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

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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 10 B-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 complex karyotypes including t(9;22) (q34;q11.2) and additional chromosome aberrations, and two had complex 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 called 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 assays. In conclusion, the detection of IKZF1 alterations results were shown to be able to identify patients who subsequently relapsed better than gene expression-based assay. In conclusion, the detection of IKZF1 alterations results were shown to be able to identify patients who subsequently relapsed better than gene expression-based assay.

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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 line 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 i(8;21)(q21;22) 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 diagnosis and treatment.

Objective: To validate FISH assay for detection of t(8;21)(q21;q22) in leukemia. Two analysts scored 500 interphase cells (200 per analysis) and 200 metaphase cells. The results of 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 green fluorophore. 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%), 2G2R (3,1%) and 3G2R (2,34%). The signal patterns and their cutoffs for blood samples were 1F2G1R (2,34%), 2G1R (3,1%), 1G2R (2,34%) and 3GIR (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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**Conclusion:**
We determined that 5% PtSer and 5% PtEtn liposomes showed highest increases in TAFI 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 Oxygen Synthase Oxigenase Domain on Human Apo AI Derived Discoidal Lipid Particles**

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**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 (HDL) 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 eNOS/nanodisc complex formation were prepared by mixing phosphatidylcholine/detergent micelles and apo AI engineered protein with and without eNOS/nanodisc 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 AKTA FPLC system using Tris-buffer at 0.5 ml/min. eNOS/nanodisc concentration in nanodisc was verified by the characteristic ferrous heme-CO adduct absorbing at 444 nm via an extinction coefficient of ε444 = 76 mM-1 cm-1. The activity of eNOS/nanodisc was determined using a Spectra max plus 384 plate reader based on the Griess reaction. Catalysis of NO production from N-hydroxyarginine (NOHA) and H2O2 by eNOS/nanodisc was assayed in 96-well microwells. Samples were run in triplicate (50 µl final volumes) and the assay plate was read at 540 nm. Results: The specific activity of eNOS/nanodisc or free eNOSoxycy was 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 (R2 = 0.9979). The detected specific activity of nanodisc-bound eNOSoxycy (49.0 ± 1.3) nmol/min/mmol was > 50% lower in comparison to the free eNOSoxycy enzyme (132.4 ± 2.4) nmol/min/mmol. **Conclusions:** The analyzed data showed a decrease in NO generation by the nanodisc-bound eNOSoxycy in contrast to free eNOSoxycy. 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**

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**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 HbS 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 α-globin gene was the (IVS II-1 A→T) type with frequency of (6%), IVS 1-5 (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**

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**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-lymphophyctic leukemia, plasma cell leukemia, chronic lymphophytic 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 nondividing cells. This is relevant to diseases with usually low mitotic
Hematology/Coagulation

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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 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 assay 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 use on 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 assay

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<th>Analyte Concentration</th>
<th>0.109g/L</th>
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<th>0.388g/L</th>
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<tr>
<td>Within run precision (%)</td>
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<td>1.7%</td>
<td>1.1%</td>
<td>1.7%</td>
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<tr>
<td>Between run precision (%)</td>
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<td>1.9%</td>
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<tr>
<td>Between day precision (%)</td>
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<td>8.6%</td>
<td>4.0%</td>
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<td>2.4%</td>
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A-186

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 assay 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 use on 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 an A1AT Optilite assay

