studies were performed according to CLSI guideline EP05-A2 and followed the scheme of 20 x 2 x 2 testing. 55 samples (PT_{sec}: n = 10, PT_{INR}: n = 9, APTT: n = 7, fibrinogen: n = 9, AT: n = 7, D-dimer: n = 6, and FVIII: n = 7) covering important medical decision points and the total CRR were used. The complete data set contained 13.200 results (4.400 for each instrument).

In addition, performance data for the Sysmex CS-2500 System regarding linearity and limit of quantitation (LOQ) for fibrinogen, AT, D-dimer, and FVIII were determined according to CLSI guidelines EP06-A and EP17-A2 respectively.

Results: Analysis of method comparison data by Passing-Bablok regression and difference plot revealed very good agreement of the Sysmex CS-2500 System to the Sysmex CS-2100*i* System, showing slopes between 0.990 and 1.026 and correlation coefficients ≥0.998. CVs for between device precision varied from 0.00 to 6.46%, with a median CV of 1.07% (depending on application, sample, and instrument).

Conclusion: Method comparison results for the two systems were in very good agreement. Precision for the new devices/reagents combination showed low CV values and linearity, and LOQ proved to be adequate for use of the Sysmex CS-2500 System in the clinical routine (in combination with the reagents/applications tested). Combined with enhanced functionality and ease of use compared to the Sysmex CS-2100*i* System, the Sysmex CS-2500 System provides improved performance, quality, and efficiency to clinical laboratories.

The products/features mentioned here are not commercially available in all countries. Due to regulatory reasons, their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.

Sysmex is a trademark of Sysmex Corporation.

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Genome-wide analysis of molecular characterization and classification in Myelodysplastic syndromes (MDS)

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Background: Myelodysplastic syndromes (MDS) contain a group of bone marrow disorders with massive variability of cytogenetic abnormalities of significant prognostic and therapeutic development importance. The molecular pathogenesis of the syndromes is poorly understood. Conventional metaphase cytogenetics (MC) reaches 10% sensitivity combination with Fluorescence In Situ Hybridization (FISH), can detect only around 50% of primary MDS, and are the focus of attention at clinical research society.

The International Prognostic Scoring System (IPSS) is the most widely used prognostic scoring system, which incorporates karyotype (MC & FISH). IPSS has been the most critical in prognostication system with clinical needs to develop innovative scoring systems, as well as continuing efforts to improve itself.

The availability of advanced molecular techniques, such as SNP-array, has allowed the discovery of additional genetic mutations for improving better diagnosis of MDS, as well as developing an enhanced prognostic system to guide therapy selection.

Objective: To discover novel MDS specific genomic mutations and develop a classification model for a better prediction of the individual prognosis for MDS.

Methods: Bone marrow aspirates (total N=208) were collected from MDS patients with chromosomal abnormalities detected on standard metaphase karyotyping. Sample preparation and microarray analysis were followed by manufacture's manual. Segmented copy number variations were calculated from SNP-array data using manufacture's power tool suite. They were further processed to illustrate the large scale genomic alterations (>4 Mbp) and clusters. Novel alterations were identified using statistical algorithms developed in house.

Results: Total 7269 chromosomal deviations were observed with 3510 segment gain abnormalities and 3759 loss abnormalities from the dataset. The most of the gain abnormalities were detected at chromosome 8 and chromosome 1, meanwhile chromosome 5 and chromosome 7 were harbored the most of the losses.

Through detailed analysis of the deviations, thousands genes were affected including 225 proto-oncogenes or tumor-suppressor genes. The well-studied MDS associated

genes, such as ASXL1, CBL, DNMT3A, EZH2, KRAS/NRAS, RUNX1, SETBP1, SRSF2, TET2, TP53 and U2AF1 were in the distressed gene list. Aberrant methylation of tumor-suppressor genes are drivers of MDS pathogenesis. Many epigenetic regulation associated genes were discovered in this study also.

Subsequently, eight sub-groups were proposed via an in-depth analysis by clustering of genome-wide alterations. The sub-groups are clustered and characterized by genetic segment variations. Such classification system demonstrates significant insights into underlying molecular mechanisms in disease development, and provide a promising dynamic tool during the disease course to monitor disease development in real time.

Conclusion: MDS is a biologically heterogeneous clonal disease. Molecular mutations in many pathways have been identified. It can be proposed that potential every MDS patients carries at least one pathogenic mutation in one gene.

Recurrent genetic mutations have been used for risk stratification, treatment selection and therapy response monitoring. The proposed classification model could be one of the highly relevant independent prognostic parameters in individual risk assessment.

This study demonstrates that SNP-array based genetic mutation analysis provides a better understanding of the molecular pathogenesis of MDS. However, more clinical studies are needed to support the proposed classification model for clinical practice.

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Reporting Of Critical Test Values for Hematological Parameters. A Large-Scale Laboratory Results

