# Tuesday, August 1, 2017

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

Hematology/Coagulation

## A-282

### Effects of 100-km ultramarathon on haematological variables in runners with hepatitis B virus carriervariables in runners with hepatitis B virus carrier

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**Background:** Ultramarathon is known to cause immediate post-race erythropoietin response, followed by substantial sports anemia. Liver and kidney are the two major organs to produce erythropoietin (EPO). Kidney and Liver injury with augmented production of many cytokines may influence EPO synthesis and response. The aim of the study is to explore whether haematological change might be different between hepatitis B (HBV) carrier and non-HBV ultramarathon runners.

**Methods:** Blood samples were collected from eight asymptomatic HBV carriers and eighteen non-HBV individuals who finished a 100-km ultramarathon race. For each subject, the samples were collected at three different times: (1) one week before race, (2) immediately following the race and (3) 24 hours after the race. Samples were analyzed and compared between these 2 groups for red blood cells (RBC) counts, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV) and plasma EPO levels.

**Results:** HBV carrier runners had a less variation on hematological change. The Hct, RBC counts, and Hb values in HBV carrier group were only slightly elevated immediately following the race and dropped to a lesser extent 24 hours after the race, compared to those of non-HBV subjects. There was no difference on change of MCV values in both groups. In HBV carrier runners, plasma EPO levels were relatively higher at baseline, and increased significantly in the same fashion in response to ultramarathon.

**Conclusions**: This is the first study to explore how hematological change specifically for ultramarathon runners with HBV carrier runners. The hemoconcentration by the end of the run was due to EPO production. Ultramarathon increased EPO production in both HBV carrier and non-HBV runners. Although HBV carrier runners have an increase of EPO immediately following the race, their change on Hct, RBC count, and HGB values or Hct had a less variation, implying that HBV carrier runners might have EPO hyporesponsiveness.

**Keywords:** EPO, hepatitis B carrier, hyporesponsiveness, hemoconcentration

## A-284

### Performance evaluation of Nanopia® PAI-1 for measurement of plasminogen activator inhibitor-1

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**Background:** Plasminogen activator inhibitor-1 (PAI-1), produced from the vascular endothelium, inhibits fibrinolytic reactions intravascularly. PAI-1 forms complexes with tissue type plasminogen activator (t-PA)—the fibrinolysis factor—resulting in t-PA losing its activity. PAI-1 levels are useful to determine the state of coagulation and intravascular fibrinolysis and are elevated in disseminated intravascular coagulation, sepsis, etc. This study evaluated the performance of Nanopia® PAI-1—a newly developed general-purpose reagent from Sekisui Medical—as well as the correlation between Nanopia PAI-1 and the LPIA-tPAI test from LSI Medience Corporation. **Methods:** A precision study was conducted using the CP3000 analyzer. Within-day reproducibility was evaluated by measuring two concentration-control samples ten consecutive times. Between-day reproducibility was evaluated by measuring two concentration-control cryopreserved samples for ten days. To evaluate dilution linearity, we prepared eight dilution series of high-concentration samples (2000 ng/mL), using physiological saline, and measured nine series of samples, including blanks, in duplicate. A correlation study between Nanopia PAI-1, using the CP3000 analyzer, and the LPIA-tPAI test, using the LPIA-NV7 analyzer, was conducted with 50 patients' plasma samples. **Results:** Within-day coefficients of variation (CVs) were 4.23% and 2.96% and between-day CVs were 5.11% and 2.93% for low-concentration control and high-concentration control, respectively. The graph of dilution linearity showed a convex upward curve up to 2000 ng/mL. It was inferred that there was linearity up to 300 ng/mL. Hence, we examined the precise linearity. We prepared ten dilution series of high-concentration samples (350 ng/mL), using physiological saline, and measured 11 series of samples, including blanks, in duplicate. As a result, good linearity that passed through a point near the origin was obtained. The correlation study (n = 50, range 8.9 to 184.3 ng/mL) showed good correlation ($r^2 = 0.966$). The regression formula was: $y = 0.92x + 6.58$. **Conclusion:** This study demonstrated good precision and correlation. It showed that high-concentration samples exceeding 300 ng/mL, which is the upper measurement limit of Nanopia PAI-1, can be measured accurately by dilution. Based on these considerations, it seems plausible that Nanopia PAI-1 has sufficient performance in routine laboratory tests.

## A-286

### Preliminary results from an international comparative laboratory field study using BAY 94-9027, a site-specifically PEGylated recombinant factor VIII product

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**Background:** Accurate measurement of factor VIII (FVIII) activity in patients with hemophilia A is important for patient monitoring and treatment decisions. Discrepancies in results using different assays or reagents to measure prolonged–half-life factor products have been recognized and highlight that effective monitoring of patient response to these products may require adjustments in clinical laboratory practices. A global field study was conducted to assess the ability of clinical laboratories to measure BAY 94-9027 activity in spiked hemophilic plasma samples using their in-house or specific assays. BAY 94-9027 is a prolonged–half-life FVIII product site-specifically conjugated with a 60-kDa polyethylene glycol molecule (2×30 kDa branched). **Methods:** In this 2-part study, laboratories received sample sets (3–4 per laboratory) of 26 blinded samples in randomized order for analysis. Each set consisted of triplicate test samples of BAY 94-9027 or a comparator (antihemophilic factor [recombinant] plasma/albumin-free method [rAHF-PFM (Advate®); Shire]) spiked at low (<10 IU/dL), medium (10–50 IU/dL), and high (50–100 IU/dL) concentrations in pooled hemophilic plasma. Normal control plasma and unspiked hemophilic plasma in triplicate were positive and negative controls, respectively. Two additional blinded samples matching 2 of the other 24 samples in the set were included in each set to decrease predictability of the sample sets. Laboratories analyzed test samples using their in-house assays, reagents, and standards (part 1). An additional sample set was provided if laboratories used both the one-stage and chromogenic assays. In part 2, all laboratories tested 2 additional sample sets using 2 activated partial thromboplastin time kits (Pathromtin® [Siemens] and HemosIL® SynthASil [Instrumentation Laboratory]) previously shown to accurately measure BAY 94-9027 and full-length FVIII. FVIII recovery and FVIII levels were primary and secondary endpoints, respectively. Results were analyzed for intra- and interlaboratory variation. **Results:** 52 laboratories in North America, Europe, and Israel participated in the field study. In part 1, 49 laboratories tested samples using the one-stage assay, 16 used the chromogenic assay, and 13 used both assays. The reagents routinely used for measuring FVIII activity varied among participating laboratories. Mean FVIII recovery ranged from 75.1%–103.2% for BAY 94-9027 and 94.6%–114.7% for rAHF-PFM across all concentrations and reagents using the one-stage assay. As expected based on previously published data, the PTT-A (Stago) and HemosIL® APTT-SP kits (Instrumentation Laboratory) underestimated BAY 94-9027 at all concentrations. More accurate one-stage results were generated using the Pathromtin® and HemosIL® SynthASil kits as shown in part 2 of the study. For the chromogenic assay, mean FVIII recovery ranged from 104.4%–117.1% for BAY 94-9027 and 87.7%–107.8% for rAHF-PFM across all concentrations. Interlaboratory variability was low for measurement of BAY 94-9027 with chromogenic assays. **Conclusions:** Results from this global field study indicate that chromogenic assays are an accurate method for measurement of plasma FVIII levels of BAY 94-9027. FVIII activity in patients receiving BAY 94-9027 can also be accurately monitored using many commonly

used one-stage assay kits without need of a conversion factor. Understanding the limitations and advantages of specific assay kits is important for choosing the correct systems to measure FVIII products in clinical practice.

## A-288

### Performance evaluation of a new generation of automated analyzer for pleural and peritoneal fluids cytology

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**Background:** Body fluids are generally sent for urgent analysis. Traditional manual cytology is a time-consuming and low precision procedure, subjective and prone to interoperator variability. Therefore, the use of an automated analyzer improves the TAT, reducing the time to report a preliminary result to the clinician. The objective of this study was to evaluate the performance of the recently installed Sysmex XN-3000 and compare it with the Sysmex XE-5000, which has been in use for some years in our laboratory for pleural and peritoneal fluids cytological analysis.

**Methods:** We studied 108 pleural and peritoneal fluids. All samples were sent in an anticoagulant-treated tube and analyzed up to 2 hours after collection. The laboratory routine included automated total and differential cell counts (Sysmex XE-5000 and Sysmex XN-3000) and manual differential counts (cytocentrifuged air-dried hematological staining of May-Grunwald). Sysmex XN-3000 validation protocol included precision, carryover, linearity studies and comparison with traditional microscopic differential counts and with the analyzer in use (Sysmex XE-5000). Simple linear regression (least-square method), paired t-test, and kappa agreement were used to the statistical analysis.

**Results:** Sysmex XN-3000 met all the requirements for analytical quality regarding precision (CVs < desirable specifications for imprecision) and linearity (r>0.99). Carryover effect was minimal (<0.1%). Sysmex XN-3000 demonstrated a strong correlation with microscopy regarding WBC differential counts (r=0.95 for both MN and PMN), with an agreement of 93% (kappa=0.813, p<0.0001). Comparison between both analyzers revealed no significant differences from a clinical or statistical point of view. Sysmex XN-3000 WBC and RBC counts were highly correlated with that of the Sysmex XE-5000 reference method (r>0.98 in both cases). An excellent agreement between both analyzers was also observed for mononuclear cells (MN) and polymorphonuclear cells (PMN, r=0.99 in both cases).

**Conclusion:** Our data demonstrated that the performance of both analyzers is equivalent, allowing both to be interchangeable without impact on the final report. Additionally, Sysmex XN-3000 showed strong correlation and agreement with traditional microscopy.

## A-289

### Evolution in the incidence of monoclonal gammopathies in a southern Spain tertiary hospital in the last thirteen years

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**Background:**

Monoclonal gammopathy (MG) is the most common plasma cells disorder. It affects around 3% of the population older than 50 years. The great majority of MG are monoclonal gammopathies of undetermined significance (MGUS), which is a premalignant disorder defined to present less than 3 g/dL of serum monoclonal protein, less than 10% of clonal bone marrow cells and absence of end-organ damage. MGUS is easily detected in laboratory tests and should be monitored because 1% of MGUS per year progress to Multiple Myeloma (MM).

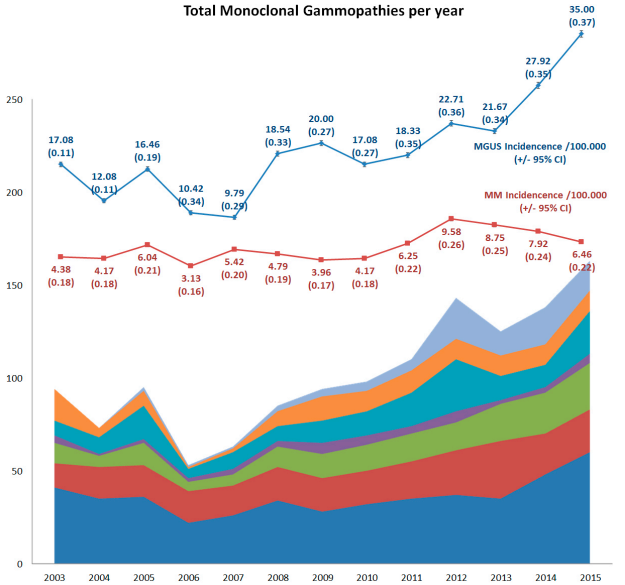
Incidence of MGUS and MM is not always easy to determine, but there is a general perception of an increasing incidence that can be attributed to different causes. One is the aging of the population. Another reason is the contribution of clinical laboratories, which count on new determinations (free light chains) or improved techniques in electrophoresis, nephelometry or immunofixation, allowing them to support the diagnose of MGUS that years before remained undiagnosed.

