Tuesday, July 28, 2015

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

Hematology/Coagulation

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Evaluation of Multiplex Ligation-Dependent Probe Amplification as a method for detection of IKZF1 (Ikaros) deletions in B-cell precursor acute lymphoblastic leukemia

J. Rodrigues[1], C. Campos[1], P. Silva[1], J. C. d. Neto[2], A. P. Dutra[2], M. A. D. Pianovski[3], E. K. Carbone[4], A. Ferreira[1], E. Mateo[1], J. G. Assumpção[5]. *[1]Hermes Pardini Institute, Vespasiano, Brazil, [2]Hospital Santa Casa de Misericórdia, Belo Horizonte, Brazil, [3]Hospital Erasto Gaertner, Belo Horizonte, Brazil, [4]Hospital Pequeno Príncipe, Belo Horizonte, Brazil, [5]Laboratório BIOCOD Biotecnologia Ltda., Belo Horizonte, Brazil*

The IKAROS (IKZF1) gene encodes a transcription factor that belongs to the family of zinc-finger DNA binding proteins associated with chromatin remodeling. IKZF1 transcription factor is crucial for many aspects of hematopoiesis. The expression of this protein is restricted to the fetal and adult hemo-lymphopoietic system and it functions as a regulator of lymphocyte differentiation. Total or partial deletions of IKZF1 are frequent in B-precursor acute lymphoblastic leukemia (pB-ALL), especially in adults positive for BCR-ABL. IKZF1 deletions have been associated with a poor prognosis in terms of overall survival and frequency of relapse. The aim of this study was to evaluate of IKZF1 deletions in B-ALL using different methodologies. A total of 34 non-consecutive pB-ALL patients, including 25 children and 9 adults, were enrolled in this study. Ten patients (3 adults) had been previously shown by RT-PCR to be BCR-ABL carriers. Bone marrow DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). IKZF1 deletions were identified using the SALSA P-202 B1 Multiplex Ligation-Dependent Probe Amplification (MLPA) assay (MRC-Holland). The analysis was performed using the GeneMarker v2.6.2 software. In 11 cases, DNA was also evaluated by Comparative Genomic Hybridization analysis (180K aCGH/SNPs; Agilent Technologies) and the data were analyzed with Agilent CytoGenomics Edition 2.9.2.4 software. MLPA analyses revealed that IKZF1 deletions occurred in 10 of pB-ALL cases. Deletions were present in 60% of the BCR-ABL positive cases, and in 17% of the BCR-ABL negative cases only. The extension of the deletions was variable, the most common ones comprised exons 4 to 7 (30%) or exons 1 to 7 (30%). Whole-gene deletions including all exons occurred in a single case, a finding consistent with the loss of a short arm of chromosome 7 and presence of a long arm isochromosome [i(7)(q10)] seen in the karyotype. Among IKZF1 deletion carriers, 4 had a normal karyotype, 4 presented complexes karyotypes including t(9;22) (q34;q11.2) and additional chromosome aberrations, and two had complexes karyotypes with aberrations other than t(9;22). aCGH confirmed the absence of IKZF1 deletions in 10 cases and presence of a deletion in one. In this latter case, the initial aCGH software

call indicated a deletion of exon 5 and its 5´intron, however a close analyses showed that the deletion extended to exon 7, as reported in MLPA results. Therefore in our study, IKZF1 deletions were associated with BCR-ABL fusion gene and comprised mainly exons 4 to 7, corroborating literature data. MLPA has the disadvantage of not detecting deletions affecting minor subclones (<20% of the cells), however their results were shown to be able to identify patients who subsequently relapsed better than gene expression-based assyas. In conclusion, the detection of IKZF1 alterations by MLPA commercial kit was relatively easy to perform, and allowed a good definition of partial gene deletions since the kit has two probes for each exon. Care should be taken during the technique standardization because some interpretation issues exist, such as low amplification of one probe only or amplification values close to the cut-off points.

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Increased Von Willebrand Factor and High Circulating Placental Growth Factor Correlate with Inflammation and Iron Overload in Patients with Compound Heterozygous Sickle Cell and Beta-Thalassemia

I. Papassotiriou[1], K. Larrisi[1], M. Politou[2], V. Komninaka[3], F. Apostolakou[1], C. Skevaki[1], E. Petridou[4], E. Terpos[5], E. Voskaridou[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Laboratory of Hematology and Blood Bank Unit, "Aretaieio" Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, [3]Thalassemia and Sickle Cell Disease Center, "Laikon" University General Hospital, Athens, Greece, [4]Department of Microbiology, "Aghia Sophia" Children's Hospital, Athens, Greece, [5]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece*

Background: Sickle cell disease (SCD) is characterized by pathological polymerization of hemoglobin, increased red cell rigidity and poor microvascular blood flow. Thus, hemolytic anemia, vaso-occlusion and vasculopathy are the hallmarks of its clinical presentation. Several other factors contribute to the clinical variability, which is present in SCD, including leukocyte dysfunction, platelet interactions with endothelial cells, pro-inflammatory cytokines, oxidative stress, reduced nitric oxide (NO) availability and hemostatic activation. Placental growth factor (PlGF) plays an important role in both inflammation and neoangiogenesis. Recently it has been reported that markers of iron overload are associated with high plasma level of PlGF and early mortality. Furthermore, in SCD patients, hemolysis can lead to a prothrombotic state by increasing the activity of von Willebrand factor (vWF). ADAMTS-13 is a member of the ADAMTS (A-Disintegrin-Metalloprotease- ThromboSpondin-type-1-repeats) family that cleaves vWF. Recent observations suggest that SCD patients suffer from an acquired ADAMTS-13 deficiency primarily because Hb competitively binds and blocks the proteolysis of vWF, leading to the accumulation of ultra-large VWF multimers in circulation and on endothelium. The aim of this study was to evaluate inflammation, endothelial dysfunction and angiogenesis in patients with compound heterozygous SCD and beta-thalassemia (HbS/βthal) and explore possible association with iron overload and other disease features.

Patients and Methods: Eighty-nine adult caucasian patients with HbS/bthal were included in the study, while 20 apparently healthy individuals served as controls. Patients with HbS/βthal divided in two groups: group A included 49 patients under hydroxycarbamide (HC+) treatment and group B included 40 patients without hydroxycarbamide (HC-) treatment. Along with hematology and blood chemistry parameters determination, measurements of circulating high-sensitivity C-reactive protein (hs-CRP), vWF, D-Dimers, ADAMTS-13, hs-Troponin-T (hs-TnT) and PlGF were measured in patients with HbS/βthal and controls using immunoenzymatic techniques.

Results: Levels of hs-CRP, vWF and PlGF were elevated in patients with HbS/βthal compared to controls (6.9±5.3 vs. 0.4±0.4mg/L, 170.1±78.3 vs. 85.3±22.1IU/dL and 20.2±7.6 vs, 15.3±2.4pg/mL, respectively, $p<0.001$), while ADAMTS-13 levels were decreased in patients with HbS/βthal compared to controls (965.2±244.0 vs. 1144.2±187.0pg/mL, $p<0.001$). No significant differences were found for the above parameters between patients of groups A and B. Hs-CRP correlated positively with vWF, PlGF and ADAMTS-13 ($p<0.01$). PlGF levels in patients with HbS/βthal correlated positively with markers of hemolysis such as reticulocyte counts, LDH and with uric acid levels ($p<0.01$). Ferritin levels correlated positively with hs-CRP, vWF, PlGF, D-Dimers and with markers of hemolysis ($p<0.01$). Furthermore, only 6 patients had slightly increased hs-TnT levels and almost all patients had pathologic D-Dimers levels.

Conclusions: Our findings demonstrate that patients with HbS/βthal have a significant degree of endothelial dysfunction as assessed by increased vWF. The increased levels of the D-Dimers in almost all patients indicate the activation of coagulation and fibrinolytic systems even in the steady state of the disease. Iron overload and inflammation along with reduced circulating ADAMTS-13 contribute, at least partially, to the increased levels of vWF. Similarly, inflammation and iron overload enhance the production of angiogenesis markers, such as PlGF suggesting a possible pathogenetic role for iron load in SCD biology.

