BLOOD SAFETY
POSTER ABSTRACTS COMPILATION
2018

• THERAFLEX MB-Plasma
• THERAFLEX UV-Platelets
• SSP+

ISBT TORONTO 2018
## THERAFLEX MB-Plasma

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## THERAFLEX UV-Platelets

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## SSP+

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BACKGROUND:
Methylene Blue (MB) is one of the widely used methods of plasma inactivation and considered as safe and effective. However, observations from the French haemovigilance data have raised the question of an increased rate of allergic reactions in plasma recipients a decade ago. We aim at retrospectively reviewing these reactions in addition with the data from the last 10 years.

AIMS:
We aim at retrospectively reviewing these reactions in addition with the data from the last 10 years.

METHODS:
Haemovigilance reports of the AFSSAPS and later the ANSM are considered along with those of the EFS. Rates of adverse reactions along with their 95% confidence intervals and their time trends are computed.

RESULTS:
Period of 2008-2011: rate of allergies to MB plasma was higher (8.9/100,000 units) compared to other types of plasma (5.6/100,000 units). The rate of allergic reactions of grade 3-4 and high imputability was decreasing over the time for MB plasma (3.9/100,000 units in 2009) while maintained for other types of plasma (5.1/100,000 units), achieving the same level during 2010-2011 (32.9/100,000 units; 34.7/100,000 units, respectively), for allergic reactions of all grades and imputability 2-3. In addition, the 95% confidence intervals for these reactions to all types of plasma overlap during the entire observed period (no statistical difference). Period of 2012-2016: number of allergic reactions to all types of plasma, all grades and imputability 2-3, has significantly increased compared to the previous years (48.5/100,000 units; 36.3/100,000 units; 34.0/100,000 units; 58.0/100,000 units and 67.6/100,000 units, respectively).

SUMMARY / CONCLUSIONS:
1) a reporting bias could have taken place as a result of a strengthened biological exploration of allergic reactions to MB plasma only; this bias, if it is the case, would invalidate the comparability of the MB and non-MB groups.
2) Until 2008, MB plasma was exclusively produced by apheresis in France, which results in more allergies than whole blood plasma (Saadah N. et al. Br J Haematol 2018). Update of the French data does not confirm the previous conclusions, which were not identified in the ISTARE database either. In conclusion, there is no longer evidence point in considering that the MB plasma is responsible for a greater number of allergies.
MERS CORONAVIRUS IS EFFICIENTLY INACTIVATED IN HUMAN PLASMA BY MB/LIGHT USING THE THERAFLEX MB-PLASMA TECHNOLOGY

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2 Institute of Virology, Philipps-Universität, Marburg, 3 MacoPharma, Langen, Germany

ISBT Congress 2018, Toronto, P-324.

Background:
Middle East Respiratory Syndrome coronavirus (MERS-CoV) was first identified in 2012 and was most likely a zoonotic transmission event from camels. MERS-CoV causes severe lower respiratory tract infections that can result in pneumonia and multiorgan failure, particularly in patients with underlying comorbidities and the elderly. Most cases of MERS-CoV infections have arisen in the Middle East, particularly the Kingdom of Saudi Arabia and in the Republic of Korea but two documented cases in travelers have been reported in the United States. Thus, it can be expected that MERS-CoV will continue to emerge in Western countries being imported by travelers entering the countries or returning home.

Aims:
This study aimed to investigate the efficacy of the THERAFLEX MB Plasma system to inactivate MERS-CoV in human plasma. The THERAFLEX MB Plasma system (Macopharma) uses methylene blue (MB) in combination with visible light for reduction of pathogen infectivity in plasma.

Methods:
Leukodepleted plasma was prepared from whole blood using standard blood banking technology. Plasma units (n=2) were spiked with virus suspension (10% v/v). MB/light treatment was done according to the manufacturer’s instructions using the Macotronic B2 illumination device. Samples were taken after spiking (load and hold sample) and after illumination with different light doses (30, 60, 90 and 120 (standard) J/cm²). The titer of MERS-CoV (strain HCoV-EMC, Ron A. Fouchier) was determined as tissue culture infective dose (TCID50) by endpoint titration on Vero E6 cells (ATCC CCL-22).

