### ORAL PRESENTATIONS

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### POSTER PRESENTATIONS

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Cryopreservation of Buffy Coat Platelet Concentrates Photochemically Treated With Amotosalen and UVA Light

P Sandgren

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BACKGROUND: Storage time for platelet concentrates (PC) in most countries is limited to 5 or 7 days. Cryopreservation is considered an encouraging approach for extended platelet (PLT) storage, bridging inventory shortages of conventionally stored PLTs. PLTs cryopreserved (CPPs) in 5% dimethyl sulfoxide (DMSO) are currently in clinical development. Preservation of PLT functions and avoidance of transmission of infectious agents via CPPs remain a challenge. The INTERCEPT™ Blood System (Cerus Europe BV) uses a photochemical treatment (PCT) with amotosalen and UVA light to inactivate pathogens and leukocytes. Consequently, INTERCEPT-treated CPPs may lower the patient safety risks. However, a comprehensive characterization of INTERCEPT-treated CPP is essential prior to clinical studies.

AIMS: The objective of this study was to analyze potential effects of the INTERCEPT treatment on CPPs as compared to untreated CPPs. Functional, phenotypic and apoptotic properties of such PLTs were analyzed.

METHODS: Eight buffy-coat (BC)-PLT units were PCT treated (Test) and eight untreated BC-PLT units served as controls. Test units were exposed to 3 J/cm² UVA light in the presence of 150μM amotosalen. Unbound amotosalen was removed by adsorption in the CAD (Compound Adsorption Device) during 15 h under agitation. Test and control CPPs were resuspended in a small volume of 5% DMSO after centrifugation at 1200xg for 10 minutes transferred into a 300mL DEHP plastic bag and centrifuged. All supernatant was removed. The bags containing approximately 10mL CPPs were frozen in cardboard boxes at -80°C for approximately 1 month. The CPPs were thawed in a thawing bath maintained at 37°C for 3 minutes and then resuspended in 200mL plasma. Functional, phenotypic properties as well as degree of apoptosis and ROS activity of all CPPs were analyzed pre-freezing, on day 0 (post freezing and thawing) and day 1.

RESULTS: After thawing, all CPPs show a number of biochemical and ultra-structural changes as compared to the pre-freezing data. Interestingly, no significant differences were observed in PLT content post-thaw (202 ± 31 vs 172 ± 38 ×10⁹/unit) and LDH activity (26.5 ± 3.6 vs 27.4 ± 67.0 % total) between control and test CPPs. Freeze–thaw recovery was (73±0.1% vs 66±0.1%). Similarly, no significant differences were observed after thawing in the percentage of cells showing apoptotic properties (JC-1 59.3 ± 6.2 vs 63.9 ± 2.7%), surface expression of P-Selectin (68.2 ± 5.8 vs 64.5 ± 4.0), Gp1ba and PECAM-1 comparing test and control. Although differences in the energy generation (ATP) and ROS activity were observed, ADP, Collagen and Thrombin-induced PAC-1 expression remained consistent after thawing between groups. Respectively, control and test CPPs show no major differences during clot formation (ROTEM MCF 71.5 ± 2.7 vs 80.8 ± 0.5). Sustained aggregates were observed in the control CPPs after thawing only.

SUMMARY/CONCLUSIONS: Overall, CPPs show a number of ultra-structural rearrangements questioning their functional integrity. However, our data indicate that PCT-CPPs exert hemostatic potential in vitro, not different to untreated CPPs. Therefore, the use of PCT is feasible and may prevent CPPs from being a potential source of infection.
Effect of Pathogen Inactivation on the Quality and miRNA Profile of Platelet Concentrates During Storage Under Standard Blood Banking Conditions

Niels Árni Árnason¹, Ragna Landrò¹, Óttar Rolfsson¹, Björn Harðarson¹, Sveinn Guðmundsson¹, Ölafur E. Sigurjónsson¹,³

¹. The blood bank, Landspitali-The National University Hospital of Iceland; 2. Center for systems biology, University of Iceland; 3. School of Science and Engineering; Reykjavik University

BACKGROUND: The Platelet has a central role in hemostasis and represents an integral part of transfusion medicine. Platelets can be stored in gas permeable plastic containers under agitation for a maximum of 5-7 days. Two main reasons for this limited storage time are a risk of pathogen contamination and an onset of platelet storage lesions (PSL). PSL is a collective term of variety of factors that contribute to the deterioration of platelet quality during storage. To reduce the risk of pathogen contamination, methods have been developed that render pathogens inactive in platelet concentrates prior to storage. The most widely used method is the INTERCEPT™ pathogen inactivation method, which is based on cross-linking nucleic acids using amotosalen and UV light.

MicroRNAs (miRNA) are small none coding 19-24 nucleotide long RNA molecules that serve as post transcriptional regulators of gene functions by binding to mRNA’s and facilitating translation inhibition or degradation of mRNA. Several publications support the notion of miRNA being important in platelet function. Changes in the regulation of specific miRNA’s during storage have been reported as well as perturbation effects related to pathogen inactivation methods.

AIM: To investigate the effects of INTERCEPT treatment on selected miRNAs in buffy coat generated platelets stored for 7 days under standard blood banking conditions.

METHODS: Platelet concentrates were produced using the double dose (DD) buffy coat (BC) method. Using a pool and split strategy 4 identical single dose units where generated that originated from 24 whole blood donors. Each sister unit received different treatment (INTERCEPT/Control-SSP+/Irradiated in plasma/Control-Plasma). In vitro quality of platelets were monitored on day 1, 2, 4 and 7 during storage using 20 different quality control (QC) parameters and miRNA were analysed using QPCR a that we selected from previous miRNA array study and the literature based on their roles in platelet biology and storage.

RESULTS: A notable difference was found in several QC parameters relating to treatment but overall the four groups where compatible in their performance in QC analysis. Out of the 30 miRNA tested only three showed a significant difference at one or more time points as a result of different treatment. On day 7 miR1260a and miR1260b where downregulated in groups Control-SSP+, Control-Plasma and Irradiated, Control-Plasma respectively when compared to day 1 baseline samples. miR-96-5p was downregulated on day 2 and 4 in the INTERCEPT group compared to Control-SSP+.

