of all tubes. Among the eight clinics which have more than fifty patients, Chest Disease Clinic was used the highest number of tubes. The first day median level of hemoglobin was reduced from 12.7 g/dL to 11.7 g/dL in eight days hospitalized patients and from 12.5 g/dL to 11.7 g/dL in nine days hospitalized patients.

Results: Blood loss from laboratory diagnostic testing is highly associated with changes in hemoglobin levels for inpatients and may contribute to anemia especially in high risk group patients. For this reason, unnecessary test requirements should be prevented and minimum test repetition time should take into consideration in order to reduce blood loss arise from laboratory diagnostic testing.

P10 – Haematology 2

P10-01

State of plasma and platelets hemostasis factors in patients with unstable angina

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Methods and results: We studied 100 patients defined clinically as unstable angina (M/F = 60/40, 61 ± 12.2 years). Analyzed blood tests (platelet count, MPV- mean platelet volume, PDW – platelet distribution width), cardiac markers (CK-MB, myoglobin, troponin), biochemical analysis (C-reactive protein, BNP), coagulation blood tests, platelet aggregation test were taken at admission. Statistical analysis for the study was conducted using Statistica 6.0 (StatSoft. Inc,USA). The combined primary end point (all-cause mortality, nonfatal myocardial infarction, recurrent UA, urgent percutaneous coronary intervention or coronary artery bypass grafting) at 6 months occurred in 36 (36%). In these patients, the mean baseline MPV was greater than that in those without a primary outcome (9.1 ± 0.6 *vs*. 8.9 ± 0.7 fl, $P < 0.05$), PDW was less (13.4 ± 1.2 *vs*. 13.8 ± 1.1 %). In patients with adverse outcomes fibrinogen (6.3 ± 0.4 *vs*. 4.6 ± 0.5 g/L, $P = 0.02$), D-dimers (660 ± 24.1 *vs*. 382.2 ± 13.0 ng/mL, $P = 0.001$), BNP (103.5 ± 7.9 ng/mL *vs*. 61.6 ± 9.3, $P < 0.001$), von Willebrand factor (vWf) (186.2 ± 14.3 *vs*. 160.4 ± 20.1%, $P = 0.03$), spontaneous and induced (ADF, adrenalin) platelet aggregation ($P \leq 0.05$) were greater compare to patients without. The criteria for 6 months adverse events were MPV > 9.0 fL, fibrinogen > 6.4 g/L, D-dimer > 640 ng/mL, BNP > 110 ng/mL, vWf > 180%.

Conclusion: MPV can be used as a risk biomarker in prognosticating the 6 months outcomes for unstable angina. Patients adverse outcomes in unstable angina have increased level of fibrinogen, D-dimer, BNP, vWf, spontaneous and induced platelet aggregation

P10-02

Evaluation of platelet parameters for differential diagnosis of thrombocytosis

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Background: There are two types of thrombocytosis: primary and secondary. Primary thrombocytosis (PT) is caused by a chronic myeloproliferative disorders (CMPD) and secondary thrombocytosis is called reactive thrombocytosis (RT) since it is associated with inflammatory states. Differential diagnosis of thrombocytosis is not always obvious. In automated blood cell analyzers various platelet parameters can now be measured. In this study, we analyzed whether 6 platelet parameters can be used for the differentitation of PT from RT.

Materials and metods: The platelet counts, mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), mean platelet mass

(MPM), mean platelet component concentration (MPC) and large platelets (LPLT) were studied in 40 patients with RT, 18 patients with PT and 60 normal control. Platelet parameters were measured by ADVIA 2120 (Bayer Diagnostics, USA). Significance was determined using the Mann-Whitney test.

Results: Patients with CMPD had significantly higher MPV, PDW, L-PLT, PCT, MPM than those with reactive thromboytosis. There was no significance difference in MPC between patients with PT and RT, but in both groups MPC was significantly lower than in control group. Also, there was no difference in MPM between patients with primary thrombocytosis and control group.

Conclusion: The platelet parameters are useful for the differential diagnosis of thrombocytosis. High MPV, PDW, and L-PLT with high platelet counts suggest primary thrombocytosis.