Results:
After spiking a MERS-CoV titer of 5.95 (bag no.1) and 6.13 (bag no.2) log10 TCID50/mL was received in the plasma units. Already with the lowest tested light dose of 30 J/cm² MERS-CoV was inactivated down to the detection limit of the system (2.67 log10 TCID50/mL), resulting in log10 reduction factors of ≥3.3 (bag no.1) and ≥3.5 (bag no.2).

Summary / Conclusions:
Our results demonstrate that the THERAFLEX MB-Plasma procedure is an effective technology to inactivate MERS-CoV in contaminated plasma units.

EBOLA VIRUS IS EFFICIENTLY INACTIVATED IN HUMAN PLASMA BY MB/LIGHT USING THE THERAFLEX MB-PLASMA TECHNOLOGY

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2 Institute of Virology, Philipps-Universität, Marburg, 3 MacoPharma, Langen, Germany

ISBT Congress 2018, Toronto, P-325.

Background:
Ebola virus (EBOV) disease is a severe, often fatal illness, with a death rate of up to 90% caused by EBOV, a member of the filovirus family. The recent outbreaks of EBOV disease in West Africa had devastated Guinea, Liberia, and Sierra Leone, causing almost 30,000 human infections with over 11,000 fatalities.

Aims:
This study aimed to investigate the efficacy of the THERAFLEX MB-Plasma system to inactivate EBOV in human plasma. The THERAFLEX MB-Plasma system (Macopharma) uses methylene blue (MB) in combination with visible light for reduction of pathogen infectivity in plasma.

Methods:
Leukodepleted plasma was prepared from whole blood using standard blood banking technology. Plasma units (n=2) were spiked with virus suspension (10% v/v). MB/light treatment was done according to the manufacturer’s instructions using the Macotronic B2 illumination device. Samples were taken after spiking (load and hold sample) and after illumination with different light doses (30, 60, 90 and 120 (standard) J/cm²). The titer of Zaire EBOV (strain Mayinga-76) was determined as tissue culture infective dose (TCID50) by endpoint titration on Vero E6 cells (ATCC CCL-22).

Results:
After spiking an EBOV titer of 6.85 (bag no.1) and 6.99 (bag no. 2) log10 TCID50/mL was received in the plasma units. Already with a light dose of 30 J/cm² (bag no. 1) or 60 J/cm² (bag no. 2) EBOV was inactivated down to the detection limit of the system (3.96 log10 TCID50/mL), resulting in log10 reduction factors of ≥4.7 for both bags.

Summary / Conclusions:
Our results demonstrate that the THERAFLEX MB-Plasma procedure is an effective technology to inactivate EBOV in contaminated plasma.
INACTIVATION OF YELLOW FEVER VIRUS IN PLASMA AND IN PLATELET CONCENTRATES FOLLOWING TREATMENT WITH THE THERAFLEX PATHOGEN INACTIVATION (PI) TECHNOLOGIES

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ISBT 2018 Congress, Toronto, P-440.

Background:
Yellow fever virus (YFV) is endemic to tropical and subtropical areas in South America and Africa, and is currently a major public health threat in Brazil. Although a live, attenuated vaccine is available, for some individuals residing in endemic areas and for many travelers to countries endemic for YFV, affected areas still experience significant morbidity and mortality. Most YFV infections are asymptomatic, but when symptoms occur, they can include fever, muscle pain with prominent backache, headache, loss of appetite, and nausea or vomiting. Transfusion-transmission of the yellow fever vaccine virus has been demonstrated, indicative of potential for viral transfusion transmission. The Australian Red Cross Blood Service (Blood Service) restricts donations from individuals who have been vaccinated against YFV for four weeks post vaccination and from individuals diagnosed with YFV infection for three months post-complete recovery. An alternative approach to manage the potential YFV transfusion-transmission risk is the use of pathogen inactivation (PI) systems, such as THERAFLEX MB-Plasma and THERAFLEX UV-Platelets.

Aims:
To investigate the efficacy of the THERAFLEX MB-Plasma and THERAFLEX UV-Platelets systems to inactivate YFV spiked into plasma or buffy coat-derived platelet concentrates (PCs).

Methods:
YFV was spiked into plasma or PCs units (n=3 per blood component). Spiked plasma units were treated using THERAFLEX MB-Plasma system (visible light doses: 20, 40, 60 and 120 (standard) J/cm²) in the presence of methylene blue (MB; approximately 0.8 μmol/L). Spiked PCs were treated using THERAFLEX UV-Platelets system (UVC doses: 0.05, 0.10, 0.15 and 0.20 (standard) J/cm²). Samples were taken prior to the first and after each illumination dose and tested for residual virus using a modified plaque assay (normal and large-volume plating methods). For each PI system the level of viral reduction was determined.