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Background: Critical test values can be defined as a life threatening state that requires an urgent reply, which is an important post analytical process for a clinical laboratory. The aim of this study is to evaluate reporting of the critical test values for hematological parameters in a large-scale university hospital. **Methods:** One-year data (2015) of hematology tests from Central and Emercency Laboratories of Hacettepe University Medical School was evaluated. Data comprised all reported, non-reported and dropped calls for critical test values. Among hematology parameters hemoglobin (Hb) (<7 g/dL and >20 g/dL), hematocrit (Hct) (<20% and >60%), platelet (Plt) (<20000/uL, and >100000/uL,) were taken references. Depending on the decision made by clinicians in our hospital, the critical test reporting for white blood cell (WBC) count has not been performed. Only verbal reports were taken into account. **Results:** During 2015, total 12.483 critical test reporting were performed in total 1.369.283 hematology test results (0.92%) in Hacettepe University Laboratories. Critical value reporting percentage was 94.8% for total tests whereas dropped call was only 4.2%.. Based on analytic test, reporting percentage was 92.87% for hemoglobin, 94.6% for hematocrit and 91.5% for platelets. The largest part of the reporting was to Adult and Pediatric Hematology Departments (80%) followed by Nephrology, Cardiology, General Surgery and Gynecology Departments, and Intensive Care Units (ICUs). Reporting percentage was found >98% for Hematology, Pediatric ICU, Adult Emergency Department (ED) and Neurosurgery, and >95% for Pediatric ED, Pediatric ICU, Newborn ICU, General Surgery, and Pediatric Surgery. The lowest reporting percentage was found for Pediatric Hematology and Pediatric Bone Marrow Transplant Unit as 41.8% and 40.3%, respectively. For inpatient clinics the mean percentage was 95.3% whereas for outpatient clinics it was 89.7%. The mean and median reporting times were 26.38 min. and 15 min. for hemoglobin; 25.62 min and 13.5 min for hematocrit, and 25.18 min and 14 min for platelets, respectively. For all, delay was mostly due to lack of the name of the doctor who ordered the test, the changes in secretariat of that clinic or coincidence with the lunchtime. **Conclusion:** Based on these results, the reporting percentage of critical test values for hematological parameters in our hospital was adequate, but it needs to be improved for pediatric hematology and transplant units. It would be better to shorten the median reporting time, in particular for ED and ICUs. Furthermore, clinical laboratories may determine their own recording policy by collaboration with clinics.

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Use of Sigma-Based QC to Monitor Hematology and Coagulation Testing in an Expanding Multi-Instrument, Multi-Site, Integrated Healthcare System

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Background: The Geisinger Medical Laboratories support an integrated healthcare system in Central PA which, over the past three years, has expanded from 11 to 17 testing sites, including 6 hospitals, 3 specialty clinics, 8 regional clinic laboratories and a core reference laboratory. The objective of this project was extension of our enterprise-wide Westgard sigma statistics and OPSpecs chemistry QC program to routine hematology and coagulation assays, including hemoglobin, hematocrit, RBC count, WBC count, platelet count, and prothrombin time. We performed monthly monitoring of sigma statistics for 1) ongoing evaluation of hematology and coagulation instruments at all integrated partner sites, and 2) monitorIng and verification of performance after conversion to system standard instruments (Sysmex, Inc. and Diagnostica Stago). **Methods**: Prior to performing sigma calculations and standardized QC evaluation, we validated systemwide reference ranges and standardized control materials and lots. The Sysmex sites used tri-level Sysmex QC materials with peer data derived from company provided Insight reports. Instrumentation included XN-9000 (3), XN-3000 (4), XN-2000 (2), XE-5000 (3), XT-4000 (1), and XS-1000 (10). The coagulation sites used the same lot of Neoplastine activation reagent and same lots of bi-level Stago QC materials for prothrombin time assays. The Stago instrumentation included the Compact (11), Evolution (6), Satellite (3), and Start4 (3). Peer data were autogenerated for the system. **Results**: Sigma values were calculated, tabulated, and graphed monthly for the instruments and control materials listed above. Across platform average sigmas and ranges for 2015 were: Hgb 13.5 (8.6 to 15), Hct 6.0 (5.2 to 8.1), RBC 8.9 (6.5 to 13.5), platelets 10.9 (4.8 to 15.1), WBC 12.3 (8.4 to 15.7), and prothrombin 8.0 (6.4 to 9.6). To better illustrate the time and specific instrument type-related data we developed a clustered scatterplot graph that allows visualization of sigma performance over time by site and by instrument. The data show steady performance with minimal variation among instrument types which, despite minor variations in averages and ranges, was consistently above the threshold of acceptablility. As expected, the lowest sigmas were observed for the hematocrit determination. **Conclusions:** All instruments yielded consistent world class performance with average Sigmas in excess of 6, and QC precision well within CLIA error limits. Overall this level of performance allows us to use the 1-3s rule throughout, with concomitant reduction of approximately two-thirds of false QC flags and associated workflow stoppage. Monitoring sigmas over time yields an additional dimension of assessment of integrated process stability in comparison to the single "slice in time" view afforded by one-time measurements. In addition, the improved graphical representation (dashboard) allows a cleaner look at the data across the system to better manage multiple instruments and identify nonrandom excursions.

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GloCyte: A New Automated Technology for Cerebrospinal Fluid (CSF) Cell Counts

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Background: GloCyte is a new semi-automated analyzer that uses fluorescent microscopy and digital image analysis to enumerate total nucleated cells (TNC) and erythrocytes (RBC) in CSF. This study aimed to (1) compare the GloCyte with manual and Sysmex XN methods and (2) determine the clinical impact of replacing the manual method by either automated method.

Methods: 57 samples from 39 patients with a variety of benign and malignant conditions were sequentially analyzed by hemocytometer, GloCyte, and Sysmex XN body fluid mode (Sysmex) within 4 hours of receipt in the laboratory. The average of duplicate manual cell counts using all 9 squares of Levy-Neubauer hemocytometers was used as the "gold standard", and compared to the Sysmex and GloCyte counts. Cytospin smears were reviewed by a pathologist and correlated with cell counts and clinical history. To assess precision, 4 samples were analyzed by all methods by 5 different technologists.

Results: Pearson correlation and Passing-Bablok regression estimates (confidence intervals) for TNC are shown in the table. In comparison to the manual method, Sysmex had a positive proportional bias, whereas GloCyte did not. When 0-5 TNC/μL was used as the reference range, sensitivity was 97% for GloCyte and 100% for Sysmex. Specificity was 91% for GloCyte and 70% for Sysmex. Eight samples had RBC/TNC ratios that suggested traumatic tap or intra-cranial hemorrhage by all methods. 19 samples were from patients with meningitis or encephalitis, all of which would have been similarly classified by all 3 methods. The precision for GloCyte and Sysmex were consistently better than manual counts, with Sysmex slightly better than or equal to GloCyte.