The aim of this study is to determine the incidence of MGUS, MM and its different types in the reference population of a tertiary hospital in southern Spain between 2003 and 2015.

**Methods:**

In a retrospective study, we determined the total number of MG and its different types diagnosed in our hospital between 2003 and 2015. We calculated the incidence per 100.000/year of MGUS and MM, with 95% confidence intervals. Our reference population, in 2015, was 480.851.

**Results:**




| | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| kappa/lambda | 0 | 0 | 2 | 1 | 1 | 3 | 4 | 5 | 6 | 22 | 13 | 20 | 16 |
| IgA/lambda | 17 | 5 | 8 | 1 | 2 | 8 | 13 | 11 | 12 | 11 | 11 | 11 | 11 |
| IgA/kappa | 8 | 9 | 18 | 5 | 9 | 8 | 12 | 13 | 18 | 28 | 13 | 12 | 23 |
| IgM/lambda | 4 | 1 | 2 | 2 | 3 | 3 | 6 | 5 | 4 | 6 | 2 | 3 | 5 |
| IgM/kappa | 11 | 6 | 12 | 5 | 6 | 11 | 13 | 14 | 15 | 15 | 20 | 22 | 25 |
| IgG/lambda | 13 | 17 | 17 | 17 | 16 | 18 | 18 | 18 | 20 | 24 | 31 | 22 | 23 |
| IgG/kappa | 41 | 35 | 36 | 22 | 26 | 34 | 28 | 32 | 35 | 37 | 35 | 48 | 60 |

**Conclusion:**

The aging of population and the higher sensitivity of laboratory techniques for diagnosing of MG is reflected in the incidence of MGUS, which increased from 17.04 cases per 100.000 in 2003 to 35.00. MM incidence in our area did not increased in parallel.

## A-291

### The Growth Differentiation Factor-15 Levels Are Increased in Patients with Compound Heterozygous Sickle Cell and Beta-Thalassemia, Correlate with Hepcidin-25-Ferritin Molar Ratio and with Markers of Hemolysis, Angiogenesis, Endothelial and Renal Dysfunction

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**Background**: The clinical manifestations of Sickle Cell Disease (SCD) include episodes of vascular occlusion, chronic hemolytic anemia and frequent infections. SCD is also characterized by the presence of chronic inflammation manifested by leukocytosis and monocytosis and increased circulating levels of pro-inflammatory cytokines and chemokines. Growth Differentiation Factor-15 (GDF-15), also known as macrophage-inhibitory-cytokine-1 (MIC-1) or non-steroidal-anti-inflammatory-drug-activated-gene (NAG-1) is a member of the transforming-growth-factor superfamily. Expression of the GDF-15 gene in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury. Also GDF-15 has been proposed as an erythroblast-derived factor, although not erythroblast specific, mediates Hepcidin-25 suppression under conditions of increased erythropoietic activity, and high levels of GDF15 associate with ineffective erythropoiesis and may reflect a certain type of

bone marrow stress or erythroblast apoptosis. **Aims**: The aim of this study was to evaluate the GDF-15 levels in patients with compound heterozygous HbS and beta-thalassemia (HbS/$\beta^{thal}$) and to explore possible associations with disease features, such as Hepcidin-25 production, hemolysis, inflammation, endothelial dysfunction and angiogenesis. **Methods**: Seventy-five adult Caucasian patients with HbS/$\beta^{thal}$ were included in the study, while 20 healthy individuals served as controls. Patients with HbS/$\beta^{thal}$ divided in two groups: group A included 36 patients under hydroxycarbamide (HC+) treatment and group B included 39 patients without hydroxycarbamide (HC-) treatment. Along with hematologic and blood chemistry parameters determination, measurements of circulating levels of GDF-15, hepcidin-25, hs-CRP, vWF-antigen, hs-TnT and Placental Growth Factor (PlGF) were performed in both patients with HbS/$\beta^{thal}$ and controls using immunoenzymatic techniques. **Results**: GDF-15 levels were elevated in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.0001$). Regarding hydroxycarbamide treatment, GDF-15 levels were elevated in (HC+) patients compared to (HC-) patients ($p=0.002$), or 30/36 vs 21/39 patients had elevated GDF-15 levels ($p=0.002$). In contrast, Hepcidin-25 levels were significantly lower in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.01$). In addition, a markedly low Hepcidin-25/Ferritin molar ratio was observed in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.001$). Whilst, no direct correlation was found between GDF-15 and hepcidin-25 levels, a significant negative correlation between GDF-15 levels and Hepcidin-25/Ferritin molar ratio was detected in patients with HbS/$\beta^{thal}$ ($p=0.002$). GDF-15 levels also correlated significantly with markers of erythropoiesis, such as Hb, HbF, ferritin and reticulocytes ($p<0.05$), with markers of hemolysis, such as LDH and uric acid ($p<0.05$), and with markers of endothelial dysfunction and angiogenesis such as vWF-antigen and PlGF ($p<0.05$). Surprisingly, no correlation was found between GDF-15 and hs-CRP levels. GDF-15 and $eGFR_{(Cystatin\text{-}C\text{-}based)}$ correlated negatively ($r=-0.421$, $p<0.001$). **Conclusions**: These findings demonstrate a multifactorial role of GDF-15 in patients with HbS/$\beta^{thal}$ as it correlates with erythropoiesis, hemolysis, angiogenesis, endothelial and renal dysfunction. Interestingly, the higher GDF-15 levels measured in patients treated with hydroxycarbamide may reflect possible drug induced sub-clinical cardiotoxicity, although this has not been described to-date. To this end, our knowledge is restricted only to doxorubicin-induced cardiotoxicity, where GDF-15 up-regulation seems to be more sensitive than that of hs-TnT, LDH and NT-proBNP. Further studies will reveal the role of GDF-15 in the biology of HbS/$\beta^{thal}$.

## A-293

### Traditional osmotic fragility test comparison with flow cytometric osmotic fragility test under the same protocol and definition of single NaCl concentration that provides an accurate hereditary spherocytosis screening by flow cytometric analysis

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Introduction

In the Traditional Osmotic Fragility test (TOF) the lysis of red blood cell (RBC) is measured colorimetrically in different tubes with decreasing concentrations of NaCl. In the Flow Cytometric Osmotic Fragility test (FCOF) the fraction of not-lysed RBCs is counted in a single tube spiked with deionized water. Although both tests are wildly accepted for hereditary spherocytosis (HS) screening, their equivalence in the same conditions is unknown. Moreover, some flow cytometers can not overlay analysis of before and after deionized water spiking. Thus, the aims of this study was to compare the FCOF with the TOF using multiple tubes with decreasing concentrations of NaCl in different pre-analytic conditions and to find a single NaCl concentration for each tested condition that allows an accurate HS screening by FCOF.

Methods

Twenty adult subjects (10 females) with HS (cases) and 20 healthy individuals (controls) matched 1:1 by gender and age were recruited to the study. To confirm the laboratorial diagnosis of HS or not, all subjects were submitted to complete blood count, reticulocyte count, blood smear. Haptoglobin, bilirubin, lactate dehydrogenase and direct antiglobulin tests were also performed. TOF (performed in E-225D, CELM) and FCOF (performed in FACSCanto II, Becton-Dickinson) were executed in fresh/incubated (24 h at 37°C degrees) heparinized/EDTA whole blood by using the 17-tube method with NaCl concentrations ranging from 1 to 0.1 g/L. Whole blood was diluted by 1:5000 for FCOF and 1:200 for TOF. Osmotic fragility curves and Median Corpuscular Fragilities (MCF) were used to compare all tested conditions and ROC curves were used to define the (%) lysis cut-off that provided a reliable discrimination between cases and controls.

Results

Laboratorial diagnosis of HS could be confirmed in all cases and none of the controls. The average age was 37.3±13.2 years for cases and 36.9±12.7 years for controls ($p=0.24$). The osmotic fragility curves in FCOF assumed the traditional sigmoid shape observed in the TOF and the curves of cases and controls were clearly distinguished in all tested condition. Incubation for 24h at 37°C degrees increased the discrimination between cases and controls in both heparinized and EDTA whole blood. The MCF for cases was significantly different from controls in all tested condition. However, the MCF values varied by technology, anticoagulant and incubation time. NaCl concentrations and (%) lysis cut-off that allowed reliable discrimination between cases and controls in FCOF were of 6g/L and 21.5% for heparinized fresh blood, 7g/L and 33.5% for heparinized incubated blood, 6g/L and 22.5% for EDTA fresh blood and 7.5g/L and 37.5%) for EDTA incubated blood, respectively. The sensitivity and specificity were 100% (95%CI 83 - 100%) for all instances, except for incubated heparinized blood, which the specificity was 95% (95%CI 75 - 99.8%).

Conclusion

The FCOF with multiple tubes and decreasing concentrations of NaCl was feasible and could discriminate cases and controls as well as TOF. Moreover, we found single NaCl concentration that allows for a reliable HS screening by using FCOF in heparinized/EDTA and fresh/incubated whole blood.

## A-294

### Development of a Wearable Device to Monitor Heparin Anticoagulation Therapy.

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**Background.** Heparin anticoagulation therapy has a narrow therapeutic window and is the second most common medication error. The partial thromboplastin time (PTT) monitors heparin, but suffers from long turnaround times, a variable reference range, and limited utility with low molecular weight heparin (**LMWH**). Here, we describe a photoacoustic imaging technique to monitor heparin anticoagulation therapy in real time and catheter than can monitor heparin.

**Methods.** We first surveyed five phenothiazinium dyes at five concentrations for their sensitivity to heparin and found that 0.4 mM methylene blue offered the highest signal to background ratio. *In vitro* experiments used fresh human blood stabilized with sodium citrate. First, 10 uL of 1 to 400 U/mL heparin was added to 90 uL of fresh human blood followed by 20 µL of 2 mM methylene blue. Samples were loaded into capillary tubes and imaged with a Visualsonics LAZR photoacoustic scanner from 680 – 900 nm. For *in vivo* experiments, mice (n=3) were injected with 100 µL of 0 or 200 U/mL of heparin dissolved in sterile PBS by tail vein. Thirty minutes later, the animals were injected with 100 µL of 50 mM methylene blue via tail vein. Blood was collected via cardiac puncture and imaged within 4 hours. We covalently linked methylene blue derivatives to a polyurethane catheter.

**Results.** Initial experiments showed strong correlation between heparin concentration and signal ($R^2>0.90$) with stability for at least 15 minutes. The signal increased within 20 seconds of heparin addition. We showed that heparin concentrations as low as 1 U/mL in blood produced statistically significant signal increases versus heparin-free samples ($p<0.02$), and the signal decreased with protamine sulfate treatment. This approach also has utility with LMWH with a detection limit of 0.1 mg/ml. The *in vivo* experiments showed a 2.8-fold photoacoustic signal increase in animals treated with MB versus PBS ($P<0.0001$). The order of addition was important—animals injected with MB first followed by heparin showed little signal. We also used the catheter to measure heparin in human blood with a detection limit of 1 U/mL.

**Data Validation.** The data was validated by comparison to the PTT and protamine sulfate treatment. Mice without heparin treatment had PTT values of 30.1 ± 8.9 s and photoacoustic signal of 16,870 ± 1200 a.u. Mice treated with 200 U/mL (100 µL) heparin had PTT values over 400 s and photoacoustic signal of 40,320 ± 7460. This shows that the signal is indicative of a functional response. Next, we showed that the photoacoustic signal decreased when animals were treated with protamine sulfate—a known heparin antagonist. Mass analysis showed that 10 g of protamine were needed to neutralize 1 U of heparin, which is the clinically used dose (see Supplementary). We also correlated the photoacoustic signal from six human samples to the PTT and showed a Pearson's R of 0.86.