Results:
Treatment of plasma with THERAFLEX MB-Plasma system resulted in an average of 4.77 log₁₀ reduction in YFV infectivity at the standard visible light dose. Residual viral infectivity reached the detection limit of the assay at 40 J/cm². Similarly, for PCs treated with THERAFLEX UV-Platelets system, an average of 4.8 log₁₀ reduction in YFV infectivity was observed at the standard UVC dose, with residual viral infectivity at the limit of detection of the assay at 0.10 J/cm².

Summary / Conclusions:
Our study suggests the THERAFLEX MB-Plasma and the THERAFLEX UV-Platelets systems can efficiently inactivate YFV in plasma or PCs. The observed reduction in viral infectivity after treatment with these PI systems was similar to that for other arboviruses, including dengue, chikungunya, Zika and West Nile viruses. Studies examining the threshold concentration to elicit disease are needed in order to determine whether the level of reduction in viral infectivity by these PI systems is sufficient to prevent transfusion-transmission. Nonetheless, given the reduction levels observed in this study, these PI systems could be an effective option for managing YFV transfusion-transmission risk in plasma and PCs.
COMBINING UVC-PATHOGEN INACTIVATION AND COLD-STORAGE: A NOVEL APPROACH TO IMPROVE PLATELET SAFETY AND EXTEND THE SHELF-LIFE

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1Research and Development, Australian Red Cross Blood Service, Alexandria, 2Proteomics Core Facility, University of Technology Sydney, Sydney, Australia

PLATELET SAFETY AND EXTEND THE SHELF-LIFE: COLD-STORAGE: A NOVEL APPROACH TO IMPROVE COMBINING UVC-PATHOGEN INACTIVATION AND COLD-STORAGE

Background: Alternatives to room-temperature (RT) storage of platelets are of interest to increase the shelf-life and safety profile of these components. Cold-storage (2-6°C) may facilitate an extension of the shelf-life of platelets, while pathogen inactivation (PI) reduces the risk of pathogen transmission. Separate investigations into both cold-storage and ultraviolet C (UVC)-PI have shown that these two storage modalities differentially affect aspects of platelet quality.

Aims: The aim of this study was to determine the impact of combining UVC-PI and cold-storage (cold-PI) on in vitro platelet quality during platelet storage.

Methods: A pooled and split design was used to generate platelets for four study arms: RT, cold, RT-PI, and cold-PI (n=8 in each arm). On day 1, platelets were left untreated or PI-treated using the THERAFLEX UV-Platelets System (MacoPharma). One unit from each pair was then stored at RT (20-24°C) or refrigerated (2-6°C). In vitro quality and function were tested over 9 days. Data was analysed using two-way ANOVA to assess the combined effect of treatment and storage, where p<0.05 was considered significant.

Results: Combining UVC-PI treatment and cold-storage reduced platelet glycolytic metabolism. PI treatment (0.07 ± 0.01 mmol/10^11plts/day) accelerated glucose consumption compared to untreated RT platelets (0.05 ± 0.01 mmol/10^11plts/day), whilst cold storage (0.04 ± 0.01 mmol/10^11plts/day) had the opposite effect. Cold-PI platelets (0.04 ± 0.01 mmol/10^11plts/day) had a glucose consumption rate equivalent to cold platelets. Similar results were observed for lactate production. Whilst both RT-PI (47 ± 10%) and cold-storage (35 ± 3%) impaired the hypotonic shock response (HSR) compared to RT controls (59 ± 11%), combined PI treatment and cold-storage resulted in complete abrogation of this response by day 5. Combined PI treatment and cold-storage also led to increased externalisation of phosphatidylserine (annexin-V binding) and activation of the GP Ib/IIa receptor (PAC-1 binding) above the levels seen with the individual treatments. Aggregation responses (ADP and collagen) were enhanced in the cold-PI platelets compared to both RT groups, but this was primarily mediated by the cold-storage.

Summary / Conclusions: Cold-storage of UVC-PI platelets reduced PI-induced acceleration of glycolytic metabolism. However, combining cold-storage and PI results in additional phenotypic and functional changes compared to each treatment individually. Further work is required to understand the impact of these changes would affect their clinical efficacy.