Conclusion: GloCyte TNC counts have less variability than manual counts and appear to be more accurate than Sysmex. Replacing manual TNC and RBC counts with either automated method would improve consistency of results without compromising diagnostic accuracy.

Regression Estimates for GloCyte and Sysmex TNC Compared to Manual Method			
	R	**Intercept**	**Slope**
GloCyte	0.988	0.00 (-0.22 - 0.95)	1.049 (1.000 - 1.238)
Sysmex	0.980	0.356 (-0.462 - 0.959)	1.205 (1.091 - 1.667)

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Genomic-wide analysis of molecular characterization and classification in Myelodysplastic syndromes (MDS)

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Background: Myelodysplastic syndromes (MDS) contain a group of bone marrow disorders with massive variability of cytogenetic abnormalities of significant prognostic and therapeutic development importance. The molecular pathogenesis of the syndromes is poorly understood. Conventional metaphase cytogenetics can detect only around 50% of primary MDS, and are the focus of attention in clinical research. The International Prognostic Scoring System (IPSS) is the most widely used prognostic scoring system, and has been the most critical parameter in prognostication system. An improved or innovative scoring system is much needed in clinical setting. The availability of advanced molecular techniques, such as SNP-array, has allowed the discovery of additional genetic mutations for better diagnosis of MDS, as well as developing an enhanced prognostic system for proper therapeutic intervention.

Objective: To discover novel MDS specific genomic mutations and develop a classification model for a better classification MDS on molecular level.

Methods: Bone marrow aspirates (total N=208) were collected from MDS patients with chromosomal abnormalities detected on standard karyotyping. Sample preparation and array analysis were followed by manufacture's manual. The whole-genome SNP-array used in this study features more than 2.6 million copy number markers and 743,304 SNP markers. Segmented copy number variations were calculated from the SNP-array data using manufacture's power tool suite. They were further processed to illustrate the large scale genomic alterations (>4 Mbp) and clusters. Novel alterations were identified using statistical algorithms developed in house.

Results: 1) Total 7269 chromosomal alterations were observed with 3510 segment gain abnormalities and 3759 loss abnormalities from the dataset. Some of the variations were observed at the first-time in MDS studies. 2) The most of the gain abnormalities were detected in chromosome 8 and chromosome 1, meanwhile chromosome 5 and chromosome 7 harbored the most of the losses. 3) Through detailed analysis of the deviations, thousands genes were affected including 225 proto-oncogenes or tumor-suppressor genes. The well-studied MDS associated genes, such as ASXL1, RUNX1, TET2 and TP53, were in the distressed gene list. 4) It is known that aberrant methylation of tumor-suppressor genes drives MDS pathogenesis. Many epigenetic regulation associated genes were discovered in this study also. 5) Subsequently, eight sub-groups were proposed via an in-depth analysis by clustering of genome-wide alterations. The sub-groups are clustered and characterized by genetic segment variations. For example, sub-group 1 primarily features the segment loss of chromosome 5 & 7, and sub-group 8 highlights the gain mutations at chromosome 8.

Conclusion: MDS is a biologically heterogeneous clonal disease. Recurrent genetic mutations have been used for risk stratification, treatment selection and therapy response monitoring. The proposed novel classification model could be one of the highly relevant independent prognostic parameters in individual risk assessment and monitoring disease development in real time. Further studies are needed to support the proposed classification model for clinical practice.

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Performance Evaluation of a New INNOVANCE Heparin Assay* for the Quantitative Determination of Both Unfractionated Heparin and Low-molecular-weight Heparin Using a Hybrid Calibration Curve

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Background: Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) are frequently used as prophylactic and therapeutic anticoagulants. They considerably accelerate the inactivation of thrombin and coagulation factor Xa by antithrombin. Therefore, they are used in prevention and treatment of venous thromboembolism, in certain types of coronary artery syndrome, and in thrombotic stroke. **Objective:** The objective of this study was the performance evaluation of the INNOVANCE Heparin assay against a similar assay and on different analyzers. The INNOVANCE® Heparin assay* from Siemens Healthcare uses a hybrid curve that enhances patient safety by eliminating errors due to sample mix-up in treatment with either UFH or LMWH. **Methods:** For performance testing of the assay, we conducted multicenter studies with 313 UFH and LMWH samples on the BCS® XP System (Siemens Healthcare) and compared the results with those of the HemosIL Liquid Anti-Xa assay on the ACL TOP system (Instrumentation Laboratories, USA). We measured both UFH and LMWH samples. The precision of the new assay was determined by testing both plasma pools and controls covering the entire measuring range over 20 days in two runs with two single determinations (20 x 2 x 2 scheme). Additionally, we tested the assay with 171 samples on the Sysmex® CS-2100*i* System* in comparison to the BCS XP System.

Results: The overall correlation between the INNOVANCE Heparin assay on the BCS XP System and the HemosIL Liquid Anti-Xa assay was high, with a correlation coefficient of 0.981, slope of 1.10, and intercept of 0.01. Both repeatability and total precision were below 7% for investigated controls and pools on the BCS XP System. Comparability of results obtained on the BCS XP System versus the Sysmex CS-2100*i* System was excellent (correlation coefficient: ≥0.998; slope: 1.00; intercept: 0.05).

Conclusion: The INNOVANCE Heparin assay is well suited for the measurement of both UFH and LMWH. It demonstrated excellent precision, correlated well with other commercially available assays, and showed excellent comparability among different analyzers.*Not available for sale in the U.S. The products/features mentioned here are not commercially available in all countries. Due to regulatory reasons, their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.