P10-03

Evaluation of in-house normal pool plasma for partial thromboplastin time mixing study on an ACL TOP

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Background: Normal pool plasma plays a critical role on the safe outcome on testing prolonged partial thromboplastin time (PTT). Mixing study for qualitative inhibitor detection is commonly used on routine laboratory testing, allowing detection or exclusion of an inhibitor as differentiation from primary factor deficiency.

Materials and methods: In-house normal pool plasma (In-house) was obtained from 50 healthy donors immediately after collection and centrifugation (3000 rpm; 10 minutes), properly mixed and stored at - 60 °C. Commercial normal pool plasma from ILTM Normal Control® (IL) and StagoTM Pool Norm® (Stago) were used for result comparison. 78 samples with prolonged PTT (ratio > 1.20) were properly mixed in equal volume with the normal pool plasmas and evaluated for PTT. Correlation was evaluated by pearson's correlation factor. Mean and standard deviation (SD) were analyzed for all groups. T-Test was performed for group comparison.

Results: Mean ± SD for the mixing tests of the three groups as ratio: In-house – 1.16 ± 0.13, IL – 1.23 ± 0.17, Stago – 1.14 ± 0.16. T-Test analysis between groups: In-house *vs*. IL – 0.0052, In-house *vs*. Stago – 0.4765, IL *vs*. Stago – 0.0016. All groups showed a good pearson correlation: In-house *vs*. IL – 0.7683, In-house *vs*. Stago – 0.8883, IL *vs*. Stago – 0.8349.

Conclusions: A critical evaluation of T-Test and correlation between in-house normal pool indicates great similarity of results compared to those obtained with the commercial plasma pools tested, thus suggesting that a careful selection of donors allows preparing in-house normal pool plasma that fits the quality demands of routine laboratory mixing PTT tests.

P10-04

Comparison of two methods for measuring FVIII levels in hemophilia A patients

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Background: Correct determination of FVIII activity is essential for the assessment of severity of hemophilia A, as well as for patient-tailored treatment strategy. The most commonly performed assay for measuring FVIII activity is one-stage clotting assay. Several groups of authors have reported discrepancies between one-stage and chromogenic assay for FVIII determination.

Materials and methods: We have compared the one-stage and chromogenic FVIII assay (Siemens, Marburg, Germany) in 100 hemophilia A patients (56 severe and 44 non-severe) and 101 healthy male subjects.

Results: Good correlation between assays was obtained in healthy subjects as well as in severe and non-severe group of patients (coefficients of correlation 0.607; 0.904 and 0.875, respectively), but statisticaly significant difference was found in all groups. According to Bland & Altman the mean differencies between assays in healthy subjects, severe and non-severe patients were 17.6%, 47.8% and 61.7%, respectively. Usually, the results with chromogenic assay were lower than results with one-stage clotting assay. Similar correlation with clinical parameters (age at first joint bleed, number of joints with hemophilic arthropathy, number of annual joint bleeds and annual FVIII consumption) was found: coefficients of correlation between 0.568 and 0.688 for one-stage and between 0.521 and 0.619 for chromogenic assay.

Conclusions: Two assays mostly show good correlation but clinically important discrepancies could be observed. It is recommended that all hemophilia centres have available both types of assay. Chromogenic assay should be used in the cases of normal aPTT and one-stage FVIII activity with presence of personal or familiy history of bleeding.

P10-05

Design and application of lupus anticoagulant diagnostic algorithm

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Background: Lupus anticoagulant (LA) diagnostics is highly complicated. Laboratory's responsibility is to select appropriate assays as recommended by certain published guidelines. Results must be interpreted properly, which is not an effortless and easy task. Aim of study was to design LA diagnostics algorithm, implement it in routine work of local laboratory, evaluate difference of diagnostic capabilities between single assays (aPTT, dRVVT) and complex of methods (assays plus indexes) used in new LA algorithm.