SUMMARY/CONCLUSIONS: The INTERCEPT treatment does not change the quality or significantly alter the miRNA profile of platelet concentrates generated and stored using standard blood banking condition.
Pathogen Inactivation of Zika, Dengue and Chikungunya Viruses In All Blood Components

D Musso1, F Santa Maria1, A Laughhunn2, M Lanteri3, A Stassinopoulos3, M Aubry1

1. Unit of Emerging Infectious Diseases, Institut Louis Malardé, Papeete, French Polynesia; 2. Microbiology, Cerus Corporation, Concord, United States; 3. Scientific Affairs, Cerus Corporation, Concord, United States

BACKGROUND: Pathogen inactivation (PI) is a proactive mitigation strategy available to safeguard the blood supply from the threat posed by emerging infectious agents like Zika virus (ZIKV). The blood bank industry recently reacted to the risk associated with documented ZIKV transfusion-transmitted infections (TTIs). TTIs have been reported for other arboviruses including all dengue virus (DENV) serotypes but not for chikungunya virus (CHIKV). However accidental laboratory infections from infectious samples and the detection of CHIKV RNA-positive blood samples from asymptomatic donors confirmed the risk for CHIKV TTIs. Donor screening is not sufficiently effective to reduce risk as most DENV and ZIKV infections are asymptomatic. Licensed arbovirus NAT assays are not available in endemic areas and NAT assays, even if multiplexed, cannot detect all arboviruses that may cocirculate in tropical and subtropical countries. PI on the other hand is a proactive strategy designed to inactivate pathogens in blood components by inhibiting nucleic acid replication, transcription, and translation. Licensed PI systems are available for plasma and platelets and a PI system is in development for red blood cells (RBCs). Previous published studies reported the inactivation of DENV and CHIKV to the limit of detection using amotosalen and ultraviolet A light (S-59/UVA) for the treatment of plasma and platelets. Moreover, data have been reported for the inactivation of ZIKV to the limit of detection in plasma using S-59/UVA and in RBCs after chemical treatment using amustaline and glutathione (S-303/GSH).

AIMS: We investigated the efficacy of S-59/UVA to inactivate high level of ZIKV titers in platelets and the efficacy of S-303/GSH to inactivate high level of ZIKV in RBCs.

METHODS: Platelet units were spiked with high levels of ZIKV titers and RBCs units were spiked with high levels of DENV and CHIKV titers. For each virus, infectivity levels (expressed in log_{10} TCID_{50}/mL) and RNA loads (expressed in log_{10} Geq/mL) were measured before and after PI treatment using previously published methodology.

RESULTS: S-59/UVA inactivated > 4.4 log_{10} ZIKV TCID_{50}/mL (corresponding to 7.5 log_{10} Geq/mL) to the limit of detection in platelets; S-303/GSH inactivated > 6.61 log_{10} DENV TCID_{50}/mL (corresponding to 8.42 log_{10} Geq/mL) and > 5.81 log_{10} CHIKV TCID_{50}/mL (corresponding to 10.49 log_{10} Geq/mL) to the limit of detection in RBCs. In all experiments, no infectious viruses were detected after completion of the inactivation process.

SUMMARY/CONCLUSIONS: These new data combined with previous published studies show that the photochemical process using S-59/UVA inactivates ZIKV, DENV and CHIKV to the limit of detection in plasma and platelets, and the chemical process using S-303/GSH inactivates these three pathogens to the limit of detection in RBCs. These studies demonstrate a complete solution for the robust inactivation of ZIKV, DENV, and CHIKV in all blood components. As PI has broad spectrum effectiveness against bacteria, viruses and parasites, the technology is of particular interest in arbovirus endemic regions that may be impacted with other transfusion-transmitted infectious diseases.
Storage Study of Apheresis Platelets In Additive Solution After Photochemical Treatment Using a Novel Triple Storage Set

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BACKGROUND: Photochemical treatment is intended to prevent transfusion-transmitted infections by inactivating pathogens in platelet concentrates (PC). A novel triple-storage set was developed for photochemical treatment of double or triple PC re-suspended in plasma/Platelet Additive Solution (PAS) (5-12 x 10^11 platelets in 420-650 mL).

AIMS: This study evaluated in vitro function after INTERCEPT™ treatment (Cerus) of platelets collected with two different apheresis collection devices using a set with 3 storage containers (TS) compared to a set with single storage container (LV) over 7 days-storage.

METHODS: After collection on Trima Accel® (Terumo BCT) or Amicus® (Fresenius Kabi) separator, one single and one double PC were pooled in respect of separator origin. This pool was split back (2/3 and 1/3 volumes) to generate units compatible for photochemical treatment with respectively TS (Test) and LV (Control) INTERCEPT™ sets. Test units contained about 8x10^11 platelets/PC and control units 4.5x10^11 platelets/PC, both in 62% PAS. Each test unit (580 mL) was connected to a TS sets for addition of amotosalen (S-59), UVA illumination and transferred to a double Compound Adsorption Device (CAD) for agitated storage for 4 hours, and finally split into 3 identical PC. Similar process was applied to the Control units (320 ml) using LV set and 6 hours single CAD treatment. Platelet quality was evaluated in a storage study on Day 1, 3, 5, and 7 after donation by measuring ATP, glucose, sCD62p, LDH, RANTES and sCD40L concentration. All results were normalized for platelet content prior to statistical analyses (paired and unpaired Student’s t-test).

RESULTS: Day 1 volume was 182 ± 14 mL in Test vs 304 ± 10 mL in Control units. Platelet content was 2.55 ± 0.22 x 10^11 in Test vs 4.10 ± 0.43 x 10^11 in Control units. Platelets in both sets showed a normal metabolism with moderate consumption of ATP. There was no statistically significant difference for glucose concentration in the storage study for the LV set compared with TS set. However, the difference was statistically significant between the two apheresis systems Amicus® and Trima Accel®. Glucose reserves were exhausted at day 7 for units collected with Amicus® separator. There was a moderated increase of sCD62p and LDH during storage (p<0.05 between day 1 and day 7). We observed no statistically significant difference for sCD62p and LDH between Test and Control units. RANTES and sCD40L increased with storage in both arms (p<0.05 between day 1 and day 7) but results are in an acceptable range and the difference between Test and Control group was not statistically significant. However, statistical significant differences were observed between the two devices for LDH, sCD62p and sCD40L. Residual amotosalen was <0.5 μmol/L in both Control and Test units.

SUMMARY/CONCLUSIONS: The storage study of apheresis platelet demonstrates no significant difference between TS and LV sets for up to 7 days of storage. Differences between AmicusTrima Accel® and ® PC sets, independent of INTERCEPT™, were observed. These differences will be further investigated.
Evaluation of Apheresis Platelets Processed With the INTERCEPT Blood System for Platelets Triple Storage Set

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BACKGROUND: The INTERCEPT™ Blood System for platelets is intended for the ex vivo preparation of pathogen-inactivated apheresis or whole blood-derived platelet components (PCs) in order to reduce the risk of transfusion-transmitted infection and transfusion-associated graft versus host disease. Platelets suspended in platelet additive solution (PAS) can be treated with any of the three licensed INTERCEPT Platelet Processing Set configurations for platelet doses of 2.5 to 8.0 x 10¹¹ platelets. Cerus has designed a platelet processing set with three storage containers to process platelet components containing doses of 5.0 to 12.0 x 10¹¹ platelets in a volume of 420 to 650 mL of 47 to 68% plasma and 32-53% PAS.