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Assessment of trough rivaroxaban concentrations on coagulability in a nonvalvular atrial fibrillation population

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Background: Whether trough-phase rivaroxaban concentrations provide sufficient anti-coagulation needs more study. We evaluated coagulability marker levels in the trough concentration phase in nonvalvular atrial fibrillation (NVAF) patients, and the correlation between these markers and rivaroxaban concentration.

Methods: Fifty-five Japanese NVAF patients (mean age, 70 years; CHADS2 score, 2.5) received 24-week rivaroxaban treatment of either 15 or 10 mg once daily. Of these, 26 patients had no history of anticoagulant therapy (naive group) and 29 had switched from warfarin (warfarin group). D-dimer levels, prothrombin fragment 1+2 (F1+2) levels, protein C activity, and antithrombin activity were measured at 0 (baseline), 12 and 24 weeks of rivaroxaban treatment just before the patient's regular dosing time (trough phase). For 49 patients, D-dimer, F1+2, and rivaroxaban concentrations were also measured twice between 28 and 32 weeks of rivaroxaban treatment at non-trough times to achieve a range of drug concentrations for correlation anaylsis.

Results: For the naive group, D-dimer and F1+2 levels were significantly reduced ($p < 0.01$) from baseline at 12 and 24 weeks (Table). For the warfarin group, these values were unchanged for D-dimer and significantly increased ($p < 0.01$) for F1+2. Protein C activity was unchanged in the naive group and was increased ($p < 0.01$) in the warfarin group. Antithrombin activity was unaffected by rivaroxaban in either group. Prothrombin time ($r = 0.92$, $p < 0.0001$) and activated partial thromboplastin time ($r = 0.54$, $p < 0.0001$) correlated with rivaroxaban concentration, but not D-dimer and F1+2 levels.

Conclusion: Rivaroxaban in the trough phase is comparable to warfarin in reducing D-dimer levels, but suppresses F1+2 levels less. Lack of correlation between rivaroxaban concentration and D-dimer and F1+2 levels suggests that the mere presence of rivaroxaban reduces their concentrations to therapeutic levels.

	Baseline	12 weeks	24 weeks
Naive group			
D-dimer (μg/mL)	0.45	0.31*	0.24*
F1+2 (pmlo/L)	235.0	164.0*	183.5*
Protein C activity (%)	104	100	103
Warfarin group			
D-dimer (μg/mL)	0.20	0.23	0.22
F1+2 (pmlo/L)	76.0	162.0*	163.0*
Protein C activity (%)	34	94*	95*

Data were presented as median value. *$P < 0.001$ compared with baseline.

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Assessing Falsely Elevated Mean Cell Volume (MCV) Due to Sample Transport Delay

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Background: The MCV is the least stable CBC parameter and is known to be temperature and time sensitive. CBC samples are commonly transported to centralized clinical laboratories, during which time samples might not be consistently maintained under appropriate conditions to preserve sample integrity. We sought to determine whether transport-related variables might account for an apparently high rate of unexpected macrocytosis from a physician practice location.

Methods: The University Hospitals Health System is an integrated health network with a courier system to transport laboratory samples from many practice locations to the main hospital laboratory for analysis. We queried the Laboratory Information System for all CBC results and turnaround times (collect - result) (TAT) from a single physician practice for 6 months. Pearson correlation of MCV with TAT was done and a correction formula for MCV was developed based on linear regression. A sample stability study was performed to confirm the interference of time and temperature on MCV on the Sysmex XN system.

Results: The sample stability study confirmed that MCV of samples stored at room temperature (RT) significantly increased as early as 4hs post collection. 697 sample from one physician office had a mean MCV = 95.2 fl and mean TAT = 8.57 hr. By regression analysis, a strong linear relationship of TAT and MCV ($r^2 = 0.6$) was demonstrated when TAT was expressed as discrete time intervals of one hour and MCV was represented by the mean MCV for samples collected over each successive hour. Based on this analysis, a correction of MCV over TAT can be calculated as: $MCV_{corrected} = MCV_{tested} - 7.4X$ TAT. Using this correction formula, 67/103 (65%) of samples with elevated MCV corrected to normal. Investigation of transport practices revealed that samples were transported in cool-bags, but typically sat at RT for variable periods of time before pick-up and after delivery to central hubs. As a result of this investigation, CBC samples are now kept in the refrigerator at all physician practice locations until pick-up and cool-packs are monitored for temperature. A follow up study 3 months after this intervention showed the average MCV dropped from 95.2 fl to 93.9 fl while mean TAT remained the same . By regression analysis, no significant correlation was observed between MCV and TAT (r^2=0.068).

Conclusions: We determined that inappropriate sample handling during transport caused false elevation of MCV in some samples and developed a mathematical model to estimate the incidence and magnitude of the errors. This method could be applied to other healthcare systems and reference labs that rely on sample transport as a quality assurance tool to monitor sample integrity.

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Sensitivity of Screening Panels For Monoclonal Gammopathies

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Background: Monoclonal gammopathies are caused by the production of a homogeneous monoclonal immunoglobulin or immunoglobulin part by a single clone of plasma cells. We evaluated the diagnostic sensitivity of serum protein electrophoresis (SPE), selective use of immunofixation electrophoresis (IFE) and immunoglobulin free light chain (FLC) assays that are recommended for identifying monoclonal gammopathies.

Methods: We investigated the clinical diagnosis and results of the 520 samples retrospectively sent to our laboratory on the same day for SPE, serum and urine IFE, serum FLC, over a 1-year period. These samples translated into 250 unique patients: Of the 250, 179 were excluded because their monoclonal protein had been previously recognized, leaving 71 newly diagnosed patients: 27 who had a newly identified monoclonal band on SPE and 44 who had no monoclonal band detected. 36 patients of these 71 patients were clinically diagnosed as monoclonal gammopathy. The sensitivity of each test alone or in a combination was calculated according to the diagnosis confirmed with biopsy. The detection of positive bands was confirmed by two experienced investigators, a positive FLC result was defined as an abnormal FLC κ/λ ratio (normal = 0.26-1.65).