**Conclusion.** To the best of our knowledge, this is the first report to image anticoagulation therapy. We are building a wearable sensor in tandem with a smart intravenous catheter to monitor anticoagulation in real time.

## A-296

### Evaluation of the High sensitivity CRP assay for use on the Binding Site Optilite® turbidimetric analyser

E. Proctor, F. Murphy, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

C-reactive protein (CRP) is a biomarker of systemic inflammation produced by hepatocytes. It is activated by inflammatory cytokines from a wide variety of stimuli including inflammation, infection, tissue damage and neoplasty. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. Minor CRP elevation has been associated with various disorders and clinical conditions in different demographic and socioeconomic groups. These mild increases in serum CRP concentrations can have prognostic implications and can be utilised to stratify patient risk. Here we describe the performance of an immunoassay for the detection and quantification of High Sensitivity CRP on the Binding Site Optilite® analyser. A linearity study was performed according to CLSI EP06-A guidelines; the assay was linear over a measuring range of 0.2-10 mg/L at a 1/1 analyser dilution. Limit of quantitation (LoQ) based on CLSI EP17 was 0.50 mg/L. Correlation to the Roche C501 assay demonstrated good agreement using 148 clinical samples ranging from 0.23-10.2 mg/L (Passing and Bablok analysis slope y=1.00x+0.15). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on three analysers. Samples were selected to cover the medical decision point, pathological concentrations, the reference interval and the minimum dilution. The total precision coefficients of variation (CVs) were as follows: 5.6% at 0.98 mg/L, 5% at 1.55 mg/L, 4.5% at 3.0 mg/L, 4.4% at 5.4 mg/L and 3.1% at 8.5 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 8 potential drug and metabolite interferents, including ibuprofen, caffeine and intralipid at four serum concentrations (0.97, 1.49, 3.13 & 5.70 mg/L). No significant interference was observed (maximum difference in the control samples was 8.67%). In conclusion, the Optilite High Sensitivity CRP assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-297

### Performance of the High sensitivity CRP assay for use on the Binding Site SPAPLUS® turbidimetric analyser

J. Seibaka, K. Samuels, F. Murphy, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

C-reactive protein (CRP) is a biomarker of systemic inflammation produced by hepatocytes. It is activated by inflammatory cytokines from a wide variety of stimuli including inflammation, infection, tissue damage and neoplasty. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. Minor CRP elevation has been associated with various disorders and clinical conditions in different demographic and socioeconomic groups. These mild increases in serum CRP concentrations can have prognostic implications and can be utilised to stratify patient risk. Here we describe the evaluation of a High Sensitivity CRP serum assay (The Binding Site Ltd, UK) for the Binding Site SPAPLUS® analyser. A linearity study was performed according to CLSI EP06-A guidelines; the assay was linear over a measuring range of 0.5-10 mg/L at a 1/1 analyser dilution. Limit of quantitation (LoQ) based on CLSI EP17 was 0.50 mg/L. Correlation to the Roche C501 assay demonstrated good agreement using 218 clinical samples ranging from 0.49-9.99 mg/L (Passing and Bablok analysis slope y=1.00x+0.08). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on four analysers. Samples were selected to cover the medical decision point, pathological concentrations, the reference interval and the minimum dilution. The total precision coefficients of variation (CVs) were as follows: 3.6% at 1.0 mg/L, 2.2% at 1.6 mg/L, 2.0% at 3.0 mg/L, 2.5% at 5.3 mg/L and 3.5% at 8.5 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 8 potential drug and metabolite interferents, including ibuprofen, penicillin and intralipid at four serum concentrations (0.98, 1.57, 3.04 & 5.46 mg/L). No significant interference was observed (maximum difference in the control samples was -4.70%). In conclusion, the HS CRP assay for the SPAPLUS assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-298

### Performance of the CRP assay for use on the Binding Site Optilite® turbidimetric analyser

O. Nevill, J. Seibaka, K. Samuels, V. L. Poole, F. Murphy, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Detection of C-reactive protein (CRP) within serum is widely regarded as the most reliable biomarker for systemic inflammation. CRP functions by readily binding damaged cell membranes and microbial polysaccharides, and is involved in the agglutination and precipitation of invasive bacteria. It is also capable of activating the complement cascade, resulting in opsonisation and phagocytosis of cell debris and bacteria. Normal serum contains <10 mg/L CRP, an increase in circulating CRP levels can be detected within 6 hours post onset of inflammation. Moderately elevated serum levels (10 - 40 mg/L) are associated with mild inflammation and viral infections whereas high levels (>40 mg/L) are indicative of acute phase inflammation and bacterial infections. Here we describe the performance of an immunoassay for the detection and quantification of serum CRP on the Binding Site Optilite® analyser. The Optilite CRP assay displayed good agreement with the Roche Modular P CRP assay in a comparison of 193 serum samples ranging from 3.87 - 498.23 mg/L (Passing-Bablok analysis; Y=1.00x+5.56). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing five serum levels on three kit lots and three analysers over 21 days. Samples were targeted to the medical decision point, pathological concentrations, the reference interval and the minimum dilution. Total precision gave CVs of 3.9% at 9.99 mg/L, 2.9% at 22.33 mg/L, 2.8% at 63.88 mg/L, 3.1% at 146.73 mg/L and 2.8% at 258.28 mg/L. Interference testing was performed using 18 potential drug and metabolite interferents including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.50, 60.06 & 170.73 mg/L). No significant interference was observed (maximum difference from control samples was -8.69%). The assay was also observed to be linear over the measuring range of 5 - 300 mg/L at the standard 1/1 dilution using a serially diluted sample pool (recovery was ≤ ±7.4% for all samples). In conclusion, the Optilite CRP assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-299

### Evaluation of the CRP assay for use on the Binding Site SPAPLUS® turbidimetric analyser

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C-reactive protein (CRP) is a nonspecific inflammatory biomarker of hepatic origin that is commonly quantified in the detection and monitoring of infection and acute phase inflammation. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. CRP concentrations reach a peak within two days on acute phase response, having a half-life of approximately 18 hours. Here we describe the evaluation of The Binding Site CRP serum assay for SPAPLUS® analyser. The assay has been validated, using a linearity study performed to CLSI EP06-A guidelines, to have a measuring range spanning 5-250 mg/L at a 1/1 analyser dilution. A limit of quantitation (LoQ) study based on CLSI EP17 confirmed a limit of 5 mg/L. Correlation to the Roche Modular P assay demonstrated good agreement using 225 clinical samples ranging from 4.66-498.23 mg/L (Passing and Bablok analysis slope y=1.02x+3.22). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on four analysers. The coefficients of variation (CVs) were as follows: 7.8% at 8.7 mg/L, 5.7% at 13.5 mg/L, 2.6% at 60.0 mg/L, 3.6% at 147 mg/L and 6.2% at 225.2 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 18 potential drug and metabolite interferents, including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.5, 63.4 & 170.0 mg/L). No significant interference was observed (maximum difference in the control samples was -9.86%). In conclusion, the SPAPLUS CRP assay provides an accurate method for quantifying human serum CRP.

## A-300

### Evaluation of the Anti-Tetanus Toxoid Immunoglobulin assay for use on the Binding Site Optilite® turbidimetric analyser

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The serological measurement of anti-tetanus toxoid antibodies produced in response to vaccination with tetanus toxoid protein aids the assessment of a patient's immune response. Here we describe the evaluation of the Anti-Tetanus Toxoid Immunoglobulin assay for use on the Binding Site Optilite® analyser. The measuring range of the assay is 1.67 - 50 IU/mL at the standard 1/10 analyser dilution. Correlation to the Binding Site Anti-Tetanus Toxoid Immunoglobulin assay for the SPAPLUS® was performed using 115 plasma samples ranging from 1.74 - 47.72 IU/mL. This demonstrated good agreement when analysed by Passing and Bablok regression (y=0.98x + 0.51). The assay also demonstrated good agreement between serum and plasma matrices using 107 paired serum and EDTA plasma samples ranging from 1.585 - 48.363 IU/mL (Passing and Bablok analysis: y=0.98x + 0.06). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing six serum levels (2.77, 3.15, 4.27, 7.43, 8.85 and 17.04 IU/mL) on a single kit lot over three analysers and 21 days. All levels gave total precision CV values of <9%. Linearity studies were performed following the CLSI EP6-A, using a serially diluted plasma sample. The assay was linear across the measuring range (all results were within 10% of expected values). Interference testing was performed according to CLSI EP7-A2, using serum samples with anti-tetanus toxoid antibody concentrations both close to the medical decision point and at an elevated level. No significant assay interference was observed with triglyceride (1000mg/dL), Intralipid (1000mg/dL), bilirubin (200mg/L) or haemoglobin (5g/L). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and gave a limit of 0.15 IU/mL. The antigen excess capacity of the assay was determined to be equivalent to 120 IU/mL at the standard dilution. In conclusion, the Anti-Tetanus Toxoid Immunoglobulin assay for the Optilite analyser provides a reliable, accurate and precise method for quantifying anti-tetanus toxoid antibodies in serum and plasma.

## A-301

### Performance of the Total Protein assay for use on the Binding Site Optilite® turbidimetric analyser

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Quantification of total protein in serum provides a useful tool for the assessment of the synthesis and maintenance of circulating proteins. Abnormal total protein levels acts as a key indicator for multiple disease states; elevated TP levels are a marker for bone marrow disorders, liver cirrhosis and inflammation. A decrease in total protein concentration can be detected in disorders associated with defective protein synthesis, impaired kidney function, malnutrition and malabsorption. Here we describe the performance of the Total Protein assay for use on the Binding Site Optilite® Analyser. The measuring range of the assay was determined as 0.12-15 g/dL. Linearity was assessed using a serially diluted serum sample, following the CLSI approved guideline EP6-A. The assay was linear across the measuring range (all results were within 10% of expected values). Correlation to the Roche Hitachi 917 assay demonstrated good agreement using 94 clinical samples ranging from 0 - 14.3 g/dL by Passing and Bablok analysis (y=1.017x-0.038). A precision study was performed over a period of 5 days. Total coefficients of variation (CVs) were as follows: 0.77% at 5.8 g/dL, 0.57% at 7.0 g/dL, and 0.54% at 10.8 g/dL. Interference testing was performed according to CLSI EP7-A2 guidelines. No significant assay interference was observed in the presence of triglycerides (1000mg/dL), L-ascorbic acid (60mg/dL), unconjugated bilirubin (60mg/dL), conjugated bilirubin (60mg/dL) and haemoglobin (500mg/dL). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and reported a limit of 0.03g/dL. In conclusion, the Optilite Total Protein assay provides a reliable and precise method for quantifying total protein in human serum.