PLATELET ADHESION AND AGGREGATION RECEPTORS ARE ALTERED BY THE COMBINED IMPACT OF UVC PATHOGEN INACTIVATION AND CRYOPRESERVATION

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Background: Conventional storage of platelets at room-temperature (20-24°C) limits their shelf-life to 5 days. Modifications to storage, including cryopreservation (-80°C) and pathogen inactivation (PI), may facilitate extension of shelf-life and improve product safety. Cryopreservation and PI have been shown individually, although differentially, to alter the abundance, conformation and glycosylation profile of the platelet adhesion and aggregation receptors, GPIbα and GPIb/IIa. Given the pivotal roles of these glycoproteins in mediating platelet function, it was important to establish the effect of combining these storage modalities.

Aims: The aim of the study was to determine the effect of PI-treatment of platelets prior to cryopreservation, focusing on the impact on platelet glycoproteins.

Methods: Two buffy-coat derived platelet units were pooled and split to form matched pairs (n=8). One unit remained untreated and the other was UV pathogen inactivated with the THERAFLEX UV-Platelets system according to manufacturer’s instructions (MacoPharma). Both were cryopreserved at -80°C using 5%-dimethylsulfoxide and resuspended in a unit of plasma after being thawed at 37°C. Platelets were sampled for testing immediately following thawing (post-thaw) and after 24 hours of storage at room temperature (post-storage).

The abundance and conformation of GP Ibα (anti-CD42b-HP1) and anti-CD42b-AN51, respectively, and the amount of surface bound fibrinogen under resting conditions (anti-fibronectin) were measured by flow cytometry. Exposure of specific glycan residues were measured using the following lectins: Sambucus nigra (SNA) for sialic acid, Ricinus communis agglutinin (RCA) for galactose and succinylated wheat germ agglutinin (sWGA) for N-acetyl-D-glucosamine (Bacillus). Statistical comparisons were performed using paired two-sided t-tests.

Results: The post-thaw recovery of PI-treated platelets (62.6 ± 1.9%) was significantly lower than untreated platelets (71.3 ± 2.5%, p<0.0001), which declined further during storage. Post-thaw abundance of GP Ibα (HP1) and GPIib/IIa on PI-treated platelets was similar to untreated platelets (p=0.1368 and p=0.7651). However, reduced AN51 binding indicated a change in the conformation of GP Ibα on PI-treated platelets immediately following thawing (p=0.0022). GPIbα (HP1 and AN51) was reexpressed on the membrane during 24 hours of storage, although recovery was lower in PI-treated platelets (p=0.0231 and p=0.0075, respectively). PAC-1 binding to PI-treated platelets was higher than untreated platelets post-thaw (p=0.0017), and increased 3-fold during subsequent storage (p=0.0001), indicating greater platelet activation. PAC-1 binding correlated with an increase in fibrinogen binding post-thaw and post-storage (p=0.0359 and p=0.0001, respectively). Further, PI-treated platelets bound the most fibrinogen following post-storage (p=0.0309). Lectin binding, including binding of SNA to sialic acid residues, was reduced on the PI-treated platelets compared to untreated platelets immediately after thawing (p<0.05 for all lectins). However, this recovered during storage, such that both groups were similar after 24 hours.

Summary / Conclusions: PI-treatment prior to cryopreservation alters the platelet phenotype. The increased abundance of activated GPIb/IIa and fibrinogen binding may enhance aggregation of PI-treated cryopreserved platelets. However, the loss of GP Ibα and desialylation of platelet proteins could lead to more rapid clearance of PI-treated cryopreserved platelets upon transfusion.
PLASMA-REDUCED SINGLE DONOR APHERESIS PLATELET CONCENTRATES MANUFACTURED UNDER ROUTINE CONDITIONS FOR THE CAPTURE TRIAL: A ONE YEAR EXPERIENCE

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ISBT Congress 2018, Toronto, P-302.

Background:
Treatment of apheresis platelet concentrates (PCs) with UVC may enhance transfusion safety of platelets with respect to contamination with pathogens.

Aims:
For use in a clinical trial (CAPTURE; EudraCT No.: 2015-001035-20) single donor apheresis platelet concentrates were produced under routine conditions. Here we present the quality data of untreated, UVC-treated and y-irradiated platelet units.