Materials and methods: In total 68 persons were selected, 40 relatively healthy for calculation of dRVVT reference values, and 28 suspected to have positive LA. aPTT, dRVVT screen and confirm, mixing studies of aPTT and dRVVT screen were performed. dRVVT normalization ratio, Rosner index (RI) of aPTT, dRVVT screen, correction index (CI) of dRVVT were calculated.

Results: Plasmas with suspected LA gave different numbers of positive results when different interpretative breakpoints were used: aPTT RI >15% was determined in 10.7%, dRVVT screen RI > 15-17.9% and dRVVT CI > 10-75% of patients.

Conclusions: Narrower than manufacturer's dRVVT reference intervals resulted in more positive LA results, most probably meaning greater number of false positives. No statistically significant difference in sex dependant dRVVT reference values was found. LA algorithm gave much less positive LA results than merely results of single assays. Despite using different reference values LA algorithm gave the same number of positive LA results and positives were detected in the same specimen.

P10-06

Relationship between homocysteine level and MTHFR haplotype in patients with thromophilia

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Background: Increased homocysteine (Hcy) concentration is associated with increased risk for venous and arterial thrombosis. Reduced methylentetrahidrofolate reductase (MTHFR) activity, due to the presence of C677T and A1298C polymorphisms, is one of the causes of hyperhomocysteinema. In a group of patients with thrombophilia, Hcy leveles were compared between MTHFR haplotypes.

Materials and methods: Study enrolled 30 patients (12 men, 18 women). Hcy was measured using HPLC technique with fluorescent detection. PCR amplification and reverse allele-specific oligonucleotide hybridization were employed for MTHFR haplotype determination. Statistical analyses included Kruskal-Wallis and Mann Whitney U tests.

Results: The following distribution of MTHFR haplotypes was observed: 677CC/1298AA was present in 4 (median Hcy = 10.8 μM), 677CC/1298AC in 7 (median Hcy = 11.7 μM), 677CC/1298CC in 3 (median Hcy = 9.7 μM), 677CT/1298AA in 4 (median Hcy = 10.4 μM), 677CT/1298AC in 8 (median Hcy = 11.2 μM) and 677TT/1298AA in 4 patients (median Hcy = 14.6 μM). No statistically significant difference was observed between Hcy concentration corresponding to the detected haplotypes.

Conclusions: In a group of patients with thrombophilia, no significant difference in Hcy concentration, related to MTHFR haplotype, was detected. This finding further implies that the Hcy measurement has greater clinical importance than MTHFR genotyping, but such assumption has to be tested in larger studies.

P10-07

Validation of Hemoclot® Thrombin Inhibitors assay for the new oral anticoagulant dabigatran

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Background: The direct thrombin inhibitor dabigatran does not require laboratory monitoring routinely. However, under special circumstances (e.g. renal insufficiency, bleeding complication, thrombosis, major surgery) testing may be crucial. Routine coagulation assays prothrombin time (PT), INR and activated partial thromboplastin time (APTT) are of limited value. We validated a commercial assay based on diluted thrombin time (TT).

Materials and methods: Hemoclot® Thrombin Inhibitors test with control samples (Hyphen, Aniara) was evaluated for repeatability and linearity. Patient results (N = 30) were compared with Owren PT (Nycotest PT, Medinor), APTT (Actin FSL, Siemens Healthcare Diagnostics) and TT (Thrombin Reagent, Siemens). Coagulation analyser BCS XP (Siemens) was used.

Results: Intra-assay repeatability (CV%) tested with patient samples was 7.2%. Inter-assay repeatabilities with control samples were 10.1% (at 130 μg/L) and 7.9% (at 290 μg/L). The method was linear within the concentration range 50-470 μg/L (R = 0.997). Correlation between Hemoclot® and APTT was moderate (R^2 = 0.77), however there was considerable variation in APTT results, likely reflecting the clinical diversity of the patients. Even

at the highest concentration (490 µg/L), APTT was prolonged only to 62 s (reference values 23-33 s). Correlation with PT was poor ($R^2 = 0.23$) expectedly. TT exceeded the measurement range (> 140 s) already at low concentrations (< 50 µg/L).