## A-303

### All Automated Analyzers for CSF Testing are Not Created Equal

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**Objective:** The main purpose of the study was to compare the manual hemocytometer method for performing cerebrospinal fluid (CSF) cell counts with the GloCyte Automated Cell Counter for CSF and the Iris iQ200 body fluid module. **Background:** Traditionally, CSF cell counts have been performed using a manual counting chamber. Clinical laboratories are beginning to look to automated methods for increased precision, shortened turnaround time, and reduced interoperator variability. However, not all automated hematology analyzers on the market provide accurate counts for both total nucleated cells (TNC) and red blood cells (RBC). Clinicians and their patients rely on accurate results making bias an important consideration for clinical laboratories. **Methods:** CSF specimens that were obtained for clinical purposes and sent for analysis to the laboratory at Multicare Health System - Tacoma General Hospital were used for the study. In total, there were 61 specimens including 60 specimens from 45 patients and 1 spiked specimen. All 61 specimens were counted using manual and GloCyte methods. Iris counts were performed on 50 of the 61 specimens. Pearson correlation and Passing-Bablok regression analysis were used to compare methods. Clinical diagnoses were also reviewed. **Results:** There was a strong linear relationship between the manual and automated methods for TNC (R = 0.984 for GloCyte; R = 0.982 for Iris). Overall, the Iris overcounted TNCs; the analyzer had a positive proportional bias of 38% while GloCyte showed no bias. Bias was absent for cell counts ≤30 TNC/μL for both analyzers. For RBC, there was a strong linear relationship between the manual and automated methods (R = 0.998 for GloCyte; R = 0.997 for Iris). Neither automated method showed bias for RBC. **Conclusion:** This study demonstrates that not all automated analyzers are created equal. The Iris exhibited a 38% bias for TNC whereas the GloCyte did not show any bias for TNC or RBC. The absence of bias for the GloCyte shows that GloCyte and manual counts can be used interchangeably and indicates that diagnostic accuracy is not compromised. The presence of bias for Iris TNC counts warrants further studies incorporating larger samples sizes and clinical outcomes to determine if there is any impact on clinical decision making.

## A-304

### Lupus Anticoagulant: Choosing the Right Testing Strategy in the Era of New Oral Anticoagulants

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**Background:**

Antiphospholipid syndrome (aPL) is an acquired autoimmune disorder associated with arterial/venous thrombosis or pregnancy loss. The syndrome can occur in the setting of autoimmune diseases or can occur as a primary entity. Laboratory diagnosis guidelines require testing for the presence of antiphospholipid antibodies (lupus anticoagulant (LA), anticardiolipin, anti Beta 2GP1).

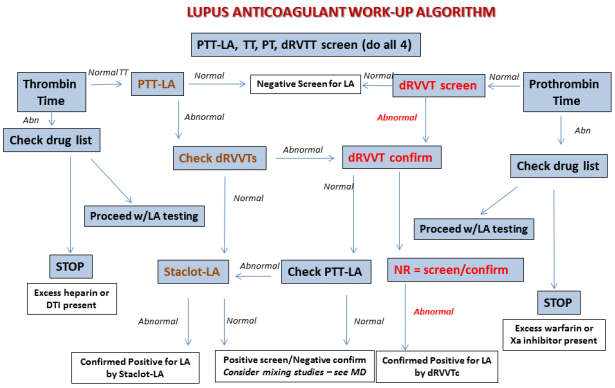
A retrospective study of our testing practices revealed a high incidence of LA false positive results. This was an impetus for us to evaluate our current testing protocol. As a result, we developed a new algorithmic approach to LA testing which significantly decreased our false positives.

Our objective was to develop a new approach to LA testing taking into consideration the current following guidelines: International Society of Hemostasis and Thrombosis (ISTH), British Committee for Standards in Haematology (BCSH), and Clinical and Laboratory Standards Institute (CLSI) to maximize detection of LA in the era of new oral anticoagulants in a cost effective way.

**Methods:** We tested 20 purchased normal plasma samples (LA negative) and established new reference ranges for the following tests: prothrombin time (PT), thrombin time (TT), lupus sensitive partial thromboplastin time (PTT-LA), dilute Russel Viper Venom screen and confirm (DRVV) times and Staclot-LA. After the implementation of the new algorithmic approach (see Figure 1), 1146 patient results tested between April 2014 to March 2015 were retrospectively reviewed.

**Results:** The following results were obtained based on our proposed algorithmic approach: DRVV positive LA 111/1146 (10%), Statclot LA positive and DRVV negative 228/1146 (20%).

**Conclusion:** The algorithmic approach to LA testing was found to be useful in decreasing our false positive rates and the cost of testing for our institution and the patients while increasing the accuracy of detection.




## A-306

### Serum free light chains in the evaluation of the response of non secretory multiple myeloma

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**Background**: Serum free light chains (sFLC) are used in the diagnosis, prognosis and therapy monitoring of patients with Multiple Myeloma (MM). Non-Secretory MM (NSMM) accounts for 1-5% of all MM cases and is characterized by the absence of detectable monoclonal proteins in serum and urine by EPS and IFX. Therefore, invasive bone marrow examinations are required for monitoring disease activity. Quantification of serum free light chains (sFLC) is a sensitive method to diagnose many of these patients. The objective of our study is to show the utility of sFLC assay also in the monitoring of a NSMM patient.

**Methods:** A 63 year old man with NSMM, in complete response after treatment with VAD (Vincristine/Adriamycin/Dexamethasone) and autologous stem cell transplant (ASCT). He was monitored regularly after ASCT to ensure remission or detect a possible relapse. sFLC were measured using the assay Freelite (The Binding Site, UK).

**Results**: During the monitoring after ASCT, sFLC lambda levels began to increase with abnormal ratio (month+46: 51.2 mg/L with ratio=0.12; month+47: 144 mg/L with ratio=0.08) suggesting recurrence of NSMM at this moment. In month+50 (lambda=572 mg/L, ratio=0.02) the bone marrow showed a 4% of plasma cells and the serum protein electrophoresis and Bence Jones proteinuria were still negative. The patient began treatment with Lenalidomide/Dexamethasone (13 cycles) achieving a reduction of sFLC lambda to 20.1 mg/L and normalization of the ratio (0.58) at month+58. Seven months after this treatment, sFLC levels began to increase again with values of 231 mg/L at month+65 (ratio=0.09), 893 mg/L at month+67 (ratio=0.01) predicting a new relapse. At month+69, the patient presented a clinical relapse with presence of new osteolytic lesions, starting a new treatment with Bortezomib/Dexamethasone.

**Conclusions**: Freelite is a noninvasive assay potentially useful for monitoring the disease activity in NSMM patients that present with abnormal sFLC. Due to its high sensitivity, this assay can predict a relapse months before evidence of clinical relapse improving monitoring and helping managing NSMM patients. Furthermore, Freelite reduces the number of bone marrow biopsies for this group of patients avoiding patient anxiety and the risk of associated complications.

## A-307

### The precision and accuracy of low Factor VIII levels by one stage clotting.

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**Background:** The safe and cost-effective treatment of severe Hemophilia requires a readily-available, robust, sensitive and precise method for low-level Factor VIII activity (FVIII:C) testing. According to guideline procedures based on one-stage clotting, patient plasma is diluted with diluent and FVIII-deficient plasma (FDP) and the Activated Partial Thromboplastin Time (APTT) provides a measure for FVIII:C. Low FVIII:C levels induce weak clots at long APTTs, which are often too imprecise and inaccurate for classification or optimal dosing. Here, we investigate the effect of plasma dilution on the accuracy and precision of low FVIII:Cs in deficient patients. **Methods:** On a STA-R Evolution, 50 µl of diluted patient plasma, 50 µl FDP and 50 µl Kaoline activator were incubated for 240 s after which 50 µl $CaCl_2$ was added and the APTT was started. The reference method, based on a 10-fold dilution of patient plasma, was compared with investigated method based on a 2-fold dilution; in the latter, the effect of manual (dilutions with FDP) versus automated (dilutions with diluent) preparation of calibrators was investigated. According to standard evaluations protocols, the precision and accuracy of samples in the range of <0.01-0.10 IU/ml was obtained and the methods were compared by Passing and Bablok regression in 22 patients with FVIII:C <0.15 IU/ml. **Results:** Relative to the reference method, shorter APTTs are acquired and a stronger response is evidenced from the calibration curves using 2-fold dilution. Unlike the reference method, there is a marked difference between calibration curves that are based on standards prepared by water-dilution and standards prepared by FDP-dilution. Herein, different APTTs are acquired at the same FVIII:C revealing the influence of the other clotting factors on the APTT in the less-diluted method. While the variance at FVIII:C = 0.09 IU/ml is similar between both methods (CV = 7-8%), better precision is found at FVIII:C = 0.01 IU/ml in the investigated method (CV = 5-8% versus 14% in the reference method). At higher concentrations of the other clotting factors, firm clots are formed within uniform clotting times; given the higher response of the calibration curve, uniformity is further enhanced upon converting APTTs to FVIII:Cs. The method comparison reveals that compositional similarity between calibrators and low FVIII:C samples is crucial at lower dilutions, hence falsely elevated FVIII:Cs are found when using calibration standards based on water-dilution ($FVIII:C_{NEW}$ = 1.49 x $FVIII:C_{REF}$ - 0.51). Proper correlation between the reference method and the 2-fold diluted method is achieved by using calibrators prepared with FDP ($FVIII:C_{NEW}$ = 0.98 x $FVIII:C_{REF}$ - 0.77). **Conclusion:** The quantification of FVIII:C by one-stage clotting is more precise using a less-diluted APTT. Considering the influence of other clotting factors on the APTT, calibrators having similar concentrations of the other clotting factors should be used. At extremely low FVIII:C levels and in the absence of interfering species, the properly calibrated and less-diluted APTT may result in higher accuracies as well.

## A-308

### Individual Erythrocyte Soret Band Absorption For Cell Type Discrimination

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Background

We sought to determine whether the hemoglobin in individual erythrocytes absorbs enough light in the Soret band to produce robust signals for detection, and whether these signals can discriminate erythrocytes from other cell types. Since hemoglobin is found in erythrocytes but not in thrombocytes or leukocytes, a robust absorption signal should discriminate among these cell types. In order to measure erythrocyte absorption it is necessary to account for all of the light intercepted by a cell. This requires a knowledge of the scattering behavior of erythrocytes, thrombocytes, and leukocytes. Using Mie scattering theory as a guide, and treating all cells and nuclei as homogeneous bodies, we attempted to measure absorption by collecting the light scattered by individual cells, including erythrocytes, into a 17 degree cone around the incident radiation, since the theory predicts that almost all of the light that is scattered by all cell types falls within this cone, and consequently only light lost to absorption will register. This technique is not subject to a limitation of standard light-scattering-based flow cytometry for blood samples; that erythrocyte light-scatter coincidence signals overlap leukocyte signals, rendering a single-dilution, non-lytic measurement impossible. A single dilution system is less expensive relative both to hardware and reagent usage than multi-dilution techniques, and so is a desirable alternative.

Methods

Blood samples were diluted in a medium that spheres and fixes erythrocytes. Erythrocyte fractions were prepared by passing whole blood through Pall Acrodisc leukocyte filters and collecting the leukocyte depleted fractions. Leukocyte fractions were prepared by back flushing used filters with $NH_4Cl$ to lyse residual erythrocytes. Whole blood and erythrocyte fraction samples were diluted 50-fold to demonstrate insensitivity to erythrocyte coincidence. Leukocyte samples were undiluted. Samples were run on a modified hematology analyzer; light source replaced by a 406 nm laser, sheath replaced by sphering and fixing diluent. Two measurements were made on each cell; light scattered over 17° and orthogonally (80-100°). 20000 cells were analyzed for each sample. Data was collected in FCS format and displayed as right angle vs. 17° (absorption) plots.

Results and Conclusions

The plots are of erythrocytes, leukocytes, erythrocytes + leukocytes, and a normal whole blood sample. They show that the erythrocyte absorption signal is robust, but that the absorption channel alone does not discriminate between erythrocytes and polymorphonuclear leukocytes which have numerous and relatively large granules that cause scattering loss outside of 17 degrees. They also show that in combination with right angle scattering intensity, these cell types are discriminated. Thrombocyte signals are below detection threshold. We conclude that 406 nm absorption by individual erythrocytes generates robust signals, distinct from leukocyte and thrombocyte signals on right angle vs. absorption plots. It applies at high erythrocyte concentration, and is therefore suitable for automated hematology analyzers requiring high sampling rates.

## A-309

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

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**Background:** The objective of this study was to compare the performance of two automated coagulation analyzers, the high-volume Sysmex® CS-5100 System and the Sysmex CA-1500 System, using Siemens Healthineers reagents. Performance characteristics of the systems for factor V deficiency (FV), factor VII deficiency (FVII), protein C deficiency clotting (PC-cl), and protein C deficiency chromogenic (BCPC) were compared.