Methods:
405 PCs were prepared from single donors with standard operation procedures (Amicus) using SSP+ (Macopharma, Mouvaux France) as additive solution from February 2017 to February 2018. UVC-PCs were treated with UVC within six hours after preparation using the THERAFLEX UV-Platelets system (Macopharma); y-PCs were y-irradiated with a minimum of 25 Gy; and control PCs (AP-PCs) were left untreated. Sampling for quality control parameters was done on day of preparation (n=44) and at the end of shelf life (AP-PCs: n=35, UVC-PCs: n=44; y-PCs: n=22). The following parameters were examined on day 0: PC volume, platelet concentrations, plasma content, residual erythrocyte and leucocyte counts. Determination of platelet concentration, pH, swirling and sterility testing was done at the end of shelf life.

Results:
Mean volumes were 344 ± 16.9mL in AP-PCs, 355 ± 8.0mL in y-PCs and 344 ± 14.9mL in UVC-PCs with platelet counts of 3.1 ± 0.3 unit, 3.2 ± 0.2 unit and 3.0 ± 0.2 unit, respectively. Residual plasma concentration ranged between 30% and 39%. Residual erythrocyte and leucocyte counts met the standard specifications for PC products in Germany. At the end of shelf life, the pH value of UVC-PCs (7.27 ±0.05) was comparable to y-PCs (7.31 ±0.08) and AP-PCs (7.29 ±0.09). Tests for bacterial contamination were negative for all tested PCs.

Summary / Conclusions:
Quality control data demonstrate that plasma-reduced UVC-treated apheresis PCs meet the standard specifications for PC products in Germany. No differences in quality control were observed between AP-PCs, y-PCs and UVC-PCs. The safety and efficacy of UVC-treated PCs is being evaluated in CAPTURE trial.
ANALYSIS OF 1000 PLATELET CONCENTRATES (PCs) USED AS CLINICAL INVESTIGATIONAL PRODUCTS FOR THE CAPTURE CLINICAL TRIAL TESTING UVC TREATED (THERAFLEX) PCs VS. UNTREATED CONTROL PCs

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Background:
Pathogen reduction technology may enhance microbial safety of platelet transfusion by reducing bacterial and viral contamination. We established the manufacturing of UVC-treated buffy coat derived platelet concentrates under routine conditions.

Aims:
The objective of this study was to evaluate potential differences in product characteristics between UVC treated (TEST group) and non UVC treated (CONTROL group) platelet concentrates (PCs) used as clinical investigational products for the CAPTURE (Clinical Assessment of Platelets Treated with UVC in Relation to Established Preparations) clinical trial.

Methods:
For this study, leukoreduced and plasma-reduced PCs were prepared from five buffy coats (BCs) using 280ml SSP+ additive solution (Macopharma). We established the preparation of TEST PCs treated with the THERAFLEX UV-Platelets system (Macopharma) within 6h after PC preparation and untreated CONTROL PCs stored in the THERAFLEX UV Storage Bag. In vitro parameters (volume, thrombocyte concentration, total protein concentration) were compared by an unpaired t-test. A p value of ≤0.05 was considered statistically significant.

Results:
UVC-treated PCs [n=445] showed no significant differences compared to untreated CONTROL PCs [n=555] regarding thrombocyte concentration [TEST 957 ± 128 x10⁹/ml, CONTROL 950 ± 127 x10⁹/ml] and total protein concentration [TEST 21.7 ± 1.4 g/dl, CONTROL 21.6 ± 1.6]. UVC treated PCs showed a small but significant [p<0.001] lower volume [TEST 353 ± 9 ml vs CONTROL 356 ± 8ml] than untreated PCs. There was no significant difference in storage time until transfusion between TEST and CONTROL PCs (total n=116). Mean age at time of transfusion was 2.6 days for TEST-PCs versus 2.5 days for CONTROL-PCs.

Summary / Conclusions:
The differences between TEST and CONTROL PCs for platelet concentration, total protein concentration and storage duration are not statistically significant. The average difference in volume between TEST and CONTROL PCs was statistically significant, however with a difference of 3 ml this is considered to be non-significant from a clinical point of view.

FELINE CALICIVIRUS, A MODEL VIRUS FOR HEPATITIS E VIRUS, IS EFFICIENTLY INACTIVATED BY