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Preclinical validation of fluorescence in situ hybridization assay for detection of the AML1/ETO translocation

S. D. L. Cueva, F. K. Marques, R. S. Ribeiro, P. C. Ângelo, E. Mateo, A. Ferreira. *Hermes Pardini Institute, Vespasiano, Brazil*

Background:The Fluorescence in situ Hybridization (FISH) is a widely used tool to study hematologic diseases. FISH can be performed in metaphase or interphase cells, which is important when dealing with leukemia cell with low proliferation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of the assay for clinical use. Clinical laboratories must independently adopt protocols to verify the performance of the assay. Rearrangements involving the AML1 and ETO genes are generated by the t(8;21)(q21;q22) translocation and are present in 30-40% of acute myeloid leukemia (LMA) subtype M2 patients. The resulting chimeric fusion protein AML1/ETO inhibits a transcription factor that acts as a tumor suppressor and, therefore, has multiple effects on the proliferation, differentiation, and viability of the leukemic cells. The rapid identification of this rearrangement allows guiding prognosis and treatment.Objective: To validate FISH assay for detection of translocation AML1/ETO following recommendations from the American College of Medical Genetics (ACMG). Methods: We used the AML1/ETO Translocation, Dual fusion probe manufactured by Cytocell®. In the familiarization phase, the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff) we estimated the false positive rate from 10 uncultured bone marrow samples and 10 uncultured blood samples from non-hematological patients with normal karyotypes who would be unlikely to harbor theAML1/ETO translocation. Two analysts scored 500 interphase cells (250 per analyst). All AML1/ETO probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function. Results: The AML1/ETO kit presents the AML1 (21q22) and ETO (8q21) probes labeled respectively with red and greenfluorophore. A normal result should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicates the presence of the translocation.The probe demonstrated 100% specificity and analytical sensitivity.In the analysis of bone marrow and blood samples, we identified three and four atypical signal patterns, respectively. We did not observe change in the cutoffs with the increase in cell count. The signal patterns and their cutoffs for bone marrow samples were 2G1R (2,34%), 1G2R (3,1%) and 3G3R (2,34%). The signal patterns and their cutoffs for blood samples were1F1G1R (2,34%), 2G1R (3,1%), 1G2R (2,34%) and 3G1R (2,34%). These cutoffs were obtained from 200-cell count. The analyses of normal and abnormal samples by FISH were in agreement with the conventional cytogenetic. Conclusion: The FISH assay for detection of the AML1/ETO translocation showed excellent reproducibility and high quality in different hybridizations, and probe specificity higher than recommended by the ACMG.

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High sensitivity Hepcidin-25 bioactive ELISA: a sensitive, fast and straightforward competitive Elisa for the quantification of Hepcidin-25 in human serum and plasma

M. Herkert[1], B. Uelker[1], T. Dudek[1], C. E. Geacintov[2]. *[1]DRG Instruments GmbH, Marburg, Germany, [2]DRG International, Inc., Springfield, NJ*

Hepcidin is an iron homoeostasis regulator peptide. The bioactive peptide Hepcidin-25 is generated predominantly in the liver by proteolytic cleavage of the C-terminal 25 amino acids of prohepcidin. Subsequent N-terminal processing of Hepcidin-25 results in smaller peptides of 20-24 amino acids that show greatly reduced activity and accumulate in the urine.

Hepcidin exerts its regulatory function by inhibiting ferroportin, the major cellular iron exporter in the membrane of macrophages, hepatocytes, and the basolateral site of enterocytes. Hepcidin-25 induces the internalization and degradation of ferroportin, resulting in increased intracellular iron stores, decreased dietary iron absorption, and decreased circulating iron concentrations.

Hepatocellular hepcidin synthesis decreases under conditions of increased demand for circulating iron like iron deficiency, hypoxia, anemia, and erythropoiesis. In contrast, hepcidin synthesis is induced by inflammation and infection.

Serum Hepcidin-25 has been shown to add value to identify and differentiate specific disease conditions. Hepcidin deficiency causes hereditary hemochromatosis, characterized by body iron overload that may progress to liver cirrhosis. In addition, low Hepcidin-25 concentration can be induced by iron loading anemias and chronic hepatitis C. In contrast, high Hepcidin-25 levels have been found in iron-refractory iron-deficiency anemia, during infection, chronic kidney disease, and after intensive exercise, explaining the high iron deficiency among athletes.

The new high sensitive DRG Hepcidin-25 assay is a colorimetric solid phase enzyme-linked immunosorbent assay (ELISA) based on the competitive binding of Hepcidin of the sample and biotinylated Hepcidin to immobilized anti-Hepcidin antibody, followed by the detection with a Streptavidin-HRP conjugate.

The total assay time is 1.5 hours. The Elisa allows the quantitative determination of Hepcidin-25 covering a measuring range form 0.15-81.0 ng/mL. Serum and plasma (EDTA, heparin, Citrate) can be used for this assay. The analytical sensitivity of the assay is 0.153 ng/ml. The test shows good reproducibility with an intra-assay precision of 6.97% (mean of 20 repeated measurements of 3 different samples) and an inter-assay precision of 12.0% (average of 40 repeated measurements of 4 different samples by two observers on 20 days with 2 different lots). The recovery was determined with 97.3% (mean of 3 samples, each spiked with 4 concentrations of Hepcidin-25; range from 88.0-108.8%). Linear dilution gave an excellent overall recovery of 97.9% (mean of 3 samples, each diluted 4-fold with dilution buffer; range from 89.1-105.2%). We found no matrix interference with haemoglobin (up to 4 mg/ml), bilirubin (up to 0.5 mg/mL) and triglycerides (up to 7.5 mg/mL). Inter-Lot precision was 7.69% (mean of 3 samples measured with 3 lots in 6 determinations).

The new assay EIA-5782) shows a good correlation to the current manual Elisa (EIA-5258; y=0.991x+0.06; r = 0.992; n=72).

Benefits of the new assay are a very straight forward procedure with ready-to-use reagents, no shaking, high sensitivity, and a short total assay time of 1.5 hours.

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Lipid effect on antithrombotic and prothrombotic activities of thrombomodulin (TM)

V. Gruzdys, X. Sun. *Cleveland State University, Cleveland, OH*

Background:

Endothelial thrombomodulin (TM) plays central roles in haemostatic balance by facilitating both thrombin-mediated protein C (PC) activation (antithrombotic) and thrombin-activatable fibrinolysis inhibitor (TAFI) activation (prothrombotic). Activated PC (APC), an anticoagulant protease, inactivates coagulation factors Va and VIIIa, thus preventing excessive coagulation. Activated TAFI (TAFIα) cleaves C-terminal lysine and arginine residues from partially degraded fibrin, suppressing plasminogen activation and thereby delaying clot lysis. It has been known that lipid membrane serves as an essential cofactor for membrane protein functions. In this study, we proposed membrane mimetic systems of TM and different lipids for defining lipid effect on TM substrate specificity for PC and TAFI activation. The research provides a unique technique to understand the biological significance and predict the effect of the lipids on TM's capacity to alter APC and TAFIα levels thus modulating progression of thrombo-inflammatory pathologies such as sepsis.

Methods:

TM was incorporated into liposomes by a reaction with swelled lipid solutions of different composition. APC and TAFIα were generated by incubation with liposomal TM and thrombin in otherwise identical conditions. APC and TAFIα levels were measured by hydrolysis of spectrozyme PCa (spectrophotometrically) and hippuryl-arginine (HPLC), respectively. In addition, time scale profiles of TAFI activation were compared by densitometric analysis of SDS PAGE and activity in different lipids. All reactions were done in triplicate independent experiments, with controls and internal standards where appropriate.

Results:

Most significant increase in APC amount was observed with 5% phosphatidylethanolamine (PtEtn) liposomes (153 ± 5%) in comparison to free TM (69 ± 8%), where TM in phosphatidylcholine (PtCho) was set as a reference (100%). Incorporation of TM into 5% phosphatidylserine (PtSer) liposomes showed a decrease in APC amount (82 ± 18%). Further decrease in APC was observed in 10% PtEtn and PtSer liposomes (70 ± 21 % and 86 ± 26%, respectively). Competitive studies using co-incubation with TAFI showed significant decreases of APC in PtCho-TM liposomes (58 ± 14%) and in 10% PtSer as well as 10% PtEtn liposomes (24 ± 21 % and 34 ± 31 %, respectively). On the other hand, increase in TAFIα was observed in 5% PtEtn-TM (207 ± 16%) and 5% PtSer-TM (234 ± 21%) liposomes compared to PtCho-TM (100 %). Reduction of TAFIα generation in competitive conditions was observed (from 207 ± 16% to 83 ± 30 %) in 5% PtEtn liposomes.

Conclusion:

We determined that 5% PtSer and 5% PtEtn liposomes showed highest increases in TAFIα levels while only 5% PtEtn resulted in APC increase. PtSer had a reducing effect on APC generation. Higher concentrations of both PtSer and PtEtn resulted in reduction of TAFI and PC activation, both in regular and competitive scenarios. This demonstrates that PtEtn has a concentration-dependent effect on TAFI and PC activation while PtSer increases TAFI activation only. Given that PtEtn and PtSer both significantly increase in cell trauma, this study suggests a link between phospholipid exposure and reduced anticoagulant and anti-inflammatory potential of the endothelium.