**Methods:** Three U.S. and one German laboratory participated in method comparison (MC) studies. Result comparability was investigated using leftover samples.† MC of the Sysmex CS-5100 System versus the Sysmex CA-1500 System was based on a total of 2269 results (sum of results over all parameters). Precision studies were performed according to CLSI guideline EP05-A3 and followed the scheme of 20 x 2 x 2 testing at three clinical sites. Twenty-two samples (FV: n = 6, FVII: n = 6, PC-cl: n = 5, BCPC: n = 5) covering important medical decision points and the total clinical reportable range CRR were used. The complete dataset contained 5209 results. In addition, performance data for the Sysmex CS-5100 System regarding limit of quantitation (LoQ) for FV, FVII, and both PC applications were determined according to CLSI guideline EP17-A2.

**Results:** Analysis of MC data was done by Passing-Bablok regression and difference plot and revealed very good agreement to the Sysmex CA-1500 System, showing slopes between 0.95 and 1.05 and correlation coefficients ≥0.984 (depending on application). CVs for within-run (repeatability) precision varied from 2.4 to 3.2% for FV, 1.3 to 2.0% for FVII, 2.5 to 3.2% for PC-cl, and 1.5 to 6.9 % for BCPC (depending on the sample).

**Conclusion:** Results for the Sysmex CS-5100 System were in good agreement with those for the Sysmex CA-1500 System. Precision for the new devices/reagents combination showed low CV values. Based on the data collected during these studies, in combination with improved functionality and ease of use, the high-volume Sysmex CS-5100 System provides high performance, quality, and efficiency to coagulation laboratories.

*Product availability may vary from country to country and is subject to varying regulatory requirements.

†Donors gave informed consent, and review boards were involved.

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## A-310

### Multicenter Study of the Mid-volume Sysmex CS-2100*i*/2500 System* Compared to the Sysmex CA-1500 System Using Siemens Healthineers Reagents

R. Barten[1], M. Kahl[1], M. Weik[1], I. Birschmann[2], C. Eby[3], J. L. Francis[4], E. Hod[5], B. Kemkes-Matthes[6], S. W. Pipe[7]. *[1]Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, [2]Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany, [3]Washington University School of Medicine, Saint Louis, MO, [4]Florida Hospital, Orlando, FL, [5]Columbia University, Medical Center, New York, NY, [6]Haemostasis Center, University Hospitals Giessen and Marburg, Giessen, Germany, [7]University of Michigan, Ann Arbor, MI*

**Background:** The objective of this study was to compare the performance of two automated coagulation analyzers, the mid-volume Sysmex® CS-2100*i*/2500 System† and the Sysmex CA-1500 System, using Siemens Healthineers reagents. Performance characteristics of the systems for factor V deficiency (FV), factor VII deficiency (FVII), protein C deficiency clotting (PC-cl), and protein C deficiency chromogenic (BCPC) were compared.

**Methods:** Three U.S. and one German laboratory participated in method comparison (MC) studies. Result comparability was investigated using leftover samples.‡ MC of the Sysmex CS-2100*i*/CS-2500 System versus CA-1500 System was based on a total of 2172 results (sum of results over all parameters). Precision studies were performed according to CLSI guideline EP05-A3 and followed the scheme of 20 x 2 x 2 testing at three clinical sites. Twenty-two samples (FV: n = 6, FVII: n = 6, PC-cl: n = 5, BCPC: n = 5) covering important medical decision points and the total clinical reportable range (CRR) were used. The complete dataset contained 5259 results. In addition, performance data for the Sysmex CS-2100*i*/CS-2500 System regarding limit of quantitation (LoQ) for FV, FVII, and both PC applications were determined according to CLSI guideline EP17-A2.

**Results:**Analysis of MC data was done by Passing-Bablok regression and difference plot and revealed very good agreement to the Sysmex CA-1500 System, showing slopes between 0.94 and 1.04 and correlation coefficients ≥0.977 (depending on application). CVs for within-run (repeatability) precision varied from 3.2 to 5.0% for FV, 1.9 to 2.5% for FVII, 2.5 to 4.8% for PC-cl, and 1.2 to 4.6 % for BCPC (depending on the sample).

**Conclusion:** Results for the Sysmex CS-2100*i*/CS-2500 System were in good agreement with those for the Sysmex CA-1500 System. Precision for the new devices/reagents combination showed low CV values. Based on the data collected during these studies, in combination with improved functionality and ease of use, the mid-volume Sysmex CS-2100*i*/2500 System provides high performance, quality, and efficiency to coagulation laboratories.

*Product availability may vary from country to country and is subject to varying regulatory requirements.

† Performance characteristics presented have been established using the Sysmex CS-2100i Automated Blood Coagulation Analyzer, which is the representative member of an instrument family. The performance can be applied accordingly to the Sysmex CS-2500 Automated Blood Coagulation Analyzer, which is a member of the same instrument family.

‡Donors gave informed consent, and review boards were involved.

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## A-312

### Loss of *Rnase1* produces coagulation abnormalities in mice

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**Background:** Use of anticoagulant drugs carries a risk of bleeding, and there is significant interest in developing new therapies that do not present this risk. This requires a better understanding of the factors that contribute to coagulation and thrombosis *in vivo*. The contact activation pathway has been a subject of recent research to this end, with studies highlighting the ability of polyphosphate and RNA to stimulate coagulation in plasma and demonstrating inhibition of the coagulation proteases factor XI (fXI) and factor XII (FXII) as possible antithrombotic therapies.

At the same time, studies in mice have demonstrated antithrombotic properties for the enzyme RNase A, a nonspecific endoribonuclease. The objective of our research is to clarify the role of RNA and RNase in regulation of coagulation *in vivo* through study of a mouse model that lacks RNase 1, the murine homolog of this RNA-degrading protein. **Methods:** *Rnase1*-null mice were generated in our laboratory, and evaluated in comparison with wild-type littermates. Plasma coagulation was evaluated *in vitro* using kinetic clotting assays with Thromborel® S or Dade Actin® FSL Activated PTT Reagent, as well as with mixing tests using human factor-deficient plasma. *In vivo* analysis of bleeding and clotting behavior was conducted using lipopolysaccharide-stimulated thrombin-antithrombin complex assay, tail-vein bleeding test, and ferric chloride-induced arterial thrombosis assay. Studies were conducted with a minimum of three biological replicates per group, and statistical significance was evaluated using Wilcoxon rank-sum test.**Results:** *In vitro* coagulation assays reveal shortened clotting times for *Rnase1*-null plasma relative to wild-type, with significantly shorter times for unstimulated plasma and when stimulated with Thromborel S. Yet, *Rnase1*-null mice did not exhibit increased thrombin-antithrombin complex formation in response to lipopolysaccharide challenge, did not bleed less than wild-type mice in a tail-vein bleeding test, and did not form thrombi more quickly than did wild-type mice in a ferric-chloride induced arterial thrombosis model. Mice that lack expression of contact pathway coagulation factors, such as fXI and fXII, also do not exhibit perturbed in vivo coagulation behavior despite prolongation of coagulation in vitro. Additionally, these factors are activated in vitro by RNA. Accordingly, and because *Rnase1*-null plasma contains significantly more RNA than does wild-type plasma, we are evaluating whether the loss of RNase 1 permits increased activation of the contact activation pathway. Indeed, preliminary factor activity assays indicate that fXII is strongly activated in *Rnase1*-null plasma, and experiments are underway to demonstrate that this is an RNA-dependent process.

**Conclusion:** Our results suggest that RNase 1 is an endogenous negative regulator of contact pathway activation in mice via RNA degradation. This finding provides insight into the function of RNA in the pathophysiology of coagulation, and could inform future development of anticoagulant therapeutics.

## A-313

### Optical platelet counts by Abbott prototype hematology analyzer show high level of agreement with CD61 immunoplatelet results

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**Background:** The CELL-DYN Sapphire (Abbott Laboratories, Santa Clara, CA) hematology analyzer is able to enumerate platelets (PLT) based on CD61 labeling in addition to optical and impedance PLT measurements. The CD61 immunoplatelet (PLTim) method is considered to be a surrogate reference method for the ICSH/ISLH recognized flow cytometric assay (CD41/CD61). We utilized this immunoplatelet assay to verify the performance of a novel optical PLT counting method on a prototype hematology analyzer (Abbott), focusing on samples with low PLT counts.

**Methods:** One hundred and sixty-two EDTA-anticoagulated samples with platelet counts of 5.1-442.8 x $10^9$/L were selected from the routine workload of the hematology laboratory. Samples were analyzed with two prototype systems and with one CELL-DYN Sapphire analyzer within eight hours of collection. PLT counts obtained with the prototype systems and the CELL-DYN Sapphire optical PLT method (PLTo) were compared to the PLTim method, as well as with each other. Agreement was evaluated using regression statistics and Pearson's correlation. **Results:** Results by all methods and on each analyzer were available for 110 samples, ranging 5.1-88.8 x$10^9$/L PLT. Pearson's correlation coefficients, slope and intercept of the regression line (Passing Bablok), and mean differences between the results are shown below:

| | n | r | Slope | Intercept | Mean difference |
|---|---|---|---|---|---|
| Prototype#1 vs PLTim | 110 | 0.98 | 0.92 | -1.6 | -2.9 |
| Prototype#2 vs PLTim | 110 | 0.97 | 0.94 | 0.22 | -2.7 |
| Prototype#1 vs Prototype#2 | 110 | 0.98 | 0.99 | -0.26 | 0.2 |
| Sapphire PLTo vs PLTim | 110 | 0.99 | 0.90 | 0.63 | -3.4 |

**Conclusion:** Results obtained by the prototype analyzers showed excellent correlation with the PLTim measurement, and also with each other. The mean difference between PLT results by either prototype system and the PLTim results was smaller than that of between the Sapphire PLTo and PLTim methods. These data demonstrate that the new prototype hematology analyzer provides results in low PLT ranges that are equivalent with those obtained with the PLTim method.