AIMS: To evaluate the processing ranges for single donor or pooled apheresis PCs suspended in PAS and treated with the INTERCEPT TS platelet set.

METHODS: Apheresis PCs (Amicus® and Trima®) were collected in 35-47% plasma and 53-65% PAS-3. One study was performed at the nominal dose (9.2 - 10.0 x 10¹¹ platelets), volume (558 – 629 mL) in 65% PAS/35% plasma using single donor apheresis collections. Three studies were performed to evaluate the high dose and high volume condition (9.7 – 11.8 x 10¹¹ platelets in 593 – 659 mL) using either single donor or pooled donations at nominal plasma content ratio or pooled donations at the high plasma content ratio. One study was performed to evaluate the low dose and low volume condition (5.5 – 5.9 x 10¹¹ platelets in 432 – 449 mL) using pooled apheresis donations at nominal plasma ratio. Input PCs (n=33) were treated with the INTERCEPT TS set by the end of Day 1 post collection; the incubation time in the Compound Adsorption Device (CAD) container ranged from 4 to 16 hours and the INTERCEPT treated PCs were stored in 2 or 3 containers. Day 5 and 7 post-donation PCs were evaluated using a panel of in vitro platelet function assays and evaluated for pH and dose per the EDQM criteria (pH >6.4 and 75% of units having a dose ≥ 2 x 10¹¹ platelets).

RESULTS: INTERCEPT treated PCs met the EDQM platelet dose requirement in 100% of the 89 INTERCEPT units evaluated and 48 of 49 INTERCEPT units evaluated met the EDQM requirement for pH after 7 days of storage (not all INTERCEPT units were evaluated for pH). Platelet dose and volume recovery post-treatment ranged from 82% to 110% and 88% to 95%, respectively. Post- CAD amotosalen levels were within the requirement of ≤2 μM amotosalen (≤0.1 to 0.3 μM) across the CAD times evaluated (9.7 to 16.5 hours). In vitro function data for apheresis PCs in PAS-3 treated in the INTERCEPT TS set were compared to data obtained from a multicenter trial evaluating single and double apheresis PCs in PAS-3 treated with the INTERCEPT DS set and demonstrated comparable in vitro function.

SUMMARY/CONCLUSIONS: The INTERCEPT Blood System for Platelets using the TS set demonstrated acceptable dose and pH according to the EDQM criteria and maintained acceptable in vitro quality through 7 days of storage.

INTERCEPT Blood System for Platelets TS kit is currently not approved for use.
Operational Validation of the Preparation of Pathogen Inactivated Double Dose Buffy Coat Platelet Concentrates

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1. The Blood bank, Landspitali - The National University Hospital of Iceland, Reykjavík; 2. The Blood bank, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland

BACKGROUND: Efficiency and cost effectiveness in the preparation of pathogen inactivated (PI) platelet concentrates (PC) require new and innovative production disposables. An “I-Platelet Pooling” (IPP) set (Kansuk) incorporating a Sepacell™ PLX 5 leukodepletion filter (Asahi Kasei) was developed to obtain a PC from up to 8 buffy coats (BC). This intermediate product can then be treated by a photochemical process utilizing amotosalen and UVA (INTERCEPT Blood System, Cerus) using Dual Storage (DS) processing sets to obtain 2 PI PC products for transfusion with less consumables and labor.

AIMS: The objective of this study was to evaluate the use of the IPP set in combination with the DS set. The study was structured in two phases, “pilot” and “routine use” evaluation of double dose (DD) INTERCEPT treated platelets.

METHODS: BC used for DD PC preparation were approximately 42 ml, 37% hematocrit and 0.8 x10¹¹ platelets. The pilot phase included the preparation of 15 DD BC PC in SSP+ (Macopharma) with the IPP set and Compomat G5 (Fresenius Kabi) with evaluation of platelet yield and recovery, plasma percentage, residual leukocytes and filtration time. The PCs were treated with INTERCEPT DS set and split in two doses. Platelet yield was measured. During routine evaluation, 50 DD BC platelets were prepared in the same way with measurements of platelet yields (pre & post INTERCEPT), residual leukocytes (n=32) and time to process 3 DD products.

RESULTS: During the pilot phase, DD PC had a volume of 410 + 11 ml and plasma ratio of 40 + 1 %. The platelet yield was 5.2 + 0.4 x10¹¹ with recovery of 72 + 7 % from the BC pool to the DD PC. WBC counts were all below 1x10⁶ with a separation and leukodepletion time of 4.5 + 0.4 minutes. After INTERCEPT treatment and split, the volume of each PC was 192 + 6 ml and the platelet yield above 2 x10¹¹ in all units (2.5 + 0.2 x 10¹¹). During routine phase, platelet volume was 399 + 12 ml and platelet yield 5.4 + 0.5 x 10¹¹ in DD PC before and 2.5 + 0.2 x10¹¹ after INTERCEPT. WBC counts in DD PC were 0.06 + 0.10 x 10⁶, all below 1x 10⁶. The time to produce 3 DD PC with 1 operator was 55 minutes.

SUMMARY/CONCLUSIONS: All units produced during the evaluation phases using the I-Platelet Pooling set met the INTERCEPT guard bands (volume, platelet content, plasma ratio) for DD PC treatment using the DS set except for 3 of 65 that were slightly above (max. 9 ml) the required volume (300–420 mL). This can be addressed by minor adjustments in pool volume. The IPP set performed as expected without WBC outliers. PC obtained post INTERCEPT met EDQM guidelines. Processing 8 BC pools allows reducing the consumables used with acceptable time for obtaining a DD PC and the same INTERCEPT processing time as required for treating a single dose. By implementing this method, the process has been simplified and the processing time has been reduced.
In Vitro Storage Quality of Triple Dose Apheresis Versus Single Dose Whole Blood Derived Photochemically Treated Platelet Concentrates

F Kahlenberg¹, A Zimmermann², A Wahler¹, J Thierbach², B Baumann-Baretti¹

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BACKGROUND: Although the risk of viral transfusion-transmitted infections (TTI) from recognized pathogens is low today, bacterial contamination and emerging pathogens remain a threat to transfusion safety. The INTERCEPT™ Blood System (Cerus) was developed to prevent TTI by inactivating pathogens utilizing amotosalen in combination with UVA light. The triple storage (TS) set has been designed to treat double or triple dose platelet donations and is currently in development.