Results: SPE, serum IFE, urine IFE and FLC assays did not perform well as single tests (72%, 86%, 44% and 75%, respectively). SPE, serum IFE, and serum FLC testing in combination is slightly more sensitive than SPE, serum IFE, and urine IFE in combination (90.6% vs. 89.6%, respectivey). Despite this observation, the difference in sensitivity for the detection of a monoclonal gammopathy comparing SPE + serum IFE + serum FLC testing versus SPE + serum IFE + urine IFE is not statistically significant (P>0.5). It can be argued that ordering only a serum sample (without a 24 h urine sample) is easier for the patient. To detect intact M-proteins, many researches use SPE, followed by serum IFE if an abnormality is detected. Although this approach is widely used, serum and urine IFE in addition to FLC provide the highest detection sensitivity (93.1%) and might be adequate without SPE, since the addition of SPE does not increase sensitivity (93.1 %). In our cohort of 520 patients, the ratio of elevated serum κ-FLC (high κ/λ ratio and high free kappa chains) in κ positive IFE samples was 67%, while the ratio of elevated serum λ -FLC (low κ/λ ratio and high free lambda chains) in L positive IFE samples was 73%.

Conclusion: In conclusion, depending on the availability of tests, combining serum IFE, urine IFE, and serum free light chain testing provides the highest sensitivity for the detection of a monoclonal gammopathy (93.1%), and addition of SPE to this panel does not increase the sensivity in detecting monoclonal gammopathies. Therefore, it is reasonable to consider IFE and serum free light chain testing as complementary tests and that each test provides important information.

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Evaluation of D-Dimer calibration verification sets to validate the linearity of the assay's analytical measurement range

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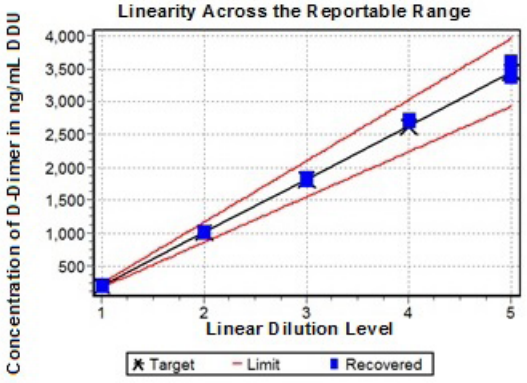
Background: Select coagulation/fibrinolysis assays were added to the College of American Pathologists (CAP) Checklist for calibration verification and Analytical Measurement Range (AMR) validation. Our objective was to address the CAP requirement for D-Dimer calibration verification and AMR validation.

Methods: D-Dimer was derived from human fibrin clots and five levels of equal delta concentrations were made in a human plasma matrix for the AMR from 150 to 3680 ng/mL, DDU, for the Instrumentation Laboratory (IL) systems and from 0.27 to 4.00 μg/mL, FEU, for the Diagnostica Stago systems. Each system used a single lot of D-Dimer reagents, calibrators and controls for these studies. We used the CLSI, EP05-A3 guidelines for Reproducibility, using the 3x5x5 (3 Lots of VALIDATE® D-Dimer x 5 days x 5 replicates/run on 1 instrument) and Precision using the 20x2x2x3 (1 Lot of VALIDATE® D-Dimer x 20 days x 2 replicates/run x 2 runs/day on 3 instruments) formats, respectively. Analyze-it, v4.60, was used for data analysis.

Linearity of sets was evaluated using MSDRx software. Three different IL systems were used at two laboratory sites.

Results: Reproducibility results for Levels 1 through 5, n = 75/Level, for the IL systems were 7.2, 4.1, 5.6, 3.7, and 4.2 total %CV. Precision results for Levels 1 through 5, n = 240 per Level, were between 2.9 and 10.0 total %CV for all Levels on the three lots of D-Dimer tested. Example of D-Dimer linearity on an IL instrument is shown below. Three different Stago systems were used at two separate laboratory sites and ran the identical study protocols. Reproducibility results were 15.5, 5.5, 4.7, 3.1, and 8.6 total %CV. Precision results were between 2.8% and 18.6 total %CV for all Levels.

Conclusion: The five level D-Dimer sets are acceptable for calibration verification for these manufacturer's claimed AMR.



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Effects of Centrifugation, Freezing, Thawing, and Re-centrifugation As Confirmed By Pro-coagulant Phospholipids on Specific Coagulation Parameters

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Background: Specific coagulation markers are generally aliquoted, stored and batch analyzed for economic efficiency or because they are sent to central laboratories. Pre-analytical factors should be carefully considered for these frozen samples. The presence of pro-coagulant phospholipids derived from circulating (platelets, white blood cells, red blood cells) and non-circulating (endothelial) cells within the vasculature may affect the results. In our study, we aimed to look for the effect of centrifugation, freezing, thawing conditions and double centrifugation or re-centrifugation -as confirmed by pro-coagulant phospholipid measurements- on specific coagulation markers.