Conclusions: Hemoclot® Thrombin Inhibitors demonstrated acceptable repeatability and linearity and seems suitable for dabigatran assessment. Here, APTT poorly estimated dabigatran concentration. Interpretation of the results depends on the clinical situation and timing. Yet, the cut-off values or safety limits in different clinical conditions remain unestablished.

P10-08

Indicators of activation of the coagulation system in children with Henoch-Schönlein purpura

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Background: Henoch-Schönlein purpura (HSP) is the most common vasculitis of childhood, affecting skin, joints, gastrointestinal tract and kidneys with clinical manifestations of bleeding tendency. The aim of this study was to evaluate global coagulation tests and D-dimer concentration as laboratory signs of activated coagulation system at first presentation of HSP.

Materials and methods: The study included 21 paediatric patients with HSP, median age 6 (range 3-16). Global coagulation tests PT, APTT, TT, fibrinogen were determined with coagulometric methods (Sysmex CA-560 analyzer, Siemens, Germany). D-dimer concentration was measured with microparticle enzyme immunoassay (Axsis Shield Diagnostics Ltd., USA) on Axsym analyzer (Abbott, USA).

Results: The results of coagulation tests expressed as median (95% confidence interval (95% CI), interquartile range (IQR)) were: PV ratio 0.96 (0.87-1.02, 0.85-1.04); APTV (sec) 26.7 (24.6-28.3, 24.0-28.5); fibrinogen (g/L) 3.7 (3.2-4.6, 3.1-4.8), TT (sec) 16.5 (16.1-17.6, 15.9-17.9), D-dimer (mg/L) 5.3 (1.8-7.7, 1.6-8.1). This study showed no alterations of PT, APTT and TT tests in HSP patients, fibrinogen was increased in 6/21 patients while D-dimer levels were increased in all 21 patients. In patients grouped according to the number of organs involved (2, 3 and 4) D-dimer concentrations were as follows: 2 organs involved: 2.9 mg/L (IQR = 1.4-7.5); 3 organs involved: 7.0 (IQR = 2.9-9.0) and 4 organs involved: 6.5 (IQR = 3.9-9.0), but there were no significant differences in D-dimer concentrations between groups.

Conclusion: Increased D-dimer concentrations in all 21 paediatric patients with HSP suggest that activation of coagulation including hyperfibrinolysis secondary to the endothelial damage to be a typical feature of HSP.

P10-09

Pre-analytical routines in coagulation testing: are guidelines followed?

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Background: It has been documented that about 70% of errors in laboratory medicine occur in the preanalytical phase. The aim of this survey was to study preanalytical conditions of routine hemostasis testing in Norwegian laboratories, and compare them with current guidelines.

Materials and methods: Hospital laboratories in Norway (N = 69) were invited to fill in a web-based

questionnaire regarding preanalytical routines for routine hemostasis testing. The first part focused on instruments and reagents for the different coagulation analyses used, and the second part focused on routines regarding venipuncture (needle gauge, citrate concentration, use of stasis, filling volume), handling of the sample before analysis, as well as routines regarding detection and handling of sample clot, high/low hematocrit, hemolysis, bilirubinemia or lipemia. The third part focused on storage and stability and the handling of samples from primary care or other hospital laboratories.

Results: 57 of 69 laboratories responded. There was good agreement regarding needle gauge, temperature in the centrifuge and type of glass used for sample collection

(3.2% Na-citrate), all more or less following the CLSI guidelines (H21-A5). However, large differences in practice were seen amongst the participants regarding centrifugation speed and duration, accepted fill volumes of the collection tubes accepted and accepted sample material (fresh or frozen plasma or citrated blood) and stability of the blood samples. In addition, there were few routines for detection of clot, pathological hematocrit, hemolysis, bilirubinemia and lipemia.

Conclusions: Wide variation is seen in preanalytical routines in hemostasis testing, often not according to the CLSI guideline.

P10-10

Characterization of blood donors with high haemoglobin concentration

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Background: The prevalence and causes of high haemoglobin concentration among blood donors are poorly described. This study aimed to characterize and develop an algorithm to manage the donors with polycythaemia.