## A-314

### Establishment of Reference Intervals for Whole Blood Luminescent Platelet Aggregometry

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**Background:** Platelet aggregometry may be employed for the diagnosis of platelet function disorders such as von Willebrand's disease and monitoring response to certain anti-platelet drugs. Though traditionally performed on platelet rich plasma, many laboratories, especially in pediatric institutions aiming to reduce blood volume collected, adapt assays for whole blood. There is a paucity of published normal range studies for whole blood platelet aggregation analysis by impedance and luminescent ATP release assays. The objective of this study is to generate reference intervals for whole blood platelet aggregometry by impedance and luminescence. **Methods:** Whole blood samples were collected by venipuncture into sodium citrate vacutainer tubes from 77 normal, drug-free donors recruited at Nationwide Children's Hospital (Columbus, OH), Seattle Children's Hospital (Seattle, WA), and St. Luke's Hospital (Boise, ID). Various concentrations of agonists were introduced to elicit aggregation, and impedance whole blood aggregometry and luminescent ATP release measured by Chronolog aggregometer according to manufacturer's recommendations (ChronoLog, Havertown, PA). Reference intervals were generated using either parametric or transformed parametric analysis, as appropriate, in EP Evaluator 10 (Data Innovations, Burlington, VE). **Results:** Reference intervals and 90% confidence intervals were generated for whole blood aggregometry by both impedance and luminescent ATP release assays in response to several agonists (see table). **Conclusion:** This study fills an important gap in the literature which currently has a paucity of reference intervals for this methodology. These may serve as guidance for laboratories running these assays.

| Agonist | Agonist Concentration | Lower Limit (confidence interval) | Upper Limit (confidence interval) |
|---|---|---|---|
| **Impedance Aggregation** | | | |
| Arachadonic Acid | 0.5 mM | 6.9 Ω (5.1-8.7) | 28.7 Ω (26.9-30.4) |
| ADP | 5 µM | 5 Ω (3.0-7.0) | 26 Ω (24-28) |
| ADP | 10 µM | 5.5 Ω (4.5-6.6) | 33.4 Ω (29.5-37.6) |
| Collagen | 1 µg/mL | 12.1 Ω (11.1-13.2) | 32.6 Ω (29.8-35.6) |
| Collagen | 5 µg/mL | 10.5 Ω (7.8-13.1) | 42 Ω (39.3-44.6) |
| **Luminescent ATP release** | | | |
| Arachadonic Acid | 0.5 mM | 0.45 nM (0.37-0.54) | 2.78 nM (2.46-3.13) |
| ADP | 5 µM | 0.11 nM (0.06-0.17) | 2.01 nM (1.58-2.53) |
| ADP | 10 µM | 0.21 nM (0.16-0.27) | 1.95 nM (1.66-2.27) |
| Collagen | 1 µg/mL | 0.43 nM (0.35-0.51) | 2.22 nM (1.96-2.50) |
| Collagen | 5 µg/mL | 0.54 nM (0.45-0.64) | 2.93 nM (2.61-3.28) |
| Ristocetin | 0.25 mg/mL | 0.0 nM (-0.7- -0.5) | 0.8 nM (0.7-0.9) |
| Ristocetin | 1.0 mg/mL | 4.3 nM (3.6-5.1) | 33.6 nM (28.3-39.8) |
| Thrombin | 1 Unit | 0.80 nM (0.71-0.90) | 3.03 nM (2.68-3.43) |

## A-318

### Protein S deficiency diagnosis: analysis from a large laboratory in Latin America

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**Background:** The diagnosis of hereditary protein S (PS) deficiency is the most difficult of the hereditary thrombophilias to document with certainty, particularly in the setting of an acute thrombosis or anticoagulant administration, especially vitamin K antagonists. Many other acquired conditions are associated with reduced levels: pregnancy, oral hormonal contraceptive use and several conditions associated with acute illnesses, in which there is an increase of C4b-binding protein. Protein S (for Seattle) is a negative regulator of coagulation. It circulates in two states: free, and bound to the complement component C4b-BP. When elevated, the C4b-BP leads to a shift of the PS from the free form (the only form that is active) to the bound (inactive) form, leading to an erroneous diagnosis. Free PS serves as a cofactor for protein C, which inactivates procoagulant factors Va and VIIIa, reducing thrombin generation. The frequency of PS deficiency may be less than 1 percent of individuals with venous thromboembolism (VTE). Free PS level (measured with an immunoassay) is probably the best screening test. The objective of this study is to evaluate the profile of total and free PS requests for the investigation of thrombophilia in a large Brazilian laboratory. **Methods:** Between October and December 2016 a survey of all samples with total and free PS was performed. The results were analyzed to identify the reduction of total and free PS and the reduction of free PS with normal total PS. **Results:** In 3227

PS applications, in only 304 (9.4 percent) of them there was also the request for free PS. Of these, in 58 there was a reduction of the total and free PS, in 53 reduction of the free PS only (in 10 samples the reduction constituted a severe deficiency). In that same period, only 851 requests (26.3 percent) were of free PS (associated or not to the requests of total PS). **Conclusion:** PS deficiency is an autosomal dominant condition, and the major clinical feature is VTE. Although the description of the best method of evaluation of PS deficiency is the dosage of free PS, a large number of requests still consider only the total PS for diagnosis. The presence of free PS reduction, with normal total PS levels, underscore the importance of the specification of free PS for the diagnosis of this thrombophilia, since only the free form is active in reducing the thrombin generation. Such a situation leads to misleading results, false negatives and manly false positives, because it is a rare condition with several acquired interfering factors. This demonstrates that a continuing education of the teams is necessary, either through information leaflets, lectures or meetings, in order to reduce the anxiety of patients and relatives and the unnecessary use of anticoagulants, which can have serious consequences for the community and health systems.

## A-319

### Performance of Hemoglobin A1c and Fructosamine on Estimating Glycemic Control in Diabetes Patients with Hemoglobin Variant Hope

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**Background:** Hemoglobin A1C (HbA1c) and fructosamine are commonly used to estimate glycemic control in diabetes patients. Hemoglobin (Hb) variant Hope is prevalent in Southeast Asian population, but has been reported in the United States. This case study aims to evaluate the clinical performance of HbA1c and fructosamine on estimating glycemic control in 4 diabetes patients with Hb variant Hope.

**Methods:** HbA1c in patients' whole blood were measured via high-performance liquid chromatography (HPLC) performed on Turbo Variant™ II (Bio-Rad) and immunoassay on DCA Vantage Analyzer (Siemens), respectively. The presence of Hb Hope was analyzed by Variant™ II β-Thalassemia Short Program and acid gel electrophoresis. Fructosamine from the same samples were quantified using spectrophotometry (ARUP Laboratories). These patients' recent fasting glucose levels (2 weeks - 3 months) were obtained through retrospective chart review.

**Results:** Spuriously elevated HbA1c (39.7%-55.3%) as determined by HPLC (Fig. 1A, D) was observed in all patients. The interference from Hb Hope was suggested based on the elevated P2 on Hb chromatograph (Fig. 1B) and intensive band corresponding to HbF on acid gel (Fig. 1C). In contrast, normal A1c% (3.5%-5.3%) from the same samples were obtained via immunoassay, which were consistent with their corresponding normal fructosamine levels (184-264 μM) (Fig. 4D). Surprisingly, patient chart review revealed that these patients encountered multiple episodes of elevated fasting glucose (110-258 mg/dL) in the past 2 weeks to 3 months, with an average glucose level of 160 mg/dL (Fig. 4D).

**Conclusion:** Our data demonstrate that Hb Hope causes significant positive bias on HbA1c HPLC assay but not HbA1c immunoassay, which displayed a good agreement with patient's fructosamine level. However, the performance of HbA1c and fructosamine in estimating glycemic control in patients with Hb Hope might be questioned, in view of the discrepancy between the suggested in-control glycemic status and the elevated fasting glucose.

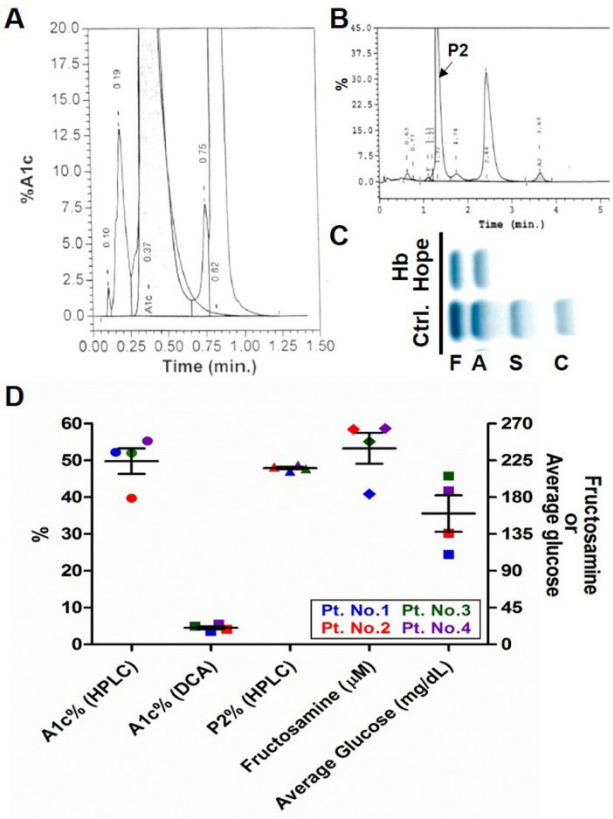


**Figure 1.** (A) HbA1c chromatograph on BIORAD Turbo Variant II. (B) Hb variant chromatograph on BIORAD variant II β-thalasima program. (C) Acid gel electrophoresis of patient with Hb Hope and loading control (Ctrl.) Hb. (D) Quantifications of A1C% by HPLC and DCA immunoassay, Hb P2% by HPLC, fructosmaine, and the average glucose in the previous 2 weeks to 3 months.

## A-320

### Discrepancies in measured fibrinogen concentration using low and high thrombin content commercial fibrinogen reagents

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**Background:** Commercial fibrinogen reagents with lower thrombin content are more susceptible to interference from direct thrombin inhibitors, anti-thrombin antibodies, and other interferants. We compared fibrinogen results between low and high thrombin content fibrinogen assays to determine the rate and magnitude of discrepant fibrinogen results in an acute care patient population.

**Methods:** As part of the evaluation of a new coagulation analyzer, we measured fibrinogen using the higher thrombin content (80 UNIH/mL) FIB 5 reagent on a Stago Compact (Diagnostica Stago); and the lower thrombin content (35 UNIH/mL) FIB C assay on an IL ACL TOP 500 (Instrumentation Laboratory). The initial comparison was done using 50 frozen samples submitted to the stat laboratory. In a follow-up experiment using 50 fresh samples, we compared FIB 5 on the Compact to both FIB C and the QFA (high thrombin content of 100 UNIH/mL) fibrinogen reagents on the IL TOP. The stat laboratory performs fibrinogen measurement primarily for patients undergoing cardiovascular surgery. The number/percent of discrepant results (>25% difference between assays) was determined.

**Results:** In the first experiment using frozen samples, 5 of 50 (10%) FIB C results were >25% lower than the corresponding FIB 5 value. For these samples fibrinogen concentration measured by the lower thrombin content FIB C reagent ranged from 74 to 350 mg/dL lower than corresponding FIB 5 (higher thrombin content) value. In the second experiment using fresh samples, 12 of 50 (24%) of FIB C values were >25% lower than the corresponding FIB 5 value, while 1 sample had FIB C value >25% greater than FIB 5. Using the higher thrombin content IL QFA reagent, 4 of 50 (8%) QFA values were >25% lower than FIB 5, while 1 was >25% higher than

the corresponding FIB 5 value. Selected chart review of patients with discrepant fibrinogen results demonstrated that 4 of the discrepant fresh sample comparisons were from 2 infants who had received topical thrombin, suggesting that anti-thrombin antibodies may have caused the discrepancies. Another discrepant result came from an adult post-myocardial infarction that was on a direct thrombin inhibitor (bivalirudin). Remaining discrepant samples had no obvious explanation.

**Conclusion:** In an acute care patient population (mostly patients following cardiovascular surgery), discrepancies between different commercial fibrinogen reagents are common. Using reagents with higher thrombin content reduces but does not eliminate discrepancies between commercial fibrinogen reagents.

## A-321

### Comparative Study of the Point-of-care Xprecia Stride Coagulation System to the BCS XP, Sysmex CS-2500, and Sysmex CA-1500 Systems

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**Background:** The objective of the study was to compare the performance of the point-of-care quantitative prothrombin time (PT) test for the monitoring of oral anticoagulant therapy with a vitamin K antagonist (VKA) using four lots of Xprecia Stride™ test strips to the results of central-lab PT/INR testing using plasma of the same samples on the BCS® XP, Sysmex® CA-1500, and Sysmex CS-2500 Systems.

**Methods:** Capillary fingerstick samples were obtained from approximately 90 patients receiving VKA therapy and 30 healthy patients at two sites. A fingerstick was used to obtain blood that produced results on four lots of Xprecia Stride test strips. In addition to the capillary fingerstick, a venous sample was obtained from each patient, which was separated into a plasma fraction. The plasma was frozen and sent to a central lab on dry ice for PT/INR testing. Method comparison and outlier removal were performed per CLSI guideline EP09-A3.