AIMS: A study was conducted to evaluate the storage quality of triple dose apheresis platelet concentrates INTERCEPT treated with a TS set (ITAPC). The results will be compared with quality control data from pooled buffy coat platelet concentrates treated in single volume sets (SV) (IBCPC).

METHODS: 6 triple dose apheresis platelet donations (9 to 11x10¹¹ platelets) from normal volunteer donors collected with Trima Accel® platform (Terumo BCT) were suspended in 40% plasma/60% SSP+ (Macopharma), for INTERCEPT processing with TS sets to obtain after split 6 x 3 treated units. Six BCPC (2 to 4x10¹¹ platelets) were processed by INTERCEPT in single volume (SV) sets. Platelet function on Day 1 and 5 was compared for the two products.

RESULTS: Platelet concentrates from triple dose donations and pooled platelet concentrates treated with amotosalen had comparable platelet content (3.2±0.2 x 10¹¹ (1of 3 units) vs 3.0±0.3 x 10¹¹). The volume of ITAPC was lower (195+3 ml (1 of 3 units) vs 263+5 ml). In vitro function was comparable. The pH (22°C) was 6.8±0.14 (D1) to 6.9±0.07 (D5) in ITAPC slightly decreased compared to IBCPC [7.0±0.03 (D1) to 7.1±0.06 (D5)].

ITAPC showed an increased expression of activation marker CD62 after stimulation with ADP (40μM) ([D1] 24.0±5.3 % without stimulation, 55.5±11.4 % stimulated, [D5] 34.5+9.5 % without to 51.4+6.6 % after stimulation). In IBCPC a mean expression of CD 62 after same stimulation increased by 43.8±9.7 % (D1) to 53.9±5.1 % (D5). Both platelet products maintained their ability to be activated by ADP.

Concentration of glucose decreased from 104.7+33.9 mg/dl (D1) to 32.4+27.0 mg/dl (D5) in ITAPC and from 119.5+7.4 mg/dl (D1) to 82.3+9.0 mg/dl (D5) in IBCPC. In comparison concentration of lactate increased in ITAPC up to 116.9±15.6 mg/dl (D5), in IBCPC up to 160.0+16.1 mg/dl (D5). All tested units were negative in bacterial cultures in both groups.

SUMMARY/CONCLUSIONS: Pathogen inactivated apheresis platelets processed using the TS set and pooled whole-blood derived platelet concentrates processed in SV set retained adequate in vitro function for up to 5 days and met the criteria of the “Council of Europe Recommendation N°.R(95) 15” as well as the German guidelines. The possibility to treat high dose apheresis platelets with as single processing set is operationally convenient.
Implementation of Double Dose Pathogen Inactivated Platelets In Routine With Productivity and Cost Optimization

MB S. Madsen

*Klinisk Immunologi, Aalborg University Hospital, Aalborg, Denmark*

**BACKGROUND:** The INTERCEPT Blood System (IBS, Cerus) utilizes a photochemical treatment with amotosalen and UVA to inactivate contaminating viruses, bacteria, parasites and leucocytes in platelet concentrates and plasma. The Dual Storage (DS) set allows the treatment of a double dose (DD) of platelets.

**AIMS:** The Blood Center of Aalborg between October 2012 and October 2014 (period 1) prepared platelet concentrates (PC) from pools of 4 buffy coats (BC) with Tacsi automated platform (Terumo BCT). After a decision to implement INTERCEPT, the production method was adapted to prepare DD PC from pools of 7 BC, first with Tacsi (November 2014 to January 2016 – period 2) then with a manual pooling set (Macopharma) (February to September 2016 – period 3). We compared QC data, productivity and cost for the 3 periods.

**METHODS:** BC prepared on Macopress separators (Macopharma) had the following average specifications during period 1: volume 62 mL, Hct 50%, plt count 1 x 10^11. For periods 2 and 3, this was adapted to: volume 45 mL, Hct 35%, plt count 1,1 x 10^11. Single dose (SD) PC were produced on Tacsi from pools of 4 BC with 300 ml SSP+ (Macopharma) during period 1. DD PC produced in period 2 from 7 BC on Tacsi or in period 3 with a manual pooling set and 280 ml SSP+ were photochemically treated with INTERCEPT DS sets to yield two pathogen inactivated PC.

**RESULTS:** Production during period 1 (4 BC, Tacsi) was 250 + 20 SD PC with 54 + 22 irradiated PC per month. During period 2 (7 BC, Tacsi, IBS), it was reduced to 142 + 13 DD PC (equivalent to 284 + 26 SD PC) without need for irradiated products. It remained stable during period 3 (7 BC, manual separation, IBS) with 144 + 16 DD PC, Platelet content per PC was 293 + 43 x 10^9 (n=258), 266 + 40 x 10^9 (n=158) and 272 + 39 x 10^9 (n=92) for the 3 periods so the impact of shifting from SD to DD and treating with IBS was a reduction of about 9% of the platelet content per PC regardless of the platelet preparation method used. Residual leucocytes met the requirement of <1 x 10^6/unit in 90% of the PC during the 3 periods. The cost of all disposables used per platelet dose when implementing INTERCEPT increased by 25% in period 2 but decreased by 7% in period 3 both compared with period 1.

**SUMMARY/CONCLUSIONS:** The production of DD BC Platelets with INTERCEPT was successfully implemented. BC characteristics were adapted (reduction of volume and hematocrit) and platelet production process optimized to obtain two doses of platelets from 7 BC instead of one dose from 4 BC with only 9% reduction in yield. The monthly production of PC became more efficient when implementing the DD method with IBS (43 %). Gamma irradiation of PC was stopped. The adoption of the DD BC production method with a manual pooling process is less expensive despite the addition of a pathogen inactivation process.
Effectiveness of Amotosalen/UVA Light Pathogen-Reduced Platelets Transfused to Adult Patients

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BACKGROUND: Platelets transfusions are an important tool for the prevention and treatment of bleeding in thrombocytopenia and / or thrombocytopathy. In contrast to decreasing red blood cell and plasma transfusions in developed countries, the incidence of platelet transfusions increases steadily. To mitigate the risk of transfusion-transmitted infections and Graft-versus-Host Disease, Amotosalen/UVA light pathogen inactivation (PI) technology (INTERCEPT Blood System, Cerus Corporation, Concord, USA) has been implemented in many blood banks in the Republic of Bashkortostan (RB) region as an additional layer of safety. However, methods for the pathogen inactivation of platelet concentrates may affect the functional state of the cells and their clinical effectiveness.