Matrials and methods: Between November 2009 and November 2011 blood donors with repeated B-Hb above the WHO limit for polycythaemia vera (10.2 and 11.5 mmol/L/ 16.5 and 18.5 g/dL for women and men, respectively) were offered consultations by a hospital-based haematologist. At consultation haematocrit, MCV, P-erythropoietin, P-ferritin, B-Hb, platelet- and leukocyte count, JAK2 V617 and JAK2 exon12 analysis were performed, in addition to other routine parameters.

Results: Among 46 donors with repeated high B-Hb, 39 had a history of smoking, which may contribute to polyglobulia. Two had PV, 5 had severe hypertension, one of them because of kidney artery stenosis, and two had diabetes mellitus. Ten donors were deferred and of the 36 donors that were not deferred, 30 donated again before May 2012, where the B-Hb was significantly lower.

Conclusion: Thus, we found a high morbidity among these donors, and recommend JAK2 V617 and JAK2 exon12 screening and clinical investigations for donors with concurrent high B-Hb, high haematocrit and iron deficiency. Also we recommend these donors to reduce smoking to reduce the risk of thrombosis in general.

P10-11

Blood donors with thalassemia trait in a blood bank outside the thalassemia belt

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Background: By far the most frequent reason for Danish blood donors to have a haemoglobin level below the level of acceptance for blood donation is iron deficiency. Hence in all low haemoglobin-

donors ferritin is measured. If haemoglobin remains low by the next visit or if the low haemoglobin cannot be explained by low ferritin, further tests are done. Five donors with mean cell volume (MCV) ≤ 78 fL, not explained by iron deficiency are described.

Materials and methods: Samples from donors with unexplained microcytaemia, were sent to Centre for Haemoglobinopathies, where Hb-electrophoresis and nucleic acid investigations were done.

Summary: With increasing globalisation donors heterozygous for thalassemia may turn up even in Nordic blood banks. Their haemoglobin level will be in the low normal area, often below the lower limit for donation, except for the α3,7 deletion where the haemoglobin level often is normal. Heterozygous thalassemia is rarely of clinical consequence, except in pregnancy where genetic counselling may be warranted. Provided that the haemoglobin level is above the limit for acceptance, people with thalassemia trait may become blood donors.

P10-12

Temporary impact of blood donation on physical performance in moderately physically active men

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Background: Donation of blood negatively affects maximal oxygen consumption (VO2max) and endurance, and donors ask about the duration and degree of reduction.

Materials and methods: Nineteen non-anaemic, physically active male blood donors age 33(24–43) were included. To determine VO2max, subjects performed a standard incremental bicycle ergometer VO2Max test. Endurance was tested using a self-paced 3 km treadmill run. Subjects were tested 2 days before and 3, 7, 14, and 28 days after donation and were drawn to analyse haematological - and iron-parameters.

Results: Haemoglobin was reduced by 9.3% at day 3. After this Hb increased, and by day 28, three of 15 donors had reached pre-donation Hb level. Ferritin declined 51% from 94.8 ± 18.4 µg/L before donation to 46.8 ± 10.4 µg/L at day 14 and remained below baseline throughout the study. VO2max declined by 6.5% from 49.7 ± 2.1 mL O_2 / kg/min to 45.6 ± 2.1 mL O_2 /kg/min at day 3 compared with before donation. Subsequently VO2max gradually increased to 49.3 ± 2.2 mL O_2 / kg/min, at day 14, thereby returning to baseline. The 3 km time trial performance declined by 5.6% from 13:55 ± 00:46 minutes before donation to 14:42 ± 01:12 minutes at day 3. At day 14 time trial performance was 13:52 ± 00:59 minutes, the same as before donation.

Summary: Haemoglobin concentration was reduced by 9.3% and ferritin by 51%. Endurance was reduced by 5.6% and VO2max by 6.1% and reached pre-donation level by day 14, It seems from our study that plasma volume expansion may partly compensate for the loss in haemoglobin.