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The Activity of Recombinant Endothelial Nitric Oxide Synthase Oxygenase Domain on Human Apo AI Derived Discoidal Lipid Particles

G. Altawallbeh[1], C. Verdi[1], M. Haque[2], D. Stuehr[2], M. Bayachou[1]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH*

Background: Cardiovascular diseases (CVDs) are the number 1 cause of death worldwide. Nitric oxide (NO), an important signaling molecule in the cardiovascular system, was recognized with a Nobel Prize in medicine in 1998. Endothelial nitric oxide synthase (eNOS) is one member of the nitric oxide generating family, and is the dominant isoform in the inner walls of blood vessels. It regulates numerous essential cardiovascular functions including vasodilation (blood pressure), inhibition of platelet aggregation and adhesion to the vascular wall, which prevents atherosclerosis and unwanted blood clots. eNOS dysfunction and disruption of nitric oxide release within the blood vessel wall is associated with the genesis of many aspects of CVD and its alarming death toll. **Purpose:** The goal is to develop a model that incorporates eNOS into miniature lipid membranes that we call nanodiscs. The concept is based on nascent discoidal high-density lipoprotein (nHDL) particles wherein the scaffold proteins that wrap around lipid bilayers are derived from human apolipoprotein A-I (apo AI). Our aim is to study the activity of the enzyme heme domain in its native microenvironment provided by this unique system and to quantify the effect of lipid membranes in the functional regulation of eNOS. This will shed light on the role of eNOS in maintaining vascular tone as well as on how eNOS dysfunction is involved in the onset of CVD and its progress. **Methods:** Nanodisc and eNOSoxy/nanodisc complex formation were prepared by mixing phosphatidylcholine/detergent micelles and apo AI engineered protein with and without eNOSoxy in a defined molar ratio. The self-assembly was initiated by dialyzing overnight and the purification was achieved by applying to a Superdex 200 10/30 gel filtration column on ÄKTA FPLC system using Tris-buffer at 0.5 ml/min. eNOSoxy concentration in nanodisc was verified by the characteristic ferrous heme-CO adduct absorbing at 444 nm via an extinction coefficient of $\varepsilon 444 = 76\ mM^{-1}\ cm^{-1}$.The activity of eNOSoxy was determined using a Spectra max plus 384 plate reader based on the Griess reaction. Catalysis of NO production from N-hydroxyarginine (NOHA) and H_2O_2 by eNOSoxy and eNOSoxy-bound nanodisc were assayed in 96-well microplates. Samples were run in triplicate (50 µl final volumes) and the assay plate was read at 540 nm. **Results:** The specific activity of eNOSoxy/nanodisc or free eNOSoxy is calculated in nmol NO/min/nmol of enzyme in the reaction volume at 37°C. The activity was determined by quantifying NO in the form of nitrite through a standard calibration curve (R^2=0.9979). The detected specific activity of nanodisc-bound eNOSoxy (49.0 ± 1.3) nmol/min/nmol was > 50% lower in comparison to the free eNOSoxy enzyme (132.4 ± 2.4) nmol/min/nmol. **Conclusions**: The analyzed data showed a decrease in NO generation by the nanodisc-bound eNOSoxy in contrast to free eNOSoxy. This study suggests that the membrane lipids affect the catalytic properties of eNOS heme domain. Clinically, since NO bioavailability correlates with endothelial dysfunction and consequently CVD, membrane lipid abnormalities could have possible implications on eNOS functionality. Future studies will test eNOS with various lipids combinations embodied in human endothelial cells.

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Molecular characterization and genotyping of alpha and beta thalassemias among anemic patients

M. K. Ahmed[1], N. H. Zakaria[2], N. Hassanien[3], H. S. Maher[4]. *[1]Molecular Biology Department - Al Borg Medical Laboratories (Head Quarter), Jeddah, Saudi Arabia, [2]Clinical Pathology Department - Faculty of Medicine - Alexandria University, Alexandria, Egypt, [3]Pediatric Department - Faculty of Medicine - Alexandria University, Alexandria, Egypt, [4]National Cancer Institute, Cairo, Egypt*

Background:-

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. Thalassemia is among the most common genetic disorders worldwide; 4.83 percent of the world's population carry globin variants, including 1.67 percent of the population who are heterozygous for α-thalassemia and β -thalassemia. In addition, 1.92 percent carries sickle hemoglobin, 0.95 percent carry hemoglobin E, and 0.29 percent carry hemoglobin C. Thus, the worldwide birth rate of people who are homozygous or compound heterozygous for symptomatic globin disorders, including α-thalassemia and β -thalassemia, is not less than 2.4 per 1000 births, of which 1.96 have sickle cell disease and 0.44 have thalassemias . Beta thalassemias are very heterogeneous at the molecular level. In most cases, mutations are single nucleotide substitutions, deletions or insertions of single nucleotides or small oligonucleotides. This disease diversity and the consequent variable degree of globin chain imbalance are the main determinants for milder phenotypes.

Method:-

One hundred patients recruited from the hematology polyclinic of private hospitals in Jeddah; KSA. DNA was analyzed using validated multiplex polymerase chain reaction (PCR) amplification of 22 Beta-globin gene mutations and 21 α globin gene mutations using biotinylated primers followed by reverse hybridization on test strip derived from ViennaLab Diagnostics GmbH Vienna, Austria. A confirmatory genetic testing was done after preliminary screening test using validated Sebia Capillary's technique for hemoglobin fractionation. Serum ferritin was determined in patients who had low mean cell volume (MCV) and low mean cell hemoglobin (MCH) to exclude presence ferrokinetic abnormalities.

Results:-

This study showed that the overall prevalence of SCD with thalassemia in KSA was 44% and according to DNA analysis the prevalence of (HbS+α-thalassemia) was 44% with HbSS in 12% and HbS in 32 % of cases; (HbS/β0-thalassemia) was detected in 4 % of cases, SCD alone was in 4% of cases respectively; while thalassemias alone was in 44% of cases; Combined both α and β thalassemias in 8% while β thalassemias alone in 12% and α thalassemias alone in 32% of cases . This study confirms presence of different combination between sickle cell gene, β0 Thalassemia and α-Thalassemia genes which means presence of a genetic variability among saudi population. The most common mutation among Beta-globin gene was the (IVS II-1 A>T) type with frequency of (6%), IVS I-5 (G>C) with a frequency of (4%), and the most common mutation among the α globin gene was deletion Δ 3.7 (-3.7 kb), found in 32% of cases. The α-thalassemia was more frequent among HbSS patients (44%) than S/β0-thalassemia patients (4%).

Conclusion:-

This study was done on small scale which did not by any means give an accurate account of the frequencies of HbS gene, β–gene, and α-gene in population of KSA. For this reason other population based studies are needed in all regions of KSA to elucidate the prevalence of SCD, alpha and beta thalassemia to build a solid scientific data which help in creating a national registry of Hemoglobinopathies and thalassemia in KSA.

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Validation of fluorescence in situ hybridization assay for detection of the t(11;14) (CCND1/IGH) translocation in a clinical laboratory

F. K. Marques, S. D. L. Cueva, P. C. Ângelo, E. Mateo, A. C. S. Ferreira. *Hermes Pardini Institute, Vespasiano, Brazil*

Background: The translocation t(11;14)(q13;q32) fuses the CCND1 and IGH genes, leading to cyclin D1 overexpression. This translocation is mainly found in mantle cell lymphoma, but also in B-prolymphocytic leukemia, plasma cell leukemia, chronic lymphocytic leukemia and multiple myeloma. Fluorescence in situ Hybridization (FISH) is a widely used tool to study hematologic diseases. FISH can be performed in dividing and nondiving cells. This is relevant to diseases with usually low mitotic

index and poor quality of metaphases, making it difficult to chromosomal analysis by conventional cytogenetic. The interphase FISH assay provides a reliable and routinely applicable tool for diagnosis of the t(11;14) translocation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of assay for clinical use. Clinical laboratories must independently adopt protocols in order to verify the performance of the assay. Objective: To validate FISH assay for detection of CCND1/IGH translocation following recommendations from the American College of Medical Genetics (ACMG). Methods: We used the CCND1/IGH Translocation, Dual fusion probe manufactured by Cytocell®. In the familiarization phase the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff) were estimate the false positive rate from 10 uncultured bone marrow samples and 10 uncultured blood samples that would be unlikely to harbor the CCND1/IGH translocation. Two analysts score 500 interphase cells (250 per analyst). All CCND1/IGH probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function available in Microsoft Excel. Results: The CCND1/IGH translocation probe presents the CCND1 (11q13) gene probe labeled with red fluorophore, and the IGH (14q32) gene probe labeled with green fluorophore. A normal result of using this probe should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicate the presence of the translocation. The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of bone marrow and blood samples were respectively identified ten and six atypical signal patterns. We didn't observe change in the cutoffs with the increase in cell count. The cutoffs obtained with BETAINV function were validated for counting 200 cells. The signal patterns and its cutoffs for bone marrow samples are 1F1G1R (5,1%), 1F1G2R (2,34%), 1F1R (1,49%), 2G1R (3,76%), 1G2R (4,43%), 1G1R (2,34%), 2G3R (1,49%), 3G2R (2,34%), 4G3R (2,34%) and 4G4R (2,34%). The signal patterns and its cutoffs for blood samples are 1F1G1R (6,26%), 2G1R (3,1%), 1G2R (3,1%), 1G1R (2,34%), 3G2R (1,49%) and 2F (1,49%). The analyses by FISH were in agreement with the conventional cytogenetic. Conclusion: The FISH assay for detection of the CCND1/IGH translocation was approved for clinical use.

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Performance of the Alpha-1-Antitrypsin assay for use on the Binding Site Optilite® turbidimetric analyser

K. MacDonald, J. R. Kerr, D. J. Matters, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Alpha-1-Antitrypsin (A1AT) is a serine protease inhibitor primarily acting on neutrophil elastase, protecting the lung from enzyme damage. Measurement of A1AT is of use in the diagnosis of several conditions including adult cirrhosis of the liver. Here we describe the performance of an A1AT assay for use on the Binding Site's Optilite analyser. Precision was determined with a CLSI protocol using 5 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers for 21 days, results are shown in table 1. Linearity was verified by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear over the range of 0.32-5.74 g/L; weighted linear regression gave y = 1.01x - 0.00 (r = 1.000) and analysis using StatisPro software in accordance with CLSI guidance confirmed linearity. This provides an assay measuring range of 0.35-5.00g/L using a 1/10 sample dilution. Interference was tested by spiking base pools at three levels (0.68, 0.89 and 1.42 g/L) with 200mg/L bilirubin, 5g/L hemoglobin, 500mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control. Interference at all analyte concentrations of <3.5% was seen with bilirubin, hemoglobin and triglycerides, for intralipid the interference was <8.40%. Correlation to the Binding Site A1AT assay for the SPAPLUS® was performed using 124 samples; 102 from disease state patients and 22 from healthy blood donors (total range 0.40-4.73g/L). This demonstrated acceptable greement when analyzed by Passing-Bablok regression; y=0.98x + 0.01. We conclude that the A1AT assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of A1AT Optilite assay

Analyte Concentration	0.565g/L	0.675g/L	0.829g/L	1.139g/L	4.328g/L
Total precision (%CV) (Acceptance <10%)	4.7%	4.2%	4.5%	3.6%	4.0%
Within run precision (%CV) (Acceptance <5%)	1.4%	0.9%	1.4%	1.1%	1.3%
Between run precision (%CV) (Acceptance <8%)	2.6%	2.7%	2.4%	1.8%	1.9%
Between day precision (%CV) (Acceptance <8%)	3.7%	3.1%	3.5%	3.0%	3.2%

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Performance of a Haptoglobin assay for use on the Binding Site Optilite® turbidimetric analyser.

E. Hardy, J. Kerr, A. Alvi, D. J. Matters, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Haptoglobin is an acid α2 acute-phase plasma glycoprotein and binds specifically to free plasma oxy-hemoglobin. The high molecular weight complex prevents filtering of hemoglobin by the kidneys. Low levels of Haptoglobin are associated with hemolytic anemias and liver disease. Here we describe the performance of a Haptoglobin assay for use on the Binding Site's Optilite analyzer. Precision was determined with a CLSI protocol using 8 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers over 21 days, results are shown in table 1. Linearity was verified by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear over the range of 0.139-4.472g/L; weighted linear regression gave y = 1.00x - 0.05 (r = 0.999). This provides an assay measuring range of 0.26-4.0g/L using a 1/10 sample dilution, with a sensitivity of 0.026g/L. The upper limit of the range is 8g/L at 1/20. Interference was tested by spiking base pools at five levels covering the range 0.1 - 5.00g/L with 200mg/L bilirubin, 2000mg/dL intralipid and 500mg/dL triglycerides and comparing with a negative control. Interference of <6.38% was seen at all analyte concentrations with bilirubin and triglycerides. Lipemia interference with intralipid was successfully detected by the blank absorbance flag utilized in this assay's parameters. Correlation to the Binding Site Haptoglobin assay for the SPAPLUS® was performed using 148 samples; 99 from disease state patients and 49 from healthy blood donors (total range 0.128g/L to 6.604g/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=0.99x + 0.01. We conclude that the Haptoglobin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of the Haptoglobin Optilite assay

Analyte Concentration	0.109 g/L	0.262 g/L	0.388 g/L	1.024 g/L	1.447 g/L	2.448 g/L	3.246 g/L	5.520 g/L
Total precision (%CV) (Acceptance <15%)	5.8%	3.4%	9.9%	5.1%	4.0%	3.2%	3.1%	5.3%
Within run precision (%CV) (Acceptance <6%)	1.3%	0.8%	1.7%	1.1%	1.7%	1.1%	1.3%	1.7%
Between run precision (%CV) (Acceptance <6%)	2.8%	1.6%	4.7%	2.9%	1.9%	1.8%	1.6%	1.6%
Between day precision (%CV) (Acceptance <10%)	4.9%	2.9%	8.6%	4.0%	3.1%	2.4%	2.3%	4.8%

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Performance of serum IgA Kappa and IgA Lambda assays for use on the Binding Site Optilite® protein analyser

L. D. Southan, A. Brown, M. D. Coley, A. Kaur, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Measurement of serum IgA Kappa (IgAκ) and IgA Lambda (IgAλ) has been shown to be of use in the detection and monitoring of monoclonal ammopathies. Elevated monoclonal protein production is indicative of an underlying abnormality such as MGUS, multiple myeloma & other disorders. International guidelines recommend SPE densitometry is performed to quantify monoclonal proteins, however monoclonal IgA can often be obscured by other proteins in the β region of a SPE gel. Turbidimetry

can be used in these instances to measure both IgA Kappa & IgA Lambda and give a more accurate representation of tumour production. Furthermore, calculation of the IgA Kappa/Lambda ratio & comparison with values found in normal subjects can give a more sensitive indication of clonality and will also compensate for any changes in plasma volume. Here we describe the performance characteristics of IgAκ and IgAλ assays (Hevylite®, The Binding Site) for use on the Binding Site's Optilite® analyser. The assays have measuring ranges of 0.18-11.20g/L for IgAκ (reference interval 0.480-2.82g/L) and 0.158-10.40g/L for IgAλ (reference interval 0.360-1.980g/L) at the standard 1/10 sample dilution, with sensitivities of 0.018g/L and 0.015g/L respectively. High samples are automatically remeasured at a dilution of 1/60 or 1/100, with upper measuring ranges of 1.80-12.00g/L (IgAκ) and 1.58-104.00g/L (IgAλ). Precision was assessed according to CLSI (EP05-A2), measuring samples at 5 concentrations, on 3 kit lots and 3 analysers over 21 days. Precision acceptance was <10% CV. Linearity was assessed by assaying a serially-diluted sample pool across the width of the measuring range and comparing expected versus observed results, with recovery required to be <10% at each level. Interference was tested by running the common interferents of triglyceride (10g/L), bilirubin (0.2g/L), haemoglobin (5.0g/L) and 17 potential drug interferents at 4 levels, acceptance being <10% difference to a negative control. Correlation to the Binding Site IgAκ and IgAλ assays for the Siemens BN™II was performed using 140 samples from normal subjects and patients with multiple myeloma as well as other monoclonal gammopathies (Range 0.043-57.46g/L κ, 0.038-20.793g/L λ). Acceptance was a Passing-Bablok regression slope of 0.9-1.1. Within-run CVs were 1.4% (0.31g/L), 2.8% (0.85g/L), 4.3% (1.63g/L), 2.0% (2.59g/L) and 1.4% (9.17g/L) for IgAK, 1.6% (0.28g/L), 3.3% (1.01g/L), 3.8% (1.86g/L), 1.8% (2.48g/L) and 1.8% (8.75g/L) IgAλ. Total precision CVs were 6.7% (0.29g/L), 3.8% (0.85g/L), 6.5% (1.65g/L), 5.1% (2.45g/L) and 4.9% (9.11g/L) for IgAK 6.8% (0.30g/L), 4.7% (0.93g/L), 9.3% (1.63g/L), 9.1% (2.32g/L) and 3.6% (8.58g/L) IgAλ. The assay was shown to be linear over the standard measuring range of the assays; y=1.00x+0.001 (R^2=0.999) IgAκ and y=1.02x+0.00 (R^2=1.000) IgAλ. No significant interference was observed at any level with the interferents studied. Correlation with the IgAκ and IgAλ BNII assays demonstrated good agreement when analysed by Passing-Bablok regression; y=1.09x+0.05 IgAκ and y=1.05x-0.02 IgAλ. We conclude that the IgAκ and IgAλ assays for the Optilite analyser provide a reliable, accurate and precise method for quantifying IgAκ and IgAλ in serum and the presence of an abnormal ratio may be useful in identifying patients with IgA myeloma.

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Evaluation of a latex-enhanced IgD assay for use on the Binding Site Optilite® turbidimetric analyser.

D. J. Matters, J. R. Kerr, A. Alvi, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Measurement of IgD is of use in both the monitoring of IgD myeloma and in the diagnosis of Hyperimmunoglobulinaemia D syndrome. Here we describe the evaluation of an IgD assay for use on the Binding Site's Optilite® analyzer. The assay time is 10 minutes and was read at end-point. The assay range is 13 - 210 mg/L using a 1/10 sample dilution, with a sensitivity of 13mg/L. The upper limit of the range is 16800 mg/L, utilizing auto-redilutions. Correlation to the Binding Site IgD assay for the SPAPLUS was performed using 93 samples including 43 from healthy donors (total range 12.355 - 14049.78mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.95x + 1.22. Precision was assessed with a protocol based on CLSI (EP05-A2) using 3 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers over 21 days, results are shown in table 1. Linearity was assessed by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was shown to be linear over the range of 12.594-244.847mg/L which exceeds the assay measuring range; weighted linear regression gave y = 1.00x - 0.30 (r = 1.000) and analysis using StatisPro software in accordance with CLSI guidance confirmed linearity . Interference was tested by spiking base pools at three levels (83.36, 133.38 and 165.46mg/L) with 200mg/L bilirubin, 5g/L hemoglobin, 2000mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control. Interference at all analyte concentration of <1.5% was seen with haemoglobin and bilirubin, for intralipid and triglycerides the interference was less than <4%. We conclude that the IgD assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of IgD Optilite assay

Analyte concentration	23.27mg/L	110.20mg/L	165.27mg/L
Total precision (%CV)(Acceptance <10%)	4.0%	3.2%	3.2%
Within run precision (%CV)(Acceptance <5%)	2.7%	2.1%	2.0%
Between run precision(%CV) (Acceptance <8%)	2.9%	1.6%	1.6%
Between day precision(%CV) (Acceptance <8%)	0.0%	1.8%	1.9%

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Multivariable Graphic and Numerical Statistical Techniques for Comparing the Performance of Two Hematology Instruments. A Practical Example with Sysmex EX-5000®and Sysmex XT4000®.