AIMS: To study the effectiveness of pathogen-inactivated platelet transfusions in the adult hospitals of the RB region.

METHODS: The answers for the questionnaire “Platelet transfusions survey” were collected from 16 hospitals in the RB region covering PI transfusion events between the 14.01.2016 and the 30.09.2016. 246 patients received 1519 Amotosalen/UVA light-treated platelet units. Main diagnoses: neoplasms – 144 patients (58,5 %), diseases of the blood and blood-forming organs – 36 patients (14,6 %), certain conditions originating in the perinatal period - 26 patients (10,6 %). For the present study 563 transfusions have been excluded:
- 522 - to patients with body surface area less than 1 m2;
- 41 - with underfill transfusion protocol.

The results were processed using descriptive statistics with a significance level of 0.05.

RESULTS: 70 (7.3%) units have been transfused to stop bleeding, in other cases 886 (92,7%) units were transfused for prevention of bleeding. In therapeutic and prophylactic transfusion recipients was no significant difference in body surface area and the average number of previous transfusions. Only the number of first platelet transfusions to control bleeding was 13.3% higher than for bleeding prevention (OR 2,14, 95% CI - 1.24 to 3,67, p <0,01). The platelet count before treatment transfusions was at 21,6% lower in the therapeutic transfusion group compared to the group of preventative transfusions. A similar ratio is maintained for the concentration of platelets after transfusion. Corrected count increment (CCI) after 24 hours in the investigated groups did not differ. The preventive transfusion group the CCI directly correlated to the concentration of platelets both before (r = 0,157; p <0,001), and after (r = 0,729; p <0,001) transfusions. In therapeutic transfusions group the CCI does not correlate with the concentration of platelets prior to transfusion and directly correlates with platelet concentration post (r = 0,748; p <0,001) transfusion.

CONCLUSION: Therapeutic transfusions were characterized by increased part of first procedures and decreased platelet counts before transfusion as well as the lack of corrected count increment (CCI) correlation with the initial concentration of platelets. The preventive transfusion group CCI directly correlated to the concentration of platelets pre- and post transfusion. Direct correlation between CCI and the initial platelet concentration indicates a lack of consumption of transfused platelets and the possible redundancy of prophylactic transfusions. No adverse transfusion reactions have been registered after 956 pathogen-reduced platelets transfusions.
Effective Pathogen Inactivation In Triple Set Kits for Platelets Suspended In Platelet Additive Solution (PASIII)

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BACKGROUND: A photochemical treatment process utilizing amotosalen and low energy ultraviolet A (UVA) light, was developed to inactivate pathogens and residual leukocytes in platelet components (PC). The INTERCEPT™ Blood System for Platelets, received CE mark for PC in 2002, and was approved in the US in 2014. Approximately 1.7 million kits have been sold since their approval for the preparation of inactivated components and comprise Small Volume (SV; 255-325mL), Double Dose (DD; 300-390mL) and Large Volume (LV; 375-420mL) kits designed to cover platelet doses between 2.9 and 8.0X10¹¹. A new Triple Storage (TS) kit that was designed to expand the dose range to 12.0X10¹¹ platelets and the maximum volume to 650mL, generating either 2 or 3 doses of pathogen inactivated PC, was validated for efficacy of Pathogen Inactivation (PI) and function.

AIMS: The objective of this study was to determine the effectiveness of PI at the worst case scenarios of high plasma content, high volume, and high platelet dose in the TS and the comparison with the results obtained for smaller platelet doses under nominal conditions.

METHODS: For each experiment, a platelet pool with 10-12x10¹¹ platelets was prepared at a final volume of ~650mL in 47% plasma/53% PASIII. These conditions result in a final concentration of amotosalen at the lower limit of the range (135μM vs. nominal of 150μM). The platelet units were inoculated with high titers of a virus or a bacterium and processed. The results were compared to control samples taken prior to the addition of amotosalen and illumination of the PC. Control and test samples were serially diluted and inoculated at the appropriate conditions to determine pre- and post-UVA pathogenic titers. Log₁₀ reduction was calculated as the difference between the mean titers in pre-UVA samples and post-UVA samples. The results (n=4) were compared with the values obtained for previous inactivation studies under nominal conditions.

RESULTS: The inactivation potential for representative viruses (bovine viral diarrhea, bluetongue and adenovirus-5) and bacteria (E. coli, K. pneumonia and S. aureus) were evaluated. The Log₁₀ reductions obtained were: for the viruses: >5.6, >5.9 and >5.8, and for the bacteria >7.0, 6.7 and >7.7, respectively. Those results compare well with the CE mark inactivation commercial claims, of the same pathogens under nominal conditions of >6.0, >5.0, >5.9 for the viruses and >6.4, >5.6 and 6.6 for the bacteria in the same medium, indicating that performance of inactivation in volumes and platelet count necessary for the TS is not affected to any significant extent, even in the extreme limits of the ranges.

SUMMARY/CONCLUSIONS: Inactivation of pathogens in platelet concentrates treated with the INTERCEPT TS kit is similar to those achieved under nominal conditions, even at extreme ranges of volume, plasma and platelet dose.
Comparison of the Efficacy of Gamma-Irradiated Platelet Concentrates (PCs) Suspended In 100% Plasma or 30% Plasma and 70% Platelet Additive Solution (PAS)

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BACKGROUND: The application of PAS may enhance infection and immunological safety of the PCs. Moreover, biochemical parameters of PCs suspended in PAS and plasma (PCpas) are more stable than in PCs suspended only in plasma (PCp). Whereas leucofiltration does not remove all leucocytes from PCS, c –irradiation of PCs minimizes the risk of transfusion-associated graft vs host disease at haematological patients. The impact of c–irradiation on the efficacy of PCpas vs PCp is unknown.

AIMS: to comparison the efficacy of transfusion of c–irradiated PCp with c–irradiated PCpas.

METHODS: We evaluated the efficacy of 50 PC transfusions in thrombocytopenic patients (25 transfusions of PCp suspended in 100% plasma and 25 transfusions of PCpas suspended in 30% plasma plus 70% of PAS). All PCs were c–irradiated (25 Gy) and leucoreduced. The median PCs collection target was 5*10¹¹ platelets. The efficacy of PC transfusions was assessed by TEG (TEG 500, Haemoscope) and Corrected Count Increment (CCI) for platelets. For TEG Peripheral venous blood samples were collected into tubes containing sodium citrate. A volume of 340 ll of citrated patient blood was loaded into each TEG cup. A volume of 20 ll of 0.2 M CaCl2 was added to re-calcify the samples. Analysed TEG parameters were maximum amplitude (MA) before (MA0) and 1 h after (MA1) PC transfusion. For exception of the influence on MA1 of fibrinogen changes, we assessed MA of functional fibrinogen before (MAFF0) and 1 h after the transfusion (MAFF1). CCIs were assessed 1 h after (CCI1) and 24 h after (CCI24) the PC transfusion. Criteria of effective PC transfusion were CCI1 > 7.5; CCI24 > 4.5; MA1 > 44 mm.

RESULTS: According to the CCI1 and CCI24 criteria, the effective transfusions were obtained in 96% and 76% of cases in the PCP group and in 100% and 72% in the PCPAS group, respectively. There were no significant differences between the groups. The MA1 was significantly higher than MA0 as well in PCP group (39 _ 3.5 mm vs 70 mm_1.8, P = 0.0001) as in PCPAS group (35.8 _ 4.8 mm vs 60 _ 3.2 mm, P = 0.0005). There were no significant differences in MA change between the groups. The MAFF0 and the MAFF1 did not differ significantly in the PCP group and in the PCPAS group (23.4 _ 1.4 mm vs 27 _ 2.2 mm, P = 0.18 and 27.5 _ 2.5 mm vs 28.3 _ 2.2 mm, P = 0.8, respectively). Thus, the increase of MA after PC transfusion was associated with platelet function and quantity increment, but not with fibrinogen level changes.

SUMMARY/CONCLUSIONS: In thrombocytopenic patients the c-irradiated PCs suspended in 100% plasma as effective as PCs suspended in PAS and plasma.
Comparison of Amotosalen/UVA Light Pathogen-Reduced Platelets In 100% Plasma Versus Amotosalen/UVA Light Pathogen-Reduced Platelets In PAS: In Vitro Functional and Survival Parameters

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INTRODUCTION: Pathogen inactivation (PI) technology for platelet concentrates (PCs) has been described to enhance blood safety by reducing the risk for transfusion-transmitted pathogens transfusion associated graft-versus host disease. Furthermore, PI technology allows the storage of PCs up to 7 days. The usage of platelet additive solution (PAS) was reported to reduce the risk for non-hemolytic transfusion reactions and enhance platelet quality during storage. However, there is not much known about the impact of the combination of PI and PAS against the combination of PI and 100% plasma on the biochemical platelet quality parameters.

AIMS: Analysis of the impact of Amotosalen/UVA pathogen inactivation technology on the biochemical parameters and functional activity of PCs suspended in 100% plasma compared to PCs suspended in 70% PAS and 30% plasma.

MATERIALS AND METHODS: Apheresis-derived PCs were either pooled in 100% plasma (PCsp, n=25) or in 30% plasma + 70% PAS (SSP+, Macopharma) (PCPAS, n=25). Each PC pool was split to obtain identical therapeutic PCs that were either control (CPCs) or treated with Amotosalen/UVA Pathogen Inactivation (INTERCEPT Blood System, Cerus B.V) (PRPCs). In vitro parameters were analyzed on day 0, 3, 5 and 7 of storage. Biochemical parameters (pH, Glucose, L-Lactic acid and Citric acid concentration) were determined in PC supernatants post centrifugation at 5000 rpm for 30 min.

RESULTS: The pH of PCs in PAS was generally more stable than the pH of PCs in 100% plasma, also in pathogen reduced units. All 4 different units were at day 7 between pH 6.6 and 7.4; within the guardbands of the EDQM Guidelines (pH 6.4-7.4). The Glucose consumption rate in PCs suspended in 100% plasma did not change significantly after pathogen inactivation at the end of the 7d storage period (in PC – 41.36 mmol (5.9 mmol/day) and 38.80 mmol (5.5 mmol/day) in PRPC). In PCPAS, the glucose consumption dropped to 23.84 mmol, and increased in PRPCPAS to 33.3 mmol. The glucose consumption rate was significantly lower in PRPCPAS compared to PRPC in 100% plasma.

Total lactate accumulation in PCs at day 7 of storage was significantly higher in PC/PRPC in 100% plasma compared to PC/PRPC in PAS. While a total concentration of 16 mMol/l was measured in PCPAS and 19 mMol/l in PRPCPAS, PCs in 100% plasma has a higher total concentration of 26 mMol/l and PRPC in 100% plasma of 24 mMol/l. The citrate content in PC storage media suspended in 100% plasma only changed slightly during the storage times, while the citrate content in PCs in PAS dropped in the beginning significantly. At day 7 of storage, a clear tendency of PC/PRPCPAS towards a lower citrate concentration (28 mMol/l) in comparison to PC/PRPC in 100% plasma (38 mMol/l) was observed.

CONCLUSIONS: More stable biochemical quality parameters have been observed in pathogen-reduced PCs in 70% PAS and 30% plasma compared to pathogen-reduced PCs in 100% plasma.
Evaluation of Pathogen Reduced (Amotosalen-UVA) Pooled Cryoprecipitate and Cryoprecipitate-Poor Plasma

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BACKGROUND: A method was developed for the production of cryoprecipitate (CP) and cryoprecipitate-poor plasma (CPP) for efficient utilization of whole blood collections and increased availability of safer products for transfusion. A process was developed to produce pathogen reduced (PR) CP and CPP using amotosalen-UVA (INTERCEPT™ Blood System, Cerus Corporation, Concord, CA) using licensed INTERCEPT plasma. An in-vitro study was performed to evaluate the production of PR CP (3 unit CP) and two units of CPP using mini-pools of 3 units of ABO-matched previously PR frozen plasmas. Levels of Fibrinogen, coagulation factor FVIII and albumin content were measured to assess quality.

AIMS: To evaluate the in vitro characteristics of pathogen-reduced cryoprecipitate and cryo-poor plasma.

METHODS: Whole blood donations of 450 mL ± 10% (CPDA) were processed following local procedures. Plasma units were frozen within 8 hours and stored at < -25°C. After thawing, 3 O positive plasma units were pooled into a standard 600 mL transfer container. The pooled plasma units were then treated with 150 μM amotosalen HCl and 3 J/cm² UVA, frozen for at least 1 hour at < -25°C and thawed for 20 – 24 hours in a 2-6°C refrigerator to precipitate cryo proteins. By routine centrifugation CPP was separated from CP, frozen and stored at < -25°C. In vitro tests were performed pre- and post-PR and post thaw.