Methods:

Blood samples were drawn from 10 non-smoking healthy donors after informed consent into citrated vacutainer tubes (Becton Dickinson, NJ, USA). None of the donors had a known coagulation defect or were treated with drugs that might affect coagulation or platelet function. Fresh samples were centrifuged at 2000xg for 15 min at room temperature once or twice and analyzed within two hours for FV, FVIII, FIX, FX, lupus anticoagulant, activated protein C-resistance (APC-R), and pro-coagulant phospholipids (Diagnostica Stago, France). At the same time, plasma samples aliquoted in polypropylene tubes (Eppendorf, Germany) were frozen at −20°C for 24 h. For observing the effects of thawing and re-centrifugation, frozen samples were thawed 24 h later in a 37°C thermostat controlled water bath in a duration of 5, 15 and 30 minutes, then either mixed by gentle inverting or re-centrifuged before analysis. Relative bias percentages from the baseline (as measured by immediate analysis after centrifuging either once or twice, according to manufacturer's suggestions) was calculated for each condition and compared with the current analytical quality specifications for desirable bias from the Westgard QC.

Results:

Phospholipid particles measured after double centrifugation as recommended by the manufacturer and the results were consistent and not affected from freezing and different thawing durations (all<±4%). Single centrifuged samples should either be analyzed immediately or centrifuged again after thawing for 5 min (-3.1% and -4.2% with respect to the baseline, respectively). Freezing significantly affected the results for FV activity for each tested condition and should be analyzed fresh. For FVIII measurements, although single centrifugation is recommended before freezing, we observed that only double centrifugation before freezing (3.8%), an additional centrifugation after thawing for 5 min (-4%) or double centrifugation without extending thawing duration more than 5 min (-7.5%) had acceptable biases (<8.9%). For FIX; analysis after double centrifugation without freezing was identical to baseline. Also, re-centrifuging after single centrifugation and thawing for 5 min (-2.3%), double centrifugation with a 5 min thawing (3.5%), and re-centrifugation after a 5 min thawing of frozen sample with double centrifugation (0.5%) gave acceptable results. FX seemed to be stable under every condition. Although double centrifugation is recommended for lupus anticoagulant assay, it was stable in each condition including single centrifugation (biases range between 0.003-0.78%). On the other hand APC-R should be analyzed fresh.

Conclusion: Increasing storage time will facilitate sample processing from off-site laboratories. In our study we demonstrated that the different storage and thawing conditions might affect coagulation testing. Laboratories should consider the pre-analytical variables accordingly during analysis and interpretation.

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Detection of hemoglobinopathies (HbS and HbC) based on the identification of hemoglobin variants by glycosylated hemoglobin analyzer.

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Background:

Structural hemoglobinopathies are due to mutations that cause changes in the DNA globin genes, leading to abnormal hemoglobin (Hb) / variants that may be either asymptomatic (Hb S / A or sickle cell trait) or associated with serious disorders such as Sickle-cell anaemia. More than 900 rare variants have been described. Most of them mimic common HB variants in hemoglobin analyzers. The most common ones are the Hb S, C, E, D and O; some of which are more common in certain geographic areas, such as Mediterranean area. The aim of our study is evaluate the benefits of screening for hemoglobinopathies based on identification of abnormal hemoglobin by glycosylated hemoglobin analyzer.

Materials and Methods:

From August 2015 to January 2016, 20.000 outpatient EDTA-blood samples were included in this study. Glycosylated hemoglobin and hemoglobinopathies detection was performed by high performance liquid chromatography (HPLC) on the analyzer Menarini Diagnostics HA-8180V and HA-8160, respectively. Samples that displayed error or borderline results were submitted to a reference hospital laboratory for further evaluation using the analyzer Biorad Variant 2, which is able to separate fractions of HbS or HBc.

According to our protocol, all the samples that displayed any anomalous band on the glycosylated hemoglobin (HbA1c) chromatograms were further examined in other specific Hematology equipment for the detection of hemoglobinopathies

Results:

During the period of August 2015 to January 2016, 20.000 samples of hemoglobin glycosylated were analyzed by the HA-8180V glycosylated hemoglobin analyzer. Thirty-seven samples displaying abnormal bands by the glycosylated hemoglobin analyzer HA-8180V were randomly selected for further evaluation by the hemoglobinopathies analyzer HA-8160. Of the 37 selected samples, 11 (29.7%) were diagnosed as sickle cell trait and the remaining 26 (70.3%) as Hb S or C heterozigous hemoglobipathies (ratio HbS /A approximately 35/65). These latter results were reported by the laboratory of Hematology with the following comment attached: "Additional test ordered by Hematology staff. Anomalous band was observed in the region S / C. Family screening for sickle cell trait is advised. Provided that the phenotype of sickle cell anemia can result from multiple combinations of molecular defects (Hb S / S, Hb S / C, Hb S / beta-logging), further confirmation by other tests (electrophoresis Hbs) is recommended".

Conclusion:

Taking advantage of the extra data provided by glycosilated hemoglobin analyzer (at no extra charge) we have described an approach for sickle cell trait screening in the daily laboratory routine. The importance of reporting on the trait or other altered hemoglobin is the possibility of further genetic counseling. In addition, information about the occurrence of this disorder in our area is also provided.

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Standardization and implementation of an eight color panel for flow cytometric immunophenotyping of bone marrow samples

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Background: Flow cytometry is a method that allows the multiparametric analysis of suspended cells. Recent advances in the use of flow cytometry made it possible to diagnose some hematopoietic pathologies by combining several antibodies in a single tube to evaluate all markers (antigens) of interest in the same sample fraction. Based on the Euroflow consortium, we have implemented an 8-color panel and new instrument standardization procedures for the immunophenotyping of bone marrow samples. **Objective:** To improve immunophenotyping processes and reduce overall time spent from instrument setup to sample acquisition and analysis by implementing new standardized procedures for instrument setup and an 8-color panel to evaluate the maturation of cell lineages in bone marrow samples. **Methods:** 20 bone marrow samples from different donors and with normal cell distribution were evaluated under two different protocols: **1)** 3- or 4-color panels manually compensated and multiple tubes per sample; **2)** 8-color panel automatically compensated and a single tube per sample. For the first protocol, three tubes were used to evaluate each sample: Tube 1, for granulocyte maturation (CD11b-FITC/CD13-PE/CD45-PC5); Tube 2, for erythrocyte maturation (CD71-FITC/CD36-PE/CD45-PC5); and Tube 3, for monocyte maturation and detection of CD34+ immature cells (CD64-FITC/CD34-PE/CD14-PC5/CD45-APC). The second protocol used a single tube to evaluate maturation of granulocytes, monocytes and erythrocytes as well as to detect CD34+ immature cells (CD36-FITC/CD13-PE/CD34-PerCP-Cy5.5/CD14-PE-Cy7/CD11b-APC/CD71-APC-H7/CD64-V450/CD45-V500). All samples were acquired on a three-laser BD FACSCanto II™ instrument with FACSDiva™ v6.1.3 software (BD Biosciences, San Jose-CA, USA). Instrument setup was performed with BD™ Cytometer Setup & Tracking (CS&T) beads and compensation was defined with BD™ CompBeads. Data files were analysed using Infinicyt v1.8 software (Cytognos S.L., Salamanca, Spain). **Results:** Following standardized procedures, automatic compensation of the 56 possible overlays from the 8-color panel (8 x 7 compensation matrix) was easily defined and in reduced time. It also hapened to be more reproducible than procedures used in the 3- or 4-color panels, therefore reducing the frequency in which a new compensation matrix needed to be defined (daily for 3- or 4-colors vs. monthly for 8-colors). Average time spent with data acquisition was measured in seconds (s) and results are expressed as mean ± SD. Protocol 1: 108s ± 148,7 ; Protocol 2: 35s ± 49,6. The expression pattern of the main cell populations was also compared by evaluating their Mean Fluorescence Intensity (MFI) and are reported as mean ± SD in Protocol 1; mean ± SD in Protocol 2. CD14+CD64+ (5.09 ±3,62 ; 4.99 ±3,60); CD71+CD36 (10.88 ±7,66; 10.14 ±7,15); CD11b+CD13 (64.49 ± 13,53; 65.12 ± 13,24); pan-leukocyte gating CD45+ (13.92 ± 10,48; 14.36 ± 10,45) and CD34+ immature cells (0.55 ± 0,45; 0.66 ± 0,45). Analysis time per sample was also improved since the number of data files was reduced from 3 tubes to 1 between protocols. **Conclusion:**These results demonstrate an improved overall productivity with the 8-color panel compared with the 3- and 4-color panel. Data quality was comparable between the protocols but the 8-color panel and its standardized procedures improved the efficiency by reducing time from instrument setup to data acquisition and analysis.

A-300

Hope Hemoglobin interfering with the measurement of Hba1c by HPLC ion exchange

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Background: Hemoglobinopathies involve genetic changes that determine hemoglobin variants which could produced clinical variations in their carriers. The main form of hemoglobin variant detection is made from the change in electric charge due to the exchange of amino acids. The Hope Hemoglobin is a variant hemoglobin. The high performance liquid chromatography (HPLC) - ion exchange, hemoglobin electrophoresis gel in alkaline pH and acid pH are used in the quantification of the anomalous fractions. Glicated Hemoglobin (HbA1c) is a molecule comprised of a glucose irreversibly bound at the terminal NH 2 (valine residue) of the beta globin chain of hemoglobin A. HbA1c is used to monitor long-term glycemic control and for diagnosing diabetes mellitus. Hemoglobin gene variants/modifications can affect the accuracy of some methods. The aim this study were demonstrated the interference and identify the variant hemoglobin in the measurement HbA1c.

Methods: Samples of 2 patients (a man, 40 years old and a japanese woman,69 years old) were sent to the laboratory for screening tests. Peripheral blood samples were collected in tubes containing EDTA at 5%. For the CBC test were used the XE-5000 hematology analyzer interconnect to the and SP.1000i analyzer (Sysmex Corporation, Japan). Serum levels of iron (Ferrozine) were performed on the Cobas 8000 P702 module and ferritin (electrochemiluminescence) in the Cobas e411 analyzer (both manufactured by Roche's Diagnostics Division, Basel, Switzerland). The Hemoglobin electrophoresis (agarose gel) in alkaline pH and acid pH using in SPIFE / REP equipment (Helena Laboratories Beaumont, Texas U.S.A.). To test the molecular DNA was isolated by QIAamp DNA Blood kit was amplified exons 1, 2 and 3, and introns 1 and 2, the beta-globin gene. The amplicons generated were subjected to DNA sequencing by the Sanger method (ABI-DNA sequencer, Model 3130 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) .The data were analyzed using the program Sequencer. The A1C on ion exchange (HPLC) Variant II and D-10 (Bio-Rad Laboratories, Inc., CA). **Results: :** The chromatograms from patient 1 and 2, it was observed A1c values of and 49.1% and 54.2% in HPLC Variant II and were confirmed in HPLC D-10. This findings showing inconsistent results with the biochemical parameters and clinical conditions. In agarose gel electrophoresis the variant fraction is faster than hemoglobin A at alkaline pH and it has a position similar to fetal hemoglobin in acid pH. In the exon 3 beta chain found a mutation where there is an exchange of G to A, position 136 with exchange of the amino acid glycine by Aspartic Acid.This features Hope hemoglobin. It has similar electrical charge to the glycated hemoglobin (A1C), performing the same retention time. Being hemoglobin with physiological changes has decreased affinity for oxygen. This mutation produce falsely elevated results because it has a similar retention time to Hba1c**.** Hope hemoglobin has increased affinity for oxygen and to be clinically silent heterozygous. **Conclusion:** Spurious HbA1c results can occur in the presence of hemoglobin variants as described in these two patients, bringing analytical challenges for the lab staff.