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<th>Analyte Concentration</th>
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<td>Total precision (%)</td>
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<tr>
<td>Within run precision (%)</td>
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<td>Between day precision (%)</td>
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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 gammopathies. Elevated monoclonal protein production is indicative of an underlying abnormality such as MGLS, 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.
can be used in these instances to measure both IgA kappa and 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 IgA and IgAκ assays (Hevytite®, 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 0.480-2.92g/L) and 0.155-10.40g/L for IgAλ (reference interval 0.360-1.980g/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). 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 IgAκ, 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) for 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% (11.1g/L) for IgAκ and 6.8% (0.30g/L), 4.7% (0.93g/L), 9.3% (1.63g/L), 9.1% (2.52g/L) and 3.6% (8.58g/L) for IgAλ. The assay was shown to be linear over the standard measuring range of the assays; y=1.00x+0.01 (R²=0.999) IgAκ and y=1.02x+0.00 (R²=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 assessed by Passing-Bablok regression: y=1.09x+0.05 for IgAκ and y=1.05x-0.02 for 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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Multivariable Graphic and Numerical Statistical Techniques for Comparing the Performance of Two Hematology Instruments. A Practical Example with Sysmex EX-5000® and Sysmex XT-4000i®

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Background: Sysmex XE-5000® and Sysmex XT-4000i® 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 simultaneously compared. We employed multivariable statistical models and their graphical representations to assess 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 XH® 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=0.21+1.1x; HCT y=0.21+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 8.5%) 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 data was consulted to recalibrate XT-4000. After recalibration, comparison with patient specimens showed no clinically significant differences for XT-4000; RBC mean bias 1.5% (minimum 0%, maximum 3%), HCT mean bias -0.8% (minimum -2.5%, maximum 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 laboratory to perform numerical and graphical data analysis simultaneously on several instruments and parameters.

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

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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 reported positive for Myeloperoxidase Deficiency (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.**

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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 was 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 tested across a range of 2.6 - 45g/L using 88 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 - 8.5g/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 patients. Correlation to the original SPAPLUS IgG4 kit (measuring range 0.030 - 8.5g/L) was established using a Passing-Bablok regression returned an agreement of y=0.999±0.003 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.

### A-192

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

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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 immunoglobulins 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 ~28.2%. Linearity was verified by analysis of 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 8.09-377.77mg/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 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 + 0.63. Correlation to the Siemens BNII albumin assay was performed using 162 samples (total range -2.12-7150mg/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.

### A-193

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

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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 prevalence 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 in 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 homozogous 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 homozogous states, and its association in the same individual was evaluated.

Results: The prevalence of the FVL mutation, searched in 3065 samples was 5.06% (n = 100) in heterozygous and 0.05% (n = 1) in homozogous state. The mutation of the prothrombin gene (1092 samples) was heterozygous in 4.39% (n = 48), homozogous 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).
Development of A Novel Device to Assess Hemostatic Function from Whole Blood


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, 64µg/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 2949 Pa (4.73% CV).

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

Heparin Interference in Anti Xa Assays for Rivaroxaban and Apixaban

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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 0.956 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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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.

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**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 R²=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.*
Evaluation of Serum IgM Hevylite® assays; IgM Kappa and IgM Lambda for use on the Binding Site Optilite® analyser

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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 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 0.10-1.94g/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 ≤±1.1% CV for IgMκ and ≤±0.5% CV for IgMλ at all levels tested. Within run precision was ≤±1.7% CV for IgMκ and ≤±3.5% 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 0.16-6.48g/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 drugs and metabolite interferences spiked into serum pools at 5 levels covering ranges of 0.10-2.49g/L for IgMκ and 0.06-1.47g/L for IgMλ, with comparison to negative controls. Acceptance was ≥99% 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 use on the Binding Site's Optilite analyser. Precision was determined following CLSI (EP05-A2) using 8 pooled sera samples in the range 0.10-1.94g/L for IgMκ and 0.06-3.90g/L for IgMλ. Acceptable agreement was seen when analyzed by Passing-Bablok regression; kappa gave a slope of y = 0.9 ± 0.01 and lambda gave y = 1.01 ± 0.00. We conclude that the Optilite IgM Kappa and IgM Lambda assays provide a reliable, accurate & precise method for quantifying IgM Kappa & IgM Lambda and show good agreement with existing turbidimetric assays.
Results:

![Graph showing differences in routine coagulation tests due to patient posture.](image)

**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 an 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.