**Results:** Passing-Bablok regression analysis showed exemplary agreement between the Xprecia Stride analyzer and the central-lab devices. The Xprecia Stride analyzer demonstrated slopes of 0.95–0.98 against the Sysmex CA-1500 System, 0.94-0.97 against the Sysmex CS-2500 System, and 0.90–0.94 against the BCS XP System.

**Conclusion:** All method comparisons between the Xprecia Stride analyzer and the laboratory devices showed good agreement. The data demonstrates that the point-of-care device can provide results that will lead to similar medical decisions across the therapeutic ranges of warfarin (VKA) monitoring. With this performance, the Xprecia Stride analyzer can be used to provide clinically relevant results in a timely manner at the point of care.

Product availability may vary from country to country and is subject to varying regulatory requirements

BCS XP and Xprecia Stride are trademarks of Siemens Healthcare; Sysmex is a trademark of Sysmex Corporation.

| Site/ Operator | Strip Lot | Xprecia Stride Analyzer vs. Sysmex CA-1500 System($r^2$) | Xprecia Stride Analyzer vs. Sysmex CS-2500 System ($r^2$) | Xprecia Stride Analyzer vs. BCS XP System($r^2$) |
|---|---|---|---|---|
| Site 1, Operator 1 | 1 | 0.966 | 0.966 | 0.966 |
| | 2 | 0.966 | 0.964 | 0.962 |
| | 3 | 0.964 | 0.966 | 0.963 |
| | 4 | 0.953 | 0.955 | 0.953 |
| Site 2, Operator 2 | 1 | 0.939 | 0.937 | 0.928 |
| | 2 | 0.948 | 0.947 | 0.939 |
| | 3 | 0.932 | 0.933 | 0.923 |
| | 4 | 0.944 | 0.943 | 0.932 |

## A-322

### New MiniCollect® 9NC Coagulation Blood Collection Tubes for pediatric sample testing

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**Background:** Drawing blood from infants or children is mostly critical, particularly when the amount needed to fill a standard coagulation tube by ensuring the correct ratio of blood to additive can't be guaranteed. The MiniCollect Coagulation Tube is intended for collection of citrate anticoagulated whole blood samples for coagulation assays and allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity.

**Methods:** Two clinical studies were carried out to compare the performance of the new pediatric tube to a standard VACUETTE Coagulation tube by taking venous blood. Altogether, 20 healthy and 75 hospitalized subjects (Laboratory Rainbach and Hospital Steyr, Upper Austria) were recruited. Informed consent was given by all donors and the study was approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, common coagulation parameters were tested using an ACL Top 500 (Laboratory Instruments). Analysis was done with the instrument's accompanying reagents (precision aPTT ≤2.5%; PT ≤ 3%, Fibrinogen ≤8%). Statistical evaluation was done by STATISTICA 13.

**Results:** Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of pediatric tubes with the new design did not reveal any clinically nor statistically significant deviations ($p<0.05$). The values in both tubes resulted in maximum deviations of 7.1% for aPTT.

**Conclusion:** From a clinical perspective, the MiniCollect Coagulation tube with the new design is substantially equivalent to a VACUETTE Coagulation tube. The newly designed tube provides an essentially enhanced blood collection device for pediatric sample testing.

## A-323

### Hematological sample testing in new MiniCollect® Blood Collection Tubes

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**Background:** Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. MiniCollect® $K_2$EDTA and $K_3$EDTA Blood Collection Tubes are used to collect, transport, store and evaluate capillary blood specimens for hematology tests.

**Methods:** Studies considering venous and capillary collection were done at Steyr Hospital and Laboratory Rainbach (Austria) using MiniCollect tubes with the old design vs. new design. Altogether, 65 hospitalized and 90 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. Complete blood counts including 15 parameters were tested using a DxH800 (Beckman Coulter, precision WBC ≤ 3%/RBC ≤ 1.5%). Comparison testing to Microtainer (BD) was done. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.

**Results:** Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design did not reveal any clinically nor statistically significant deviations ($p<0.05$). Comparing the initial values of the old and new design for venous collection, both EDTA tubes resulted in a highest deviation of 3.0% for RBC. Comparable highest deviations for initial values in relation to 48h values were obtained for $K_2$EDTA (WBC 0.4%; RBC 0.1%) and $K_3$EDTA (WBC 2.6%; RBC 0.1%). Capillary collection led to a highest deviation for WBC of 0.7% for $K_2$EDTA and of 2.2%.the $K_3$EDTA tubes.

**Conclusion:** From a clinical perspective, the MiniCollect $K_2$EDTA and $K_3$EDTA tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

## A-324

### A proposition for Total error evaluation of fetal hemoglobin

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Background: At birth, fetal hemoglobin comprises 65 to 90% of total hemoglobin concentration, and after the second trimester, this percentage decreases to less than

2%. Fetal hemoglobin (HbF) is formed by two gamma globin chains combined with two alpha globin chains and is represented by the formula α2 γ2 with expression of the genes γG γA, located on the short arm of chromosome 11. The permanent increased percentage of HbF can occur due to some hereditary abnormalities, as in: delta-beta thalassemia characterized by reduced synthesis or absence of delta and beta chains, with consequent increase in Hb F; in beta thalassemia, when syntesis of beta chains is reduced with increased of A2 and fetal hemoglobins; and, in hereditary persistence of Hb F (HPFH), a genetic disorder characterized by continuous production of HbF in adulthood. HbF also influences the clinical manifestation of other hemoglobinopathies, working as an important protective factor against sickling phenomenon, due higher affinity for oxygen.

Objective: We aimed to propose a total error limit in HbF dosage by the sum of random error with systematic error, evaluate how the results can vary and also to define a target value, for a clinical acceptable performance for this analyte, thus helping the continuous improvement of quality.

Methods: Total error of the analyte fetal hemoglobin (VARIANT ™ II - ß-thalassemia Short Program Bio-Rad®) was calculated as the sum of random and systematic errors, obtained from January 2013 to December 2016. As random error, we used coefficient of variation (CV) of the test multiplied by 1.65 to a desired confidence interval of 90%. For the systematic error we used in the calculation the results from the proficiency testing provider Control Lab® (hemoglobinopathies).Results: During this period, we obtained the medium CV of 2.76% and total error of 14.05%, for fetal hemoglobin.

Conclusion: We compared average CV observed at this study to those reported by the kit manufacturer's labeling, and found that the CV obtained was very close to the informed by Bio-Rad® (2.47%). We also noted that the results of the Proficiency Test were within the acceptable limit stated by the provider, Control Lab®. Whereas until the present moment there is no suggestion in the literature to the total error of this analyte, we conclude that a total error of 14.05% should be acceptable for fetal hemoglobin.

## A-325

### Avoiding Unnecessary Plasmapheresis in Suspected Thrombotic Thrombocytopenic Purpura Using Stat Testing of ADAMTS13 Activity in Blood

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We document a significant number of unnecessary plasma exchange procedures to treat suspected thrombotic thrombocytopenic purpura (TTP) which could have been avoided with rapid measurement of ADAMTS13 activity in blood. ADAMTS13 is a metalloproteinase that cleaves von Willebrand factor (vWF) to maintain coagulation homeostasis. Reduced ADAMTS13 activity results in large vWF multipliers, hypercoagulability, extensive microthrombus formation, and severe end-organ damage. TTP is a rare disease (annual incidence 4-11 cases per million) characterized by reduced ADAMTS13 activity. Diagnosis of TTP hinges on the ability to differentiate it from other primary or drug-induced thrombotic microangiopathies. Rapid diagnosis and initiation of therapy is paramount due to the high mortality rate if untreated (>90%) and severe pathogenic complications, including renal dysfunction, dysrhythmias, and neurological manifestations. The first-line therapy is daily plasma exchange which is both expensive and presents its own set of risks to patients.

Laboratory testing for ADAMTS13 activity is diagnostic for TTP if below 10% of expected. However, as a send-out test, turnaround time for ADAMTS13 activity is often days or weeks. Due to the high mortality of TTP when untreated, in the absence of another etiology for microthrombotic angiopathy, all patients with suspected TTP will undergo plasma exchange though less than half will have a final diagnosis of TTP upon reporting of ADAMTS13 activity (literature reports 10-45% and our present study shows 42% (5/12) of suspected cases to be true TTP).

We posit that stat testing for ADAMTS13 would benefit patients and hospitals through a reduction in the risks and high cost of therapy. To that end, we examined the effect of laboratory turnaround time on plasma usage in all patients suspected of having TTP over an 18-month period. We report that 1210 units of plasma were unnecessarily transferred while awaiting ADAMTS13 activity results, representing 76 individual apheresis encounters. Average laboratory turnaround time was 5.8 days for the three reference laboratories used during the study period. We also examined the potential costs and benefits if the laboratory offered testing of ADAMTS13 activity. Infrequency of requests for ADAMTS13 activity testing means that as a stat test, samples will not be batched, resulting in the utilization of many laboratory resources, including 1.5-4 personnel hours and a complete set of control reagents for every analyzed sample. The average price per test offered by 3 different manufacturers would result in laboratory direct costs of $362.50 per sample if no batching of samples was possible compared to reference laboratory testing at less than $200 per sample. The hospital and patient, however, would see a reduction in inappropriate plasmapheresis amounting to reduced direct costs of $17,425 per patient or $121,030 for the facility over the 18-month study period. Further, the reduction in inappropriate therapy removes unnecessary complications and expedites appropriate therapy. We conclude that availability of a rapid in-house assay for ADAMTS13 activity would reduce unnecessary plasma exchanges. Although the test will likely represent a loss of revenue for the laboratory, losses should be offset when compared to the cost-savings to the patient and hospital.

## A-326

### Age and Gender Specific Complete Blood Count Reference Intervals for a Community-based Patient Population in Ontario, Canada

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**Objective:** Establish age and gender specific reference intervals (RIs) for complete blood count (CBC) testing of community-based patients on Beckman Coulter DxH analyzers.

**Methods:** RIs were identified through a retrospective review of 84584 male and 119050 female CBC results from our regional reference laboratory information system**.** CBC results were initially partitioned by year for patients ≥ 1Y and by month for patients < 1Y. Final stratification of age and gender RIs was based on clinical and statistical significance. Statistical software (EP Evaluator) was then used to establish each RI using a central 95$^{th}$ percentile criterion. All proposed age and gender specific RIs were subsequently verified by testing *N*=20 normal patient specimens.

**Results:** Except for MCHC (RI: 308-340g/L) and MPV (RI: 7.5-11.7fL) the CBC analytes were stratified into multiple age groups.

The derived RIs revealed potential linkages between age/gender and CBC results: (1) relatively high concentrations in childhood which decrease with age and stabilize in adulthood (WBC; RDW; platelets; lymphocytes; monocytes; eosinophils; and basophils); (2) relatively low concentrations in childhood which increase with age and stabilize in adulthood (neutrophils and RBC); (3) high concentrations at birth that drop after 1M then increase with age and stabilize in adulthood (hemoglobin; hematocrit; MCV; and MCH); (4) no change with age (MCHC and MPV); (5) RBC, hemoglobin and platelets RIs drop significantly in patients >75Y; (6) Male and female <15Y CBC RIs are identical. After adulthood, male and female RIs of RBC, hemoglobin, hematocrit and MCV are significantly different and require stratification.