RESULTS: PR CP maintained levels of Fibrinogen (average 1040 mg/3 unit mini-pool) and Factor VIII (average 175 IU/unit) which met the protocol-specified mean European therapeutic targets* > 450 mg Fibrinogen and ≥ 150 IU factor VIII per unitary product. Fibrinogen levels of the PR CP 50% greater than the fibrinogen levels in current production 492 mg (current average for a pool of 3 single units transfused). PR CPP contained on average 288 ± 37 mg of Fibrinogen, 42± 14 IU of FVIII and 7.6 ± 0.6 g of albumin. PR CP is currently being used for acquired Fibrinogen deficiency replacement while as CPP is currently used for plasma exchange to treat patients with thrombotic thrombocytopenic purpura.

*For FVII content we applied the EU specifications for PR plasma and used the minimum activity of on average 50IU/unit or 150 IU/pool.

SUMMARY/CONCLUSIONS: PR jumbo CP can replace the currently used 3 single units. Pathogen reduction improves safety for patients in need of CP and CPP, it reduces risk associated with highly prevalent emerging pathogens and allows for making more CP as a source of Fibrinogen, and CPP available from whole blood donations at lower cost. A mini-pool of three CP units is equivalent to one industrial Fibrinogen concentrate, which contains 1 g of the protein. Additional applications for the use of PR CPP are feasible where albumin is indicated for transfusion.
Pathogen Inactivation of Whole Blood (WB) Derived Plasma With Amotosalen/UVA: Results From a Validation In Vitro Study

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BACKGROUND: Pathogen inactivation (PI) of plasma increases the safety of plasma transfusion. To establish an alternative to quarantine plasma a PI technology suitable for blood banks can be used. Pooling of WB plasma before PI can increase the efficacy providing that the specifications of the EDQM’s guide to the preparation, use and quality assurance of blood components for PI plasma (Factor VIII >50 U/dL; Fibrinogen-recovery >60%) and the requirements of the PI technology are met.

AIMS: The aim of the validation study was to prove the feasibility and efficacy of producing PI plasma pooled from five WB derived fresh or thawed plasma units with or without leucocyte reduction (WBC-R) and processed with the INTERCEPT Blood System™ for plasma.

METHODS: Four groups of PI plasma were produced by pooling five units each of ABO-identical WBC-R fresh plasma (group G 1) or previously thawed (G 2) or non-WBC-R fresh plasma (group G 3) or thawed (G 4). In each group at least one pool was of blood type O. Each Pool was split into two parts, each half with a maximum of 650mL was separately pathogen-inactivated with amotosalen/UVA with the outcome of 3 units of PI-plasma of each part (six units per pool).

RESULTS: Results are presented as mean±SD followed by mean values for G1, 2, 3 and 4. We produced 16 pools, four in each group, resulting in 32 PI processes and 96 end products with a volume of 200±5mL (197, 201, 200, 202mL). WBC in the pool were below 1x10E6/L in filtered plasma (G1 and G2) and below 0,1x10E9/L in the unfiltered plasma (G3 and G4). Residual amotosalen was below the threshold of 2 µmol/L in all products (0,8±0,1). Retention of coagulation factors was good with values after INTERCEPT treatment and prior to storage of: F VIII 80±16 IU/dL (83, 68, 79, 90), and fibrinogen 230±21 mg/dL (235, 231, 223, 230). Fibrinogen recovery on average was 90±5% (90, 91, 89, 92) with values of 221±20 mg/dL (222, 213, 231, 217) after treatment and 87±6% (86, 84, 93, 86) after storage for six months. F VIII retention was also very good with 75±22 IU/dl (83, 68, 79, 90) after storage. fibrinogen-recovery. Other coagulation and inhibiting factors were also well preserved.

SUMMARY/CONCLUSIONS: Each of the four groups of PI plasma met the specifications of EDQM and the requirements of the INTERCEPT Blood System™ for plasma. It is feasible, safe and efficacious to use a pool of five WB plasma units and resulting in six units after the INTERCEPT PI process with fresh and thawed plasma as well as WBC reduced and non-reduced plasma. This novel approach has also very positive economical implications.

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BACKGROUND: The INTERCEPT™ Blood System for Red Blood Cells (RBCs) inactivates pathogens and leukocytes in RBC components for transfusion using amustaline to form adducts with nucleic acids, preventing replication of contaminating pathogens and leukocytes. A Phase 3 clinical investigation in patients with thalassemia major requiring chronic RBC transfusion support is in progress in Europe. Ege University Hospital is providing study RBCs to evaluate the safety and efficacy of INTERCEPT RBCs compared to conventional RBCs.

AIMS: This study was designed to qualify Ege University Hospital Blood Bank to produce INTERCEPT RBCs for use during the Phase 3 clinical investigation with SAGM RBC prepared using manual or automated whole blood separation.

METHODS: On the day (D) of collection, D0, CPD whole blood was processed using manual (M) or automated (A; Reveos system, Terumo BCT, USA) methodologies. SAG-M RBCs were stored at 4±2°C until treatment on D1 with the INTERCEPT process. Leukocyte depleted SAG-M RBCs (Test-M: 226-286mL and Test-A: 260-321mL) were added to processing solution containing glutathione (GSH) followed by amustaline addition (final concentrations of 20mM GSH/0.2mM amustaline, based on 280mL RBC input). After 18-24 hours hold at 20-25°C, RBCs were centrifuged and the supernatant was replaced with SAG-M. INTERCEPT RBCs were stored at 4±2°C for 35 days and were sampled on D2, D14 and D35 for analysis of in vitro physical and metabolic parameters.

RESULTS: All units met the acceptance criteria for site qualification. The volume post treatment was 226-295mL (Test-M) and 250-337mL (Test-A), with a loss of 3±2g of Hb attributed to the INTERCEPT process. All units had Hb values of ≥40g ranging from 41–58g. The final Hct was 55-63%, within the 50-70% criterion. After 35 days of storage all INTERCEPT RBCs met the acceptance criteria of ≤0.8%hemolysis; hemolysis was higher in Test-M (0.45±0.18%) compared to Test- A components (0.18±0.06 %). ATP values exceeded 2μmol/g Hb.

SUMMARY/CONCLUSIONS: The INTERCEPT Blood System for RBC technology was successfully validated at Ege Blood Bank. This study demonstrated that SAG-M RBC inputs prepared using manual and automated WB separation methods are compatible with the INTERCEPT Blood System for RBC. INTERCEPT RBC units met the EDQM guidelines (18th Ed.) for leukocyte depleted RBCs in additive solution with respect to Hct, Hb content and hemolysis at end of storage. All measured in vitro parameters of INTERCEPT treated RBCs, including ATP levels, indicate suitability for transfusion.

INTERCEPT Blood System for Red Blood Cells is not approved for commercial use.
Robustness of the INTERCEPT Blood System for Red Blood Cells

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BACKGROUND: The INTERCEPT™ Blood System for Red Blood Cells (RBCs) is being developed for the inactivation of pathogens and leukocytes in RBC components for transfusion using amustaline to form adducts with nucleic acids, preventing replication of contaminating pathogens and leukocytes. The proposed EU input specifications for the RBC pathogen inactivation (PI) process are 220 to 360 mL of SAG-M RBC, collected in ACD or CPD, which meet the EDQM requirements for Hemoglobin (Hb) and Hematocrit (Hct) for RBCs in additive solution (AS). Within 48 hours of collection input RBCs are added to processing solution containing glutathione (GSH) followed by amustaline addition (final concentrations of 20mM GSH/0.2mM amustaline, based on 280mL RBC input). After 18-24 hours hold at 20-25°C, RBCs are centrifuged and the supernatant is replaced with SAG-M for storage up to 35 days.

AIMS: In vitro function was evaluated in PI treated RBCs prepared within and outside the full range of the input specifications and critical processing parameters.

METHODS: RBCs in AS were from apheresis or CPD whole blood (WB) collections; WB was held at 1-6°C or room temperature prior to separation. Input RBCs (n=160) varied by additive solution (AS-5 and SAG-M), age, Hb content, Hct, and leukocyte content. The input RBCs in AS contained 38 to 72 g Hb in 216-361mL with Hct of 57 to 69% and <1×10^6 to 2.5×10^9 leukocytes/unit; the age ranged from 1 to 3 days post collection. The processing parameter variables were GSH and amustaline concentrations as well as PI hold time and temperature and included excursions from the defined processing parameters. Input RBCs in AS were added to processing solution containing GSH followed by amustaline addition (final concentrations were 18-29 mM GSH/0.20-0.25 mM amustaline). After 16 to 28 hours hold at 16 to 27°C, RBCs were centrifuged and the supernatant was replaced with SAG-M. PI RBCs were stored for 35 days 2-6°C and were sampled for analysis of in vitro parameters. PI treated RBCs were evaluated for conformity to the EDQM quality requirements for red blood cells, leukocyte-depleted in AS, or red cells in AS after 35 days of storage.

RESULTS: All but one unit had Hb values of ≥40g ranging from 38–72g; the PI RBCs with Hb content <40 g, had an input Hb content of 38 g prior to treatment. The final Hct was 54-69%, within the 50-70% criterion. After 35 days of storage all PI RBCs met the hemolysis acceptance criteria of ≤0.8%, ranging from 0.1 to 0.7%. ATP values exceeded 2μmol/g Hb (4.1±0.7, 2.4–5.6).

SUMMARY/CONCLUSIONS: This evaluation demonstrated the robustness of the INTERCEPT treatment process within and outside the proposed range of input RBCs and processing conditions. PI treated RBC units met the EDQM guidelines (18th Ed.) for leukocyte depleted RBCs in additive solution with respect to Hct, Hb content and hemolysis at end of storage. All measured in vitro parameters of INTERCEPT treated RBCs, including ATP levels, indicate suitability for transfusion.

The INTERCEPT Blood System for Red Blood Cells is not approved for use.
Robust Inactivation of Duck Hepatitis B Virus With Amustaline/GSH In Whole Blood

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BACKGROUND: Duck Hepatitis B virus (DHBV) is a small, enveloped, dsDNA virus of the Avihepadnavirus genus, a group of viruses in the family Hepadnaviridae. DHBV can be utilized as a model virus for human hepatitis B virus (HBV). Human hepatitis B is a potentially life-threatening liver infection caused by HBV that can result in chronic infection and an increased risk of death from cirrhosis and liver cancer. Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5–10% of the adult population is chronically infected (WHO). In highly endemic areas, hepatitis B is most commonly spread through horizontal transmission via exposure to infected bodily fluids, highlighting the risk associated with blood transfusion. The inability to consistently supply blood components makes WB transfusion common, and since transfusion-transmitted diseases are prevalent in the developing world, the development of a robust WB pathogen inactivation system is desirable. The INTERCEPT Blood System for WB uses the small molecule amustaline to form covalent adducts and crosslinks within nucleic acids of leukocytes and contaminating pathogens to prevent replication. The process includes addition of 0.2 mM amustaline and 2 mM glutathione (GSH) and 24h incubation at room temperature (RT). At the conclusion of the RT incubation, the treated WB unit is suitable for storage up to 7 days.

AIMS: The objective of this study was to evaluate the inactivation of DHBV with the INTERCEPT™ Blood System for Whole Blood (WB) to support the Swiss Red Cross Humanitarian Foundation for Whole Blood Pathogen Inactivation for Africa.

METHODS: For each experiment, a single WB unit was spiked with DHBV to a final concentration of ~10^4.5 TCID_{50}/mL and treated with amustaline and GSH. A control sample was removed prior to the addition of amustaline, serially diluted up to 100,000-fold and inoculated onto duck hepatocytes to determine the pre-treatment titer, resulting in a control titer of 4.6 log_{10} TCID_{50}/mL. Each unit was then dosed with amustaline and a test sample was removed after 24 hours to determine the levels of inactivation. Test samples were diluted 1:2 to 1:10 and inoculated onto duck hepatocytes. The Limit of Detection (LOD) was determined to be <0.7 log_{10} TCID_{50}/mL. The plates were incubated for 10 days at 37°C, fixed with ethanol and the presence of viable DHBV determined by indirect IFA with a mAb to the DHBV envelope protein. Log reduction was calculated as the difference between the mean titer in pre-amustaline samples and the mean titer in the 24 hour post-amustaline samples.

RESULTS: Robust inactivation of DHBV in WB was achieved to the LOD, at >5.3 log_{10} (n=4). This corroborates previous results achieved in AS-5 red blood cells with 0.2 mM amustaline and 20 mM glutathione, resulting in inactivation to the LOD at >5.1 log_{10} of DHBV (n=4).

SUMMARY/CONCLUSIONS: Duck hepatitis B virus was inactivated to the limit of detection in WB after treatment with amustaline and GSH using the duck hepatocyte infectivity model.

The INTERCEPT Blood system for WB or RBCs is not approved for use.
INTERCEPT REGULATORY APPROVALS

**Brazil (ANVISA)**
2015 (platelets and plasma)

**United States (FDA)**
2014 (platelets and plasma)

**Mexico (COFEPRIS)**
2014 (platelets and plasma)

**Singapore (HSA)**
2014 (platelets)

**Switzerland (Swissmedic)**
2009 (platelets), 2010 (plasma)

**Germany (PEI)**
2007* (platelets), 2011* (plasma)

**France (ANSM)**
2003 (platelets), 2006 (plasma)

**CE mark, Class III**
2002 (platelets), 2006 (plasma)

* First blood center marketing authorization approved.