V. M. Genta[1], W. Tang[1], J. White[1], D. Washburn[2]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Healtcare, Norfolk, VA*

Background: Sysmex XE-5000® and Sysmex XT-4000® determine

WBC, platelets, reticulocytes, and RBC counts, HGB, HCT, and the WBC

differential for patient specimens in an 'open' and 'closed' mode. Consequently, for evaluating their performance two modes for each analyte have to be simultanously compared. We employed multivariable statistical models and their graphic representations to asses simultaneously the performance of the two instruments operating in the two modes. ***Methods***: The precision was evaluated by assaying quality control material (E-CHEK® (XE) and (XT) Lot # 2201, Sysmex) with five independent runs for each day for five consecutive days. Linearity was evaluated by assaying at least five levels of linearity material (Range Check XII® Lot # 2184, and Lot # 2192, Sysmex) with five independent runs in one day. The performance with patient specimens was assessed by assaying 52 patient specimens in parallel and within 30 minutes. The observations were transferred to Minitab® (Version16, Minitab Inc.) and analyzed with the general linear model (GLM) to compare multiple means, the polynomial regression model, their diagnostics and their graphical representations. *Results*: For the precision study the GLM showed that while for WBC, platelets, and HGB there were no statistically significant differences between instruments and modes (P>0.05) for RBC, HCT and reticulocytes showed statistically significant differences between instruments and/or modes (P<0.001). The parallel box plots by day, instrument, and mode clearly illustrated the differences between the means of instruments and modes. The polynomial regression analysis showed linear performance (pure error test, P>0.05) and statistically significant differences between instruments for RBC, HCT, and reticulocytes regression lines (P<0.001). Since the differences could have been significant for patient care, patient specimens were assayed in parallel with both instruments. The regression model corroborated the differences between instruments: RBC y=-0.2+1.1x; HCT y=0.2+1.1x; reticulocytes y=0.05+0.98x. The plots of the differences showed for XT-4000 a mean bias of 4% for RBC (minimum 0.7 maximum 7.8%), and of 5.5% for HCT (minimum 1.4 maximum 8.3%) and no clinically significant bias for reticulocytes (mean 0.01, minimum -0.5, maximum 0.4). Since the XE-5000 was considered our reference instrument the manufacturer representative was consulted to recalibrate the XT-4000. After recalibration, comparison with patient specimens showed no clinically significant differences for XT-4000; RBC: mean bias 1.5% (minimun 0%, maximum 3%), HCT mean bias -0.8% (minimum -2.5%, maximun 1.5%). *Conclusion*: These results clearly showed that the graphical and numerical multivariable statistical analysis techniques could immediately demonstrate differences between instruments performance. Further multivariate and univariate analysis on patient specimen determined that the differences were clinically significant. The secondary instrument was recalibrated, and the differences were clinically acceptable. Finally, the availability of statistical software, such as Minitab®, allowed the laboratorian to perform numerical and graphical data analysis simultaneosly on several instruments and parameters.

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Myeloperoxidase enzyme deficiency detected by peroxidase cytochemistry method available in Hematology system

L. E. Fedalto, D. C. M. G. Cerqueira. *LANAC, Curitiba, Brazil*

Background: The myeloperoxidase (MPO) is an enzyme found in white blood cells (neutrophils, monocytes and eosinophils). This enzyme is involved in the killing of several micro-organisms and foreign cells, including bacteria, fungi, viruses, red cells, and malignant and nonmalignant nucleated cells. Despite the primary role of the oxygen-dependent MPO system in the destruction of certain phagocytosed microbes, there are subjects with total or partial MPO deficiency. Infectious diseases, especially

with species of Candida, have been observed predominantly in MPO-deficient patients who also have diabetes mellitus, but the frequency of such cases is very low, less than 5% of reported MPO-deficient subjects. This study investigates the incidence of the myeloperoxidase deficiency (MPO-def) in patients of LANAC Laboratory.

Methods: Approximately 40.000 blood counts were performed from October to December, 2014, by LANAC Laboratory using the equipment ADVIA® Hematology (Siemens Healthcare Diagnostics). 22 samples were identified with MPO-def through the flags and charts released by the equipment.

Results: The report of these 22 samples contained the following observation: "Sample result suggestive of Myeloperoxidase Deficiency" because they presented the MPO Def flag, MPXI and Perox cytochemistry characteristic, revealing weak staining of the cells by hydrogen peroxide method "cytochemistry of peroxidase" available in electronic analyzers ADVIA Hematology line. In this method, leukocytes are identified based on the size and intensity of the peroxidase reaction. As neutrophils, eosinophils and monocytes are stained revealing the existing concentration of peroxidase. Other white blood cell does not contain peroxidase and thus do not stain. The cytology of available Perox therefore has a characteristic saturation area associated with the flag MPO Def, allows the analyst to suggest MPO deficiency on the report. These 22 samples represent 0.05% of this lab routine during the study period.This profile had prevalence in pregnant patients, children and patients who were making use of some anti-inflammatory.

Conclusion:

Through the peroxidase cytochemistry Methodology available in ADVIA Hematology line, it was possible to suggest in report the deficiency of the enzyme myeloperoxidase for these 22 patients. This observation certainly contributes to the clinical conduct, making this methodology an excellent alternative for clinical laboratory routine.

A-191

Evaluation of an IgG4 assay with increased measuring range for use on the Binding Site SPAPLUS® automated analyser.

C. T. Wilson, K. Sharp, P. J. Showell. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Measurement of IgG4 is useful in the detection and monitoring of primary immunodeficiency (PID) disorders, but is increasingly used to detect disorders associated with hyper-elevation of serum IgG4, notably autoimmune pancreatitis (AIP). Here we describe the evaluation of an improved IgG4 assay for use on the Binding Site SPAPLUS platform, incorporating an increased measuring range and prozone protection to facilitate measurement of AIP samples. The assay measuring range is 0.030 - 2.6g/L using a 1/20 sample dilution, with sensitivity at 1/1 of 0.003g/L and an upper limit of 13g/L using automatic sample redilution (1/100). Prozone protection is programmed to monitor reaction kinetics and identify samples with a fast, early response indicative of antigen excess. Such samples are indicated with a 'P' flag and are automatically rediluted if preset criteria are exceeded. Precision was assessed according to CLSI (EP05-A2) using 8 samples (0.02 - 4g/L), on three kit lots and three analysers over 21 days. Interference testing was carried out at five analyte concentrations with Intralipid (1%), triglyceride (1%), bilirubin (200mg/L), haemoglobin (5g/L) and a panel of 17 commonly prescribed drugs. Linearity was tested across a range exceeding the reportable range using a series of dilutions of elevated and depleted pools. Prozone functionality was challenged on three kit lots by running 60 samples with an antigen concentration up to an equivalent of 45g/L. Correlation to the original SPAPLUS IgG4 kit (measuring range 0.030 - 0.850g/L) was carried out using 229 serum samples, incorporating 72 disease state samples, of which 20 were IgG4 deficient and 34 were above the normal range, including AIP positive samples up to a level of 8.5g/L. The precision testing returned within run CV's of 0.9% (0.024g/L), 1% (0.030g/L), 4.0% (0.056g/L), 1.7% (0.298g/L), 1.2% (0.756g/L), 1.2% (0.998g/L), 1.8% (1.889g/L) and 2.2% (4.216g/L). Total precision CV's were 7.2% (0.024g/L), 6.7% (0.030g/L), 10.1% (0.056g/L), 5.7% (0.298g/L), 5.2% (0.756g/L), 6.3% (0.998g/L), 5.8% (1.889g/L) and 6.6% (4.216g/L). No significant interference (<10%) was observed with any of the chemical or biological interferents tested. Linearity was demonstrated across the range 0.024 - 2.7g/L with a correlation y=1.0092x+0.0511. Prozone protection was demonstrated to a minimum of 45g/L with three kit lots, on two occasions each on separate analysers. All elevated samples from 2.6 - 45g/L were correctly reported as >2.6g/L or 'P' flagged and auto-rediluted at 1/100. Analysis of reaction kinetics confirmed there was no undetected antigen excess. Correlation to the original SPAPLUS IgG4 kit using Passing-Bablok regression returned an agreement of y=0.99x+0.00 on a sample range from 0.016g/L to 8.5g/L. We conclude that the extended range IgG4 assay for the Binding Site SPAPLUS shows good performance and agreement with existing assays and allows accurate measurement of elevated IgG4 conditions without the possibility of false low results through prozone.

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Performance of a low level albumin assay for use on the Binding Site Optilite® turbidimetric analyser.

D. J. Matters, F. Murphy, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Serum is the predominant source for albumin present in the cerebrospinal fluid (CSF), as regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can be indicative of barrier dysfunction and/or local synthesis of immunoglobulin within the central nervous system. Early detection and treatment of nephropathy is important in preventing renal failure in insulin-dependent diabetics. Elevated urinary albumin concentration is a good indicator of glomerular damage in such patients. Increased albumin excretion is also a marker of future cardiovascular problems in non insulin-dependent diabetes mellitus and also occurs in other chronic conditions such as hypertension, malignancy and chronic obstructive airways disease. Here we describe the performance of a low level albumin assay for measurement of CSF and urine samples on the Binding Site's Optilite analyser. Precision was verified using a protocol based on CLSI (EP05-A2) testing samples spiked with purified albumin to give CSF levels of 145.49, 281.51, 439.90, 593.11 and 975.24mg/L and urine levels of 22.98, 39.04, 153.40, 275.05, 1490.18mg/L, with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers for 21 days. The acceptance criteria for total precision was a CV of <10%. For both CSF and urine samples at all levels the total precision gave CV's of ≤8.2%. Linearity was verified by assaying a serially-diluted CSF or urine sample across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear for CSF over the range of 9.23-373.11mg/L. For urine, the assay was linear over the range of 8.09-397.77mg/L. This provides an assay measuring range of 11-332mg/L at neat, with an upper limit of 16600mg/L utilizing auto-dilutions. CSF interference was tested by spiking CSF pools at two levels (159.52 & 371.26mg/L) with 200mg/L bilirubin and 5000mg/L haemoglobin. Urine interference was tested by spiking urine pools at two levels (29.33mg/L and 495.80mg/L) with 200mg/L bilirubin, 200mg/L ascorbic acid, 1000mg/L total protein and 250mg/L haemoglobin. All results were compared against a negative control. Interference at all analyte concentrations in CSF was <8% and in urine was <11%. Correlation to the Siemens BNII albumin CSF assay was performed using 124 samples (total range 30.3-1340mg/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=1.01x + 10.63. Correlation to the Siemens BNII albumin urine assay was performed using 162 samples (total range <2.12-7350mg/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=1.06x - 0.37. We conclude that the low level albumin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

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The frequence of factor V de Leiden and prothrombin gene mutation (PTM) in results from a large Brazilian laboratory database.

A. K. Zeinad-Valim, R. M. Cosentino. *DASA, Sao Paulo, Brazil*

Background: The factor V Leiden (FVL) is the most common inherited risk factor that predisposes venous thromboembolism (VTE) in Caucasian individuals. The prevalence of the mutation (G1691A) varies between different ethnic groups and geographically. The FVL is very rare in African blacks, Asians, and native populations of America and Oceania. On the other hand, the highest prevalence is found in European with allele frequency ranging from 1.4% to 7%. The presence of FVL may be associated with VTE and possibly obstetric complications. Sometimes it is assumed that the identification of a positive result will always be useful, but if positive, can lead to anxiety by the diagnosis of a genetic disease. Although thrombophilia testing can identify positive cases, the evidence that an individual has the diagnosis rarely influences the clinical management.

Objectives: To evaluate the incidence of FVL and prothrombin gene mutation (PTM) in heterozygous and homozygous states, and the association of the two mutations in 3065 samples evaluated in a Brazilian laboratory.

Methods: We analyzed 3065 samples in 28 days in 2014; 1973 with the search for FVL mutation and 1092 samples for the PTM. The prevalence of the presence of mutations in heterozygous and homozygous states, and its association in the same individual was evaluated.

Results: The prevalence of the FVL mutation, searched in 1973 samples was 5.06% (n = 100) in heterozygous and 0.05% (n = 1) in homozygous state. The mutation of the prothrombin gene (1092 samples) was heterozygous in 4.39% (n = 48), homozygous 0.18% (n = 2), and the double heterozygous (FVL and PTM) was found in the same individual in 0.097% of samples (n = 3 in 3065 samples).

Conclusion: Heterozygous FVL mutation is associated with 4 to 5 times increased risk of VTE and prothrombin gene mutation 2 to 3 times. The presence of mutations in homozygous and double heterozygous for FVL and PTM indicate a much higher risk of thromboembolic events, and the change in medical management in these individuals with a previous thromboembolic event is necessary, requiring anticoagulation indefinitely and need for antithrombotic prophylaxis during pregnancy and postpartum. The prevalence of the FVL mutation in the Caucasian population is 5%, similar to that found in our sample. In a previous study in the Brazilian population, the incidence of FVL and PTM in heterozygous states was observed in 1.2% and 0.5%, in 400 blood donors. Since the Brazilian population is mixed, we can conclude that the tested individuals at our study should correspond to a population with a higher incidence of VTE or obstetric complications.

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Development of A Novel Device to Assess Hemostatic Function from Whole Blood

E. A. Ferrante, K. R. Blasier, T. Fischer, F. Viola. *HemoSonics LLC, Charlottesville, VA*

Background: Previous studies have shown the benefit of rapid information to guide treatment of excessive bleeding in the clinic, including targeted transfusion and better outcomes. However, no point of care (POC), comprehensive, whole blood assay exists. Current tools provide limited information slowly and often require skilled technicians. HemoSonics LLC is developing the QuantraDx™ point-of-care (POC) system, which provides time critical, quantitative information on hemostatic subsystems in whole blood.

Aims: To demonstrate the ability of the QuantraDx to provide rapid and comprehensive results on the treatable causes of bleeding at the POC.

Methods: The QuantraDx applies ultrasound technology to estimate the shear modulus (stiffness) of whole blood during the process of coagulation. The device incorporates a multi-well cartridge with embedded reagents. The clotting time and stiffness values measured in each well are used to quantify the treatable components of hemostasis. QuantraDx reproducibility was assessed with kaolin and thromboplastin activation with a novel blood-derived control sample (n = 21 for kaolin, n = 28 for thromboplastin) and whole blood (n = 11 for kaolin, n = 12 for thromboplastin) from healthy volunteers. Additional whole blood samples (n = 5) were tested with varying amounts of abciximab (0, 24, 32, 64ug/ml), a potent platelet inhibitor, to challenge the device with lower stiffness clots and assess accuracy at low dynamic range threshold.

Results: Control tests show average clotting times of 3 to 5 min with kaolin or thromboplastin with 3.5 and 9.4% CVs respectively. Average clot stiffness was 690 Pa for kaolin (8.1% CV) and 781 Pa for thromboplastin (10% CV). When testing with whole blood, intra-cartridge average clotting time for kaolin activation was 3 minutes (2% CV), while average clot stiffness with thromboplastin was 2944 Pa (4.75% CV). Increasing the amount of abciximab in the sample progressively reduced clot stiffness from ~2900Pa to ~300Pa (4.5% CV), equivalent to a 90% reduction in clot stiffness.

Conclusions: The QuantraDx provides quantitative measures of clot time, overall clot stiffness and platelet and fibrinogen contribution to clot formation in 15min with low variability. A multi site clinical study is planned to further evaluate clinical performance.

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Heparin Interference in Anti Xa Assays for Rivaroxaban and Apixaban

K. Finnegan[1], G. A. Cutsforth[2]. *[1]Stony Brook University, Stony Brook, NY, [2]Diagnostica Stago Inc, Vernon Hills, NJ*

The purpose of this study was to evaluate the degree of interference from therapeutic and supra-therapeutic levels of unfractionated and low molecular weight heparins (UFH, LMWH) on rivaroxaban and apixaban assays. The package insert for the rivaroxaban and apixaban calibrators states "other anticoagulants (vitamin K antagonist, UFH, LMWH, fondaparinux, direct thrombin inhibitors...)" can interfere with the assay. Rivaroxaban and Apixaban are target specific oral anticoagulants recently approved by the FDA for use in specific indications. Rivaroxaban (Xarelto®) is approved for stroke prevention in atrial fibrillation patients, prevention and treatment of venous thromboembolism (VTE) and for VTE prevention after total hip and knee replacement. Apixaban (Eliquis®) is approved for stroke prevention in atrial fibrillation patients. The anti-Xa activities of rivaroxaban and apixaban are higher than the activities of UFH and LMWH because they are specific for Factor Xa. Therefore, measuring the new anti-Xa anticoagulants with UFH or LMWH calibrators and controls is not possible. This protocol is designed to measure the anti-Xa activity of therapeutic UFH and LMWH levels in the assays calibrated for rivaroxaban and apixaban. A total of 88 samples were analyzed. Samples were collected from the clinical laboratory identified by prolonged aPTT and samples previously run for anti-Xa assay. Samples with anti-Xa levels above the therapeutic range were purchased: 20 samples from 1.5-2.0, 10 samples from 2.0-2.5, and 10 samples from 2.5-3.0 IU/ml . Each sample was analyzed by aPTT and anti-Xa. Three calibration curves were used in the anti-Xa assay: a hybrid curve for UFH and LMWH, rivaroxaban and apixaban. Assays were performed in singlicate and the values were compared by linear regression. Linear regression analysis demonstrated that there was no correlation between the APTT and the anti-Xa results using either of the three calibration curves. When the heparin samples were assayed using either the rivaroxaban or apixaban calibration curves, heparin anti-Xa values ≤ 0.5 IU/ml produced values ≤ 25 ng/ml, the lower limit of quantification for the assay. Heparin samples ≥0.5 IU/L/ml demonstrated a linear relationship to both rivaroxaban and apixaban values with a correlation coefficient of .956 and 0.959 respectively. The conclusion of the study is that the overlap of UFH and LMWH therapy and the use of rivaroxaban or apixaban will result in anti Xa results that are due to combined anti Xa activity of the anticoagulants.

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DxH PROService Solutions: A Case Study - Proactive Monitoring of System Data

J. Colom, C. Ramirez, J. Lu, J. Castillo, S. Guntupalli, P. ONeil. *Beckman Coulter, Miami, FL*

Background:

The UniCel DxH Series of Coulter Cellular Analysis Systems provides CBC, WBC differential, NRBC and reticulocyte analysis. Turnaround time is critical and physicians require prompt and accurate results. An inoperable system directly impacts the laboratory's turnaround time and it's essential to provide a solution that efficiently analyzes, in near real time, large amounts of system data for root cause analysis.

PRO*Service* is a remote management and diagnostics system that facilitates the continuous transfer and analysis of system performance data from Beckman Coulter instruments through Beckman Coulter's PRO*Service* servers. The PRO*Service* framework is designed for real time monitoring of instrument system functions and includes large-scale, multi-dimensional data analysis over time. Incoming data is channeled through PRO*Service* applications where the output is used by Beckman Coulter Technical Service and Product Development engineers to proactively diagnose and resolve system issues.

Methods:

Instrument subsystem performance data from over 1800 DxH 800 instruments is collected, collated and analyzed through PRO*Service* applications utilizing a Hadoop server to manage, standardize, analyze and graphically present large scale instrument data analysis. The analysis identifies the primary drivers and related parameters by region over time.

Results: A data set of ≈120 million data records was analyzed. Figure 1, identifies graphically, the three key subsystem parameters that correlate to the increased calls. The data details, by parameter (Y axis), over time (X axis) and by system (red require maintenance; blue are properly maintained) specific system information for effective problem identification and resolution. The data also documents the corrective measures implemented were effective.

Conclusion: The PRO*Service* Solution provides real time system monitoring on the DxH series including expanded data analysis and identification capabilities facilitating large-scale, complex investigations. Several programs working in conjunction enable efficient, large scale data analysis and management for the proactive identification of factors contributing to system down time.

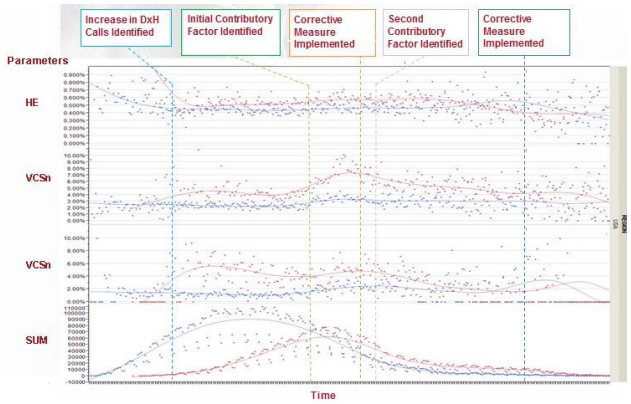

Figure 1. *PROService* System Data Analysis

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A Validation Study for the Quantitative Measurement of Prothrombin Time/International Normalized Ratio (PT/INR) test on the Xprecia Stride™ Coagulation Analyzer* for Warfarin Monitoring.

C. Lessard, I. Akunovich. *Siemens Healthcare Diagnostics, Norwood, MA*

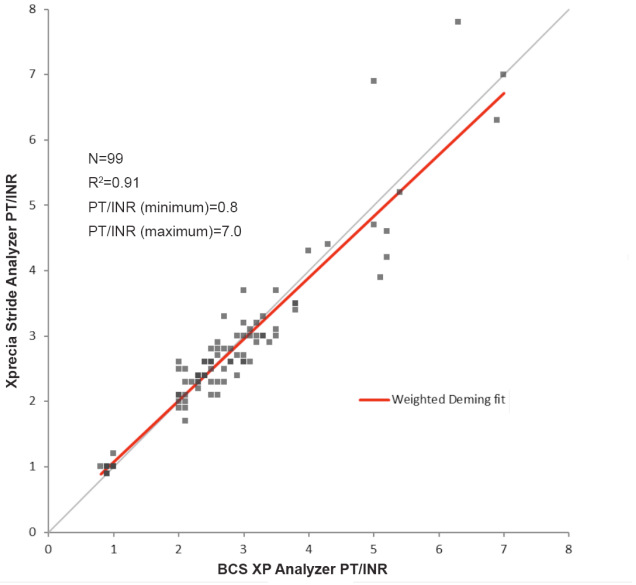
Background: The Xprecia Stride™ Coagulation Analyzer from Siemens Healthcare Diagnostics (SHD) is a novel, handheld POC device that generates rapid PT/INR results from fingerstick samples for oral anticoagulant therapy monitoring (OAT). This external validation study, conducted under the International Conference on Harmonization/Good Clinical Practice (ICH/GCP) guidelines, assessed the clinical substantial equivalence of the Xprecia Stride analyzer PT/INR test against an established laboratory hemostasis method (BCS® XP System).

Methods: Test methods were based on Clinical Laboratory Standards Institute (CLSI) guidelines. Evaluation of Precision Performance of Quantitative Measurement Methods (EP05-A2), Measurement Procedure Comparison and Bias Estimation Using Patient Samples (EP09-A3) and Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (EP28-A3c).

Results: Weighted Deming regression analysis yielded a slope of 0.95 and an intercept of 0.12, with R^2=0.91 across the range of 0.8 to 7.0 INR. Repeatability using whole blood demonstrated %CVs were ≤5.9 across the reportable range. LQC at two levels demonstrated repeatability precision % CVs that were ≤3.6 and within laboratory %CVs that were ≤7.0. The Expected Range for the PT/INR on the Xprecia Stride analyzer was 0.9 to 1.1 for subjects not on OAT.

Conclusion: The Xprecia stride analyzer PT/INR test results were substantially equivalent to the BCS XP system.

*Not available for sale in the U.S. Product availability varies by country



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Application of Procalcitonin and neutrophil VCS parameters in the diagnosis of bacterial infection in non-small cell lung cancer postoperative patients

L. Liu[1], N. Cong[2], D. Li[3], X. Song[4]. *[1]Clinical Laboratory, Shandong Cancer Hospital, Ji'nan, China, [2]Department of Surgical Oncology (Interventional Therapy), Shandong Cancer Hospital, Ji'nan, China, [3]Clinical Laboratory, Shandong Cancer Hospital, ji'nan, China, [4]Clinical laboratory, Shandong Cancer Hospital, Ji'nan, China*

Background:

We explored the predictive value of procalcitonin and VCS parameters of neutrophil in infected non-small cell lung cancer patients after operation.

Method:

To analysis treated 37 infected NSCLC patients after thoracotomy operation in Department of thoracic surgery from 2014 June to October, collected venous blood samples 1 - 3 days before operation and first days after surgery , analyzed blood cells by Beckman Coulter LH750, and collected neutrophil VCS parameters, including the mean neutrophil volume (MNV), neutrophil volume distribution width (MNV-SD), mean neutrophil conductivity (MNC), neutrophil conductivity distribution width (MNC-SD), mean neutrophil scatter(MNS) and neutrophil scatter distribution width(MNS-SD) ; and detected serum procalcitonin (PCT) concentration. Meanwhile obtained the area under the ROC curve, to evaluate the ability of each marker in the diagnosis of bacterial infection.

Results:

To compare the indicators of WBC ,neutrophil VCS parameters and serum PCT before and after the operation, the differences were statistically significant (P<0.05). Area under the ROC of MNV, MNV-SD and serum PCT was respectively 0.95,0.946, 0.892, this suggest that they have a good diagnosis effect. The positive rates were compared between Serum PCT, MNV and MNV-SD in diagnosing infection, there were not significant differences (P>0.05).

Conclusion:

The serum PCT, neutrophil MNV and MNV-SD were significant in the early diagnosis of bacterial infection in the thoracotomy NSCLC patients.

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Variant hemoglobin prevalence in Brazil and its regions: An update from a large laboratory database.

A. C. Rocha, M. D. C. Freire. *DASA, Cascavel, Brasil, Cascavel, Brazil*

Background:

Hemoglobinopathies are caused by genetic structural abnormalities of the chain of hemoglobin, affecting its functionality, that occurs when there are changes in genes encoding trainers for the formation of hemoglobin variants. The structural abnormalities of hemoglobin are among the most common genetic diseases in human populations. Brazilian population, because of miscegenation, show great diversity of gene translocations in the formation of hemoglobin, which can complicate the clinical and laboratory diagnosis of these diseases. This study aims to assess the prevalence and diversity of hemoglobinopathies in Brazilian population, and its distribution among country regions.

Methods:

We actively searched LIS database, from January to December 2014, of a national central laboratory in Brazil, that receive samples for all country regions. Hemoglobinopathy diagnose was determined by hemoglobin High-Performance Liquid Chromatography (HPLC) results, obtained by Variant II Beta Thalassemia Short Program by Bio-Rad.

Results:

From 148,317 individual hemoglobin HPLC results, 21,451 showed hemoglobinopathies, and were classified by hemoglobin found and country region.

We observed that 6.86% were suggested positive for sickle cell trace(AS) , 2.70% were suggested positive for beta thalassemia screening and 2.13% were suggested positive for C Hemoglobine trace. The incidence of hemoglobinopathies in females was higher, 13,271 (61,87%), when compared to males, 8,180 (38,13%). Northeast region showed 8,528 (5.75%) OF hemoglobin variant results, 6,178 (4.17%) of positive results were found in Southeast patients, 2,866 (1.93%) in the Central West patients, 2,753 (1.86%) in South and 1,126 (0.76%) in North of Brazil.

Conclusion:

Thus these results show the great variability of hemoglobin variants presented in Brazil, thus aiming to provide an update on regional and national hemoglobinopathy epidemiology. The higher prevalence in the Southeast and South of Brazil can be explained by the demographic formation of the area, those regions have the largest population concentration in the country, built by the great mass immigration of Africans, Italians and Oriental peoples in the process of colonization of Brazil. The Northeast region of the country has the largest prevalence of variations of S hemoglobin in the study, even as the Southeast, which can be explained because of the ethnicity of the population, mostly black, which is more propitious for developing the S hemoglobin. In turn, the North region concentrates the lower portion of the findings, which may be due to the low population of the region.

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Evaluation of Serum IgM Hevylite® assays; IgM Kappa and IgM Lambda for use on the Binding Site Optilite® analyser

J. R. Kerr, S. K. Ahaliwal, S. Kausar, F. Murphy, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Elevated monoclonal protein production is indicative of an underlying abnormality such as MGUS, multiple myeloma & other disorders. SPE densitometry is recommended to quantify monoclonal proteins however, monoclonal IgM can be obscured by other proteins on SPE gels. Measurement of IgM Kappa (IgMκ) and IgM Lambda (IgMλ) may give a more accurate representation of tumor production. Moreover, calculation of the IgMκ/IgMλ ratio and comparison with values found in normal subjects can give a more sensitive indication of clonality. Here we describe the performance characteristics of the IgM Kappa and IgM Lambda Hevylite assays for use on the Binding Site's Optilite analyser. Precision was determined following CLSI (EP05-A2) using 8 pooled sera samples in the range 0.10-3.94g/L for IgMκ and 0.06-3.90g/L for IgMλ with two duplicate runs a day on 3 kit lots and 3 analysers over 21 days. Acceptance criteria were <10% CV for total precision and <5% CV for within run precision. Total precision was ≤6.1% CV for IgMκ and ≤5.0% CV for IgMλ at all levels tested. Within run precision was ≤1.7% CV for IgMκ and ≤1.5% CV for IgMλ at all levels tested. Linearity was verified by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results. Acceptance was defined as recovery within 10% at each level. The assay was shown to be linear using StatisPro software in accordance with CLSI guidance over the range 0.14-5.80g/L for IgMκ and 0.16-4.98g/L for IgMλ. This validated measuring ranges of 0.2-5.0g/L for IgMκ and 0.18-4.50g/L for IgMλ at 1/10 with sensitivity of 0.020g/L and 0.018g/L and upper limits of 150.0g/L and 135.0g/L respectively, utilizing auto-redilutions. Interference testing was carried out using 200mg/L bilirubin, 5g/L haemoglobin, 1500mg/dL intralipid, 1000mg/dL triglycerides and 15 other common drug and metabolite interferents spiked into serum pools at 5 levels covering ranges of 0.10-2.48g/L for IgMκ and 0.06-1.47g/L for IgMλ with comparison to negative controls. Acceptance was <10% difference to the negative control. Interference was found to be acceptable for all interferents at all levels on both assays. Correlations to the Binding Site IgMκ and IgMλ assays for the SPAPLUS were performed using 263 samples on IgMκ (range 0.02-51.75g/L) and 249 samples on IgMλ (range 0.03-33.03g/L. Acceptable agreement was seen when analyzed by Passing-Bablok regression; kappa gave a slope of y=0.93x + 0.01 and lambda gave y=1.01x - 0.00. We conclude that the Optilite IgM Hevylite assays provide a reliable, accurate & precise method for quantifying IgM Kappa & IgM Lambda and show good agreement with existing turbidimetric assays.

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Complex Biological Profile of Hematological Markers and Indices Across Pediatric, Adult, and Geriatric Age: Establishment of Robust Reference Intervals based on the Canadian Health Measures Survey

J. E. Raizman[1], Y. Chen[1], V. Higgins[1], M. Nieuwesteeg[1], M. Abdelhaleem[1], S. L. Wong[2], D. Blais[2], K. Adeli[1]. *[1]CALIPER Program, Divisions of Clinical Biochemistry and Hematopathology, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada, [2]Health Analysis Division, Statistics Canada, Ottawa, ON, Canada*

Background: The Canadian Health Measures Survey (CHMS) is a project designed by Statistics Canada with the objective to collect comprehensive nationwide health information and blood specimens from children and adults in the household population. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) has collaborated with Statistics Canada to develop a comprehensive database of reference intervals for haematological biomarkers of disease across healthy pediatric, adult, and geriatric age groups. This study aimed to address the challenges of obtaining fresh whole blood specimens for analysis of hematological marker reference intervals and provides a robust dataset based on a very large cohort of children and adults.

Methods: Between 2007-2011, the CHMS collected health data from approximately 12000 respondents aged 3 to 79 and obtained fresh whole blood specimens. Sixteen hematology markers (including calculated parameters) were measured using the Beckman Coulter DxH300C or HmX analyzers on fresh whole blood. After exclusion criteria were applied and outliers were removed, statistically relevant age and gender partitions were determined and reference intervals, including 90% confidence intervals, were calculated using CSLI C28-A3 guidelines.

Results: Concentrations of hematology markers showed dynamic changes from childhood into adulthood, as well as between genders, necessitating distinct partitions throughout life. Most age partitions were found during childhood, reflecting the hematological changes that occur during growth and development. Hemoglobin, red blood cell (RBC) count, hematocrit, and indices (MCV, MCH, and MCHC) increased with age, but as expected females had lower hemoglobin and hematocrit starting at puberty. Platelet count increased with age and required multiple gender partitions during adolescence and adulthood. White blood cell (WBC) count and fibrinogen also declined with age, requiring distinct age and gender partitions. Eosinophil and basophil levels were low, as these markers were not detected in many of the participants. Upper limits, however, were highest in early life.

Conclusion: The robust dataset generated in this study has allowed observation of dynamic biological profiles of several hematology markers and resulted in the establishment of comprehensive age- and sex-specific reference intervals that may contribute to accurate monitoring of pediatric, adult, and geriatric patients. The rich dataset collected also allows for data mining using a systems biology approach, and therefore, has the potential to uncover relationships between hematology biomarker values, clinical outcomes, and risk factors for many disease states.

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Patient posture during phlebotomy influence routine coagulation testing and jeopardize patient safety

G. Lima-Oliveira[1], G. C. Guidi[1], G. L. Salvagno[1], E. Danese[1], E. J. Favaloro[2], G. Lippi[3]. *[1]University of Verona, Verona, Italy, [2]Westmead Hospital, Westmead, Australia, [3]University of Parma, Parma, Italy*

Background: Routine hemostasis testing is an essential part of the clinical decision making process in patients with both hemorrhagic and thrombotic disorders. Several lines of evidence now attest that the quality of hemostasis testing may be dramatically impaired by a number of preanalytical variables, mostly related to collection, transportation, and patient preparation. Nevertheless, the patient posture during phlebotomy is rarely regarded as a potential source of laboratory error. This study was aimed to verify if routine coagulation tests are influenced by patient position during blood collection by venipuncture.

Methods: Three citrate blood tubes were collected by venipuncture from all 19 volunteers without tourniquet application. The first tube was drawn after the volunteers remained in supine position for 25 min, the second after 20 min resting in a comfortable sitting position, and the third after 20 min stationary standing in upright position. The citrated samples were separated at 1500xg for 15 min, at 20°C, then assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen (Fib) using ACL TOP 700 (Instrumentation Laboratory). Differences on routine coagulation tests due to patient posture during blood collection by venipuncture were reported as bias (Figure 1). The significance of differences was assessed with Mann-Whitney test. Statistical significance was set at $P < 0.05$.

Results:

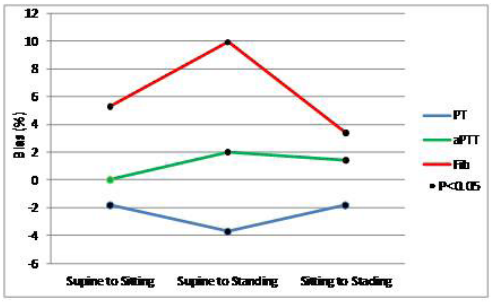

Figure 1. Differences on routine coagulation tests due to patient posture .

Conclusion: Patient posture may have a significant impact on results of routine hemostasis testing, especially if patient position is changed from supine to standing, thus is necessary to standardize the patient position for blood collection by venipuncture. A minimum period between 15 to 20 min of resting in reference position should be observed before collecting diagnostic blood samples. From a organizational perspective, this resting period could seriously impact on the workflow at blood collection sites - but this can be obviated by scheduling blood collections, thus starting an appropriate personalization of laboratory analysis.