**Conclusion:** Using lab specific patient population data to establish central 95$^{th}$ percentile intervals helps to label abnormalities appropriately within Ontario's ethnically diverse population and avoid unnecessary further investigation.

| Age Stratification of Reference Intervals | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 - 3D | 4D – 1M | 2 M - 1Y | 2 - 9Y | 10 – 14Y | 15 - 75Y | > 75Y |
| WBC (10E9/L) | 8.7 - 29.0 | 5.0 - 16.7 | | 3.7 - 13.7 | | 3.5 - 11.8 | |
| RBC (10E12/L) | 3.9 - 6.0 | 2.2 - 5.7 | 3.5 - 5.4 | 4.0 - 5.6 | | M:3.7 - 6.0<br>F:3.6 - 5.4 | M:2.9 - 5.5<br>F:3.0 - 5.2 |
| HEMOGLOBIN(g/L) | 136 - 196 | 71 - 194 | 87 - 138 | 102 - 143 | 108 - 155 | M:110 –169<br>F:101 - 152 | M:89 – 162<br>F:88 - 151 |
| HEMATOCRIT (L/L) | 0.42 - 0.64 | 0.20 - 0.59 | 0.27 - 0.41 | 0.32 - 0.43 | 0.33 - 0.47 | M:0.31 - 0.51<br>F:0.30 - 0.46 | |
| MCV (fL) | 101 - 122 | 86 - 113 | 58 - 89 | 63 - 91 | 68 - 94 | M:75 – 103<br>F:72 - 102 | |
| MCH (pg) | 29 - 39 | | 18 - 30 | 20 - 30 | 22 - 31 | 23 - 33 | |
| RDW | 12.3 - 23.7 | | | 12.4 - 18.8 | | | |
| PLATELETS (10E9/L) | 178 - 535 | | | | 165 - 424 | 136 - 400 | 109 - 429 |
| RETICULOCYTES (10E9/L) | 8 - 153 (0-3M) | | 17 - 146 (>= 4M) | | | | |
| NEUTROPHILS (10E9/L) | 0.8 - 7.2 | | | 1.1 - 8.4 | 1.3 - 7.4 | 1.7 - 8.3 (>= 14Y) | |
| LYMPHOCYTES<br>EOSINOPHILS (10E9/L) | 2.0 - 9.5<br>0.0 - 1.6 | | 2.4 - 9.6<br>0.0 - 1.1 | 1.3 - 6.4<br>0.0 - 0.9 | 1.2 - 4.2<br>0.0 - 0.8 | 0.8 - 3.4 (>= 14Y)<br>0.0 - 0.6 (>= 14Y) | |
| MONOCYTES<br>BASOPHILS (10E9/L) | 0.1 - 2.4<br>0.00 - 0.24 | | 0.2 - 1.5<br>0.00 - 0.16 | 0.2 - 1.0<br>0.00 - 0.11 | | | |

## A-327

### Estimating short- and long-term reference change values for tests of platelet function

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**Background:** To balance the risks of perioperative bleeding and thrombosis, protocols for mechanical circulatory support placement often require titration of antiplatelet agents using laboratory tests of platelet function. A relative change value (RCV) or "delta", based upon analytic and biologic variability, would be useful to define

significant changes in platelet function. Platelet function tests with higher RCVs are more likely to cross defined thresholds for high platelet reactivity due to analytic and biologic variability, rather than changes in patient condition. However, variable platelet activation occurs with each blood draw, making separate measurement of analytic and biologic variability (the traditional approach to RCV calculation) difficult for platelet function tests. We estimated short-term and long-term RCVs for two tests of platelet function to facilitate antiplatelet agent titration and monitoring. **Methods:** A total of 16 healthy volunteers (8 male and 8 female) were recruited to have arachidonic acid-induced and adenosine 5'-diphosphate (ADP)-induced platelet function measured by whole blood impedance aggregometry using Multiplate (Diapharma Group Inc., West Chester, OH) and VerifyNow (Accumetrics, San Diego, CA) devices. Study volunteers had blood drawn on 3 occasions on the first study day and returned for a single blood draw 1, 2, and 3 months after the initial measurements. All measurements were performed in duplicate following each blood draw, for a total of 96 duplicate measurements. Analytic variability ($CV_A$) was estimated from the average variability observed among the 96 duplicate measurements. Short-term RCV is a function of variability attributable to imprecision ($CV_A$) and pre-analytic factors (platelet activation with each blood draw); and was estimated from $CV_A$ and within person biologic variability ($CV_I$) observed among the 6 measurements per subject performed on study day 1. Long-term RCV is a function of total (analytic and biologic) variability and was calculated from $CV_A$ and $CV_I$ observed from the first measurement on day 1 and from measurements 1, 2, and 3 months later. Short-term and long-term RCVs were calculated according to the following equation: $RCV = 2.77*(CV_A^2 + CV_I^2)^{1/2}$. **Results:** Estimated short-term and long-term RCVs for arachidonic acid-induced platelet function by VerifyNow were 4%; compared to short- and long-term RCVs of 19% and 32% for Multiplate. Short-term and long-term RCVs for ADP-induced platelet function by VerifyNow were 16% and 23%; while short- and long-term RCVs by Multiplate were 25% and 40%. **Conclusion:** Small (~ 5%) changes in arachidonic acid-induced platelet function by VerifyNow can be interpreted as a change in patient condition or status and not a function of analytic and biologic variability. In contrast, by Multiplate changes up to 20% are anticipated due to analytic variability and effects of drawing blood. Over longer periods of time, Multiplate changes under ~30% may represent analytic and biologic variability. For ADP-induced platelet function, relevant "deltas" are approximately 20% (VerifyNow) and 25-40% (Multiplate). Lower RCVs on the VerifyNow should allow for more consistent classification of platelet function over time for any individual patient.

## A-328

### Hemoglobin Greenville-North Carolina: A Novel Hemoglobinopathy Diagnosed At East Carolina University/Vidant Medical Center.

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**Background and Objective**: Abnormal hemoglobin variants resulting in hemoglobinopathy pose serious health problems leading to wide range of morbidities throughout the world. A large number of such hemoglobin variants have been discovered. We present a novel hemoglobin variant that has not been described in literature or hemoglobin variant database. After confirmation at Mayo Clinic, Rochester, MN, the hemoglobin was registered as hemoglobin Greenville-NC in Hemoglobin Variant (HbVar) Database of Human Hemoglobin Variants.

**Methods and Results**: A sixteen year old African American female presented with positive pregnancy test with estimated gestation of 12 weeks. Patient's mother had a history of sickle cell trait and father's -hemoglobinopathy status was unknown. During routine prenatal work up, mild anemia with normocytic and normochromic red blood cells and increased red blood cell distribution width (RDW) were detected. High Performance Liquid Chromatography (HPLC) was performed as screening test for detection of hemoglobinopathy. By HPLC, hemoglobin A, A2, S and two other hemoglobins were identified. Hemoglobin A, S and other hemoglobin that migrated in between Hb S and Hb C position were confirmed by acid gel electrophoresis. The two alpha variants could not be positively characterized by hemoglobin electrophoretic methods. Molecular testing, capillary electrophoresis and mass spectrometry were performed at Mayo Clinic. Alpha globin gene sequencing identified two heterozygous mutations: *HBA1*: alpha1, 63 GCC>ACC, Ala>Thr (HGVS c.190G>A, p.A64T), a novel variant; and *HBA2*: alpha2, 48 CTG>CGG, Leu>Arg, (HGVS c.146T>G, p.L49R), hemoglobin Montgomery. Beta globin gene sequencing confirmed heterozygous hemoglobin S [*HBB*: Beta 6, GAG>GTG, Glu>Val (HGVS: c.20A>T, p.E7V)]. Mass spectrometry confirmed the novel variant mass of 15156 amu. Relative percentages by mass spectrometry of different hemoglobins in this patient were estimated to be Hb Montgomery 18%, Hb Greenville-NC 19%, and Hb S 40%. *In silico* analysis by computation methods are mixed with some showing a possible cryptic splice site enhancement and others suggest no significant effect.

**Conclusion**: The novel variant, Hb Greenville-NC, has not previously been reported in population genetics databases, and its clinical significance is unknown. It is possible that this mutation is inherited and can be identified in other family members. Hemoglobinopathies may not have clinical manifestations in heterozygotes, even when compounded by additional hemoglobinopathies. For example, our patient also had hemoglobin Montgomery. In heterozygous individuals, hemoglobin Montgomery is not associated with clinical manifestations or hematologic abnormalities. When combined with Hb S, it is expected to behave similarly to Hb S trait. The patient's mild anemia on prenatal workup may have been the result of her hemoglobinopathy, but could also have resulted from nutritional or other factors. We intend to pursue hemoglobin testing and correlation with any relevant clinical history of the patient's family members, to better determine patterns of inheritance and clinical manifestations of this novel hemoglobinopathy.

## A-329

### CELL-DYN Emerald 22 results are equivalent with those obtained with CELL-DYN 3700 and CELL-DYN Sapphire

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**Background:** The CELL-DYN Emerald 22 (E22) is a compact hematology analyzer designed for small and medium-sized laboratories. It provides a complete blood count, including a 5-part WBC differential. The analyzer combines impedance technology for cell counts with UNI-FLOW dual-angle light scatter for the differential. The goal of the study was to assess the performance of E22 in comparison with CELL-DYN CD3700 (CD37) and CELL-DYN Sapphire (SAP). **Methods:** Five hundred and one routine blood samples were tested on all three analyzers. Due to invalidations and results being outside of the analytical measuring range, results were available for analysis on 461 to 501 samples, depending on the measurand. Data were processed by Passing-Bablok regression, Pearson correlation and Bland-Altman bias plots. A subset analysis was performed on samples with WBC $<2.0x10^9$/L (n=42) and with PLT $<50x10^9$/L (n=30).

**Results:** Correlation coefficients for WBC, PLT, HGB, RBC and MCV ranged from 0.96 to 0.99 between E22 and CD37, and from 0.96 to 1.00 between E22 and SAP. In the subset analysis of cytopenic samples, the correlation coefficients for WBC and PLT were 0.98 and 0.87 between E22 and CD37, and 0.97 and 0.82 between E22 and SAP. In this subpopulation the mean (±SD) and median (inter-quartile range) differences between E22 and CD37 results were -7.36 (± 7.67) and -5.60 (8.27) for PLT, and -0.06 (± 0.13) and -0.06 (0.16) for WBC, respectively. The mean and median differences between E22 and SAP results were -0.24 (± 7.38) and 1.60 (6.33) for PLT and -0.04 (± 0.13) and -0.02 (0.15) for WBC. The predicted bias for WBC at 1.0 $x10^9$/L and for PLT at 20.0 $x10^9$/L were -0.01 and -2.47 when compared to CD37, and 0.00 and -1.70 when compared to SAP. **Conclusion:** Results generated by E22 were substantially equivalent with those generated by CD37 and SAP. WBC results in the low range were very consistent among the three analyzers. PLT results by E22 below 50 $x10^9$/L tended to be lower than those reported by CD37 and SAP. CELL-DYN Emerald 22 is a suitable backup instrument for labs using CELL-DYN 3700 or CELL-DYN Sapphire.

## A-330

### Characterization of Hematologic Malignancies with Anchored Multiplex PCR and Next-Generation Sequencing

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**Introduction:** Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutation type. We developed targeted next-generation sequencing (NGS) assays based on Anchored Multiplex PCR (AMP™) for simultaneous detection of mutations and gene expression levels relevant in hematologic malignancies.

**Methods:** AMP is a library preparation method for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification. AMP-based Archer® VariantPlex™ and FusionPlex® assays enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as internal tandem duplications (ITDs) with VariantPlex assays. MBC adapters ligated to RNA fragments prior to amplification enable relative gene expression analysis.

**Results:** We show that open-ended amplification from KMT2A GSPs enabled detection of a KMT2A-MLLT3 fusion through breakpoint identification, with reads extending 6 exons into MLLT3. We also detected a novel RUNX1 fusion, RUNX1-G6PD, in a case of acute unclassifiable leukemia. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected ITDs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples.

**Conclusions:** Our results demonstrate that AMP-based NGS enables comprehensive detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of known and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels.