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

M. Pizarro¹, C. Cepeda de Lacen², A. Laguer-Arroyo². ¹Laboratorio Clínico Chegar, Rio Grande, PR, ²Lab Care Instruments, San Juan, PR

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.

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.

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.

Reliability Proof of the Beckman Coulter DxH 500 System¹

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 weaknesses, 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:

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

P. Moez, R. Mofthah, H. Hamouda. Faculty of Medicine - Alexandria University, Alexandria, Egypt

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 anemia. 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 γγ 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 Xmn1 restriction enzyme. The mean age of SCD patients was 13.2 ± 2.4 years and controls was 10.1 ± 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 γγ gene was amplified using the primer 5’- AAC TGT TGC TTT ATA GGA TTT T-3’ and 5’- AGG AGC TTA TTG ATA GCA G-3’. Genotypes 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.
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.

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.

**Conclusions:** Nephrotic 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.

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

J. L. García de Veas Silva1, T. De Haro Muñoz2, R. Escobar Conesa2, R. Rios Tamayo2, M. Lopez Velez2, J. García Lario2, Complejo Hospitalario Universitario de Granada, Granada, Spain, Hospital Comarcal de Jarrío, Asturias, Spain

**Background:** The “International Kidney and Monoclonal Gammapathy 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 to a rapid identification of a monoclonal FLC as the possible cause of a tubular interstitial process.

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

M. R. Weik1, H. Ackermann1, M. Kahl1, H. Katsumi2, H. Kotake2, M. Slama2, Y. Tabuchi3, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, Sysmex Corporation, Kobe, Japan

**Background:** The objective of this study was to compare the performance of the Sysmex® CS-2100i 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 (PTsec: n = 302, PTinr: 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 (PTsec: n = 10, PTinr: 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 13,208 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-2100i 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-2100i 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)**

A. W. Zhang1, J. Zhang2, J. Che2, Y. Liu3, ‘Canyon Crest Academy, San Diego, CA, 1San Valley Biotechnology LLC, Beijing, China, 2Peking University People’s Hospital, Peking University Institute of Hematology, Beijing, China

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 manufacturer’s manual. Segmented copy number variations were calculated from SNP-array data using manufacturer’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 2250 proto-oncogenes or tumor-suppressor genes. The well-studied MDS associated genes, such as ASXL1, CBL, DNM3TA2, EZH2, KRAS/HRAS, 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 associated genes. 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 group 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**

O. - PORTAKAL, A. FAKBIYIK, Hacettepe University, ANKARA, Turkey

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 Emergency 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) (<20,000/µl, and >100,000/µl) 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 clinicians.

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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**


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 supports use of 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 Neoplastrine activation reagent and same lots of bi-level Stago QC materials for prothrombin analysis of method comparison data by Passing-Bablok regression 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).
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 performance stability with minimal variation among instrument types which, despite minor variations in averages and ranges, was consistently above the threshold of acceptability. 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

1University Hospitals Case Medical Center, Cleveland, OH, 2Bistat Solutions, Inc, Frederick, MD, 3Advanced Instruments, Inc., Norwood, MA

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 with the 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 were consistently better than manual counts, with Sysmex slightly better than GloCyte TNC counts have less variability than manual counts and appear clustered scatterplot graph that allows visualization of sigma performance over time. 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.

Regression Estimates for GloCyte and Sysmex TNC Compared to Manual Method

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<td>0.000 (0.422 - 0.95)</td>
<td>1.049 (1.000 - 1.238)</td>
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<td>Sysmex</td>
<td>0.980</td>
<td>0.356 (0.462 - 0.959)</td>
<td>1.205 (1.091 - 1.667)</td>
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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

S. Lange1, M. Mertz1, K. Madlener1. 1Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, 2Kerckhoff-Klinik GmbH, Bad Nauheim, Germany

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-2100i 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-2100i 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

F. Kitagawa1, S. Hiramitsu2, A. Kuno1, T. Fujita1, T. Ishikawa1, H. Naruse3, J. Ishii1, Fujita Health University, Toyoake, Japan, 2Hiramitsu Heart Clinic, Nagoi, Japan

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 to 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 analysis.