A-301

Redraw in The Hematology laboratory: Accuracy, Cost, and Necessity.

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Background: It is estimated that more than 70% of the clinical decisions for patient management are based on laboratory results; and ensuring that the laboratory delivers accurate results is very crucial to the patient and the doctor. However, producing accurate results require sometime a redraw of the sample either to confirm a result or because it is compromised. Beside the delay in reporting results which could cause delay in treatment for the patient, the redraw cost the laboratories an enormous amount of money, in addition to the frustration to the patients.

Method: more than 33,208 samples were collected for CBC from resident in Long-Term Care facilities, all tests were done using Beckman Coulter, DxH. Redraws were separated into preanalytic and analytic; they were separated further by the reason for the redraw. Statistical analysis was done using Analyse-it.

Results: 475 redraws were generated, 396 redraw were generated before analysis and 79 during analysis. More than 60% of the redraw before analysis were due to either missing lavender tube or drawing the wrong tube; 51.9% of the redraw upon analysis was due to platelets clumping, 46.8% due to failed delta and 1.3% was due to clotted specimen.

Conclusions: Preanalytical causes were responsible for the majority of the redraws. Laboratories should increase awareness of the effect of preanalytical factors on hematology results. Redraws can be reduced by developing clear procedure, enhancing employees training and periodic retraining, improve communication between laboratory sections, between laboratories and health care providers, and increase error detection by implementing and monitoring quality indicators. By lowering the preanalytical errors, patients will receive fast and accurate care, laboratory will save on the cost, and the relation between the laboratory and the requesting doctor/facilities will be enhanced.

A-302

Evaluation of the Sebia® CAPILLARYS™ 2 Flex Piercing analyzer for measurement of Hemoglobin A1c

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Background: Hemoglobin A1c (HbA1c) is integral for monitoring long-term glycemic control in diabetic patients and has also been recommended for use as a diagnostic test for diabetes. Methods for measuring HbA1c include immunoassays, ion exchange high performance liquid chromatography (HPLC), boronate affinity HPLC and more recently, capillary electrophoresis. It is imperative that methods used to measure HbA1c meet established performance goals and readily differentiate hemoglobin variants that may affect HbA1c quantitation. Objective: To evaluate the analytical performance of the Sebia® CAPILLARYS™ 2 Flex Piercing instrument for HbA1c measurement and compare HbA1c results obtained to ion exchange HPLC and boronate affinity HPLC methods. Methods: Precision studies were conducted using commercial quality control material (Sebia and Bio-Rad Laboratories) (intra-assay n=20, inter-assay n=40, 10 days). Limit of quantitation (n=32, 4 days), analytical measurement range (determined by mixing residual EDTA whole blood samples with high and low HbA1c concentrations) and analyte stability (n=10) were evaluated. Residual EDTA whole blood samples with the following characteristics were obtained: (i) samples without hemoglobin variants (non-variant, n=100); (ii) variants including HbC, HbD, HbE, HbS, HbAD Punjab samples; beta-thalassemia and high HbF samples; rare hemoglobin variant samples including Hb Wayne, Hb Athens Georgia, Hb Constant Springs, Hb Hope, Hb G Philadelphia, and Hb K Woolwhich (variant, n=95); (iii) samples provided and analyzed by the National Glycohemoglobin Standardization Program (NGSP, n=45); (iv) samples containing carbamylated, labile, and hemoglobin F peaks (interference, n=44). HbA1c was measured using Bio-Rad Variant™ II Turbo ion-exchange HPLC (VariantII), Trinity Biotech™ Ultra^2 boronate affinity HPLC (Ultra2), and Sebia CAPILLARYS 2 Flex Piercing capillary electrophoresis (Capillarys2) instruments. The NGSP sample group was also analyzed by the NGSP Tosoh G8 (Tosoh Bioscience) ion-exchange HPLC assay. Results: Imprecision studies demonstrated intra-assay and inter-assay coefficients of variation (CVs) of <2% across the measurement range using the Capillarys2. The CV at the limit of quantitation (3.8% HbA1c) was <2%. The verified analytical measurement range was 4.3-13.0% (slope=0.98, y-intercept =0.11, r^2=0.999). HbA1c was stable in whole blood stored ambient (20-25^{o}C), refrigerated (2-8^{o}C), and frozen (-80^{o}C) for 3, 7, and 60 days, respectively. Comparisons to the ion-exchange and boronate affinity HPLC methods using the non-variant and NGSP sample groups demonstrated excellent agreement (non-variant: Capillarys2=1.03(VariantII) - 0.18, r^2=0.998, range=3.1-16.5% and Capillarys2=0.99(Ultra2)-0.25, r^2=0.997, range=3.7-16.8%; NGSP: Capillarys2=1.02(VariantII)-0.12, r^2=0.998, range=4.6-10.4% and Capillarys2=1.08(Ultra2)-0.64, r^2=0.996, range=4.6-10.4%) with bias ≤0.3 units at 4.0, 5.6, and 6.5% HbA1c concentrations. Overall, samples with common hemoglobin variants including HbAS, HbAC, HbAD, HbAE, HbAD Punjab, beta-thalassemia, and HbF <15% demonstrated satisfactory agreement (bias ≤0.3 units) between the Capillarys2, VariantII and Ultra2 with the exception of HbAE and HbAD Punjab which were elevated by 0.7-1.4% HbA1c using the VariantII method. HbA1c measurements on the Capillarys2 were not affected by the presence of carbamylated, labile, or HbF fractions up to the manufacturer-established thresholds. Conclusion: The Sebia® CAPILLARYS™ 2 Flex Piercing instrument met established performance goals for HbA1c measurement with acceptable imprecision and bias compared to ion-exchange HPLC and boronate affinity HPLC methods. The Capillarys2 was also able to readily identify common hemoglobin variants and interferences.