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
Sensitivity of Screening Panels For Monoclonal Gammopathies

E. Uner1, R. Turkal2, O. Kara3, T. Toptas¹, T. Firatli Tugular¹, O. Sirikci1, G. Haklar1, 1Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey, 2Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey, 3Department of Hematology, School of Medicine, Marmara University, Istanbul, Turkey

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 s/k 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%, respectively). 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 hr urine sample) is easier for the patient. To detect intact M-proteins, many researchers 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 λ-FLC (high s/k ratio and high free kappa chains) in s positive IFE samples was 67%, while the ratio of elevated serum λ-FLC (low s/k 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 sensitivity 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.

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

J. Herod1, G. Currier2, L. T. Salvatore3, D. A. Martin1, D. B. Roman1, J. Springford3, M. R. Tetreault1, R. K. Ito1, 1LGC Maine Standards Company, Cumberland Foreside, ME, 2Lahey Hospital & Medical Center, Burlington, MA, 3Dartmouth-Hitchcock Medical Center, Lebanon, NH, 4Holy Family Hospital, Methuen, MA

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 mg/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 3x5x3 (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.

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

M. Zhu1, L. Sandhaus1, E. Lavall1, C. Dillman1, K. Monheim1, 1University Hospitals Case Medical Center, Cleveland, OH, 2University Hospitals Medina Health Center, Medina, OH

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 4hrs 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 (r² = 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:

\[ \text{MCV}_{\text{corrected}} = \text{MCV}_{\text{baseline}} - 7.4 \times \text{TAT} \]

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 (r²=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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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, \( n = 75 / \text{Level} \), 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, 5.3, 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

A. Yaman¹, R. Turkal², P. Vatansever³, O. Sirikci, G. Haklat⁴. ¹Department of Biochemistry, Schoo of Medicine, Marmara University, Istanbul, Turkey, ²Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey

**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 2000g for 15 min at room temperature once or twice and analyzed within two hours for FV, FVIII, FIX, FFX, 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 \(-20^\circ\text{C}\) 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 \(\leq 4\%\)). Single centrifuged samples should either be analyzed immediately or centrifuged again after thawing for 5 min (\(\leq 3.1 \%\) and \(\leq 4.2 \%\) 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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Standardization and implementation of an eight color panel for flow cytometric immunophenotyping of bone marrow samples

A. F. Silva¹, M. A. Viana², V. P. Nascimento², C. R. T. Gody³, R. Pestana², E. X. Souza³, F. V. R. Maciel¹, A. C. C. V. Soares¹, M. L. C. R. Silva¹, O. Fernandes¹. ¹Diagnósticos da America S/A -DASA, São Paulo, Brazil, ²BD Biosciences, San Jose - CA, USA, ³São Paulo, Brazil

**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) 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- to 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/CD54-PC5); Tube 2, for erythrocyte maturation (CD71-ITC/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/CD54-PerC-Cy5.5/CD14-PE-Cy7/CD11b-APC/CD71-APC/CD64-VA50/C545-V500). All samples were acquired on a three-laser BD FACS Canto™ II instrument with FACS Diva™ 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™ CompBeats. 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 happened 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 ± 148.7s; Protocol 2: 35s ± 49.6s. The expression pattern of the main cell populations was also compared by evaluating their Mean Fluorescence Intensity (MFI) and were as 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-leucocyte 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.
Hope Hemoglobin interfering with the measurement of HbA1c by HPLC ion exchange

M. E. Mendes1, N. M. Viviani1, T. L. D’Agostini1, N. J. C. Duarte1, S. C. Ferreira2, J. E. Levy2, N. M. Sumita3. 1Central Laboratory Division Hospital das Clínicas Faculdade de Medicina of São Paulo University, LIM 03 FMUSP, São Paulo,SP, Brazil., 2Fundação Pró-Sangue Hemonceto de São Paulo, São Paulo,SP, Brazil, 3Fundação Pró-Sangue Hemonceto de São Paulo, São Paulo,SP, Brazil, São Paulo, Brazil

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 NH2 (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 interconnector to the and SP.1000i analyzer (Sysmex Corporation, Japan). Serum levels of iron (Ferrizone) 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 Sequence. 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 13 and 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. Because 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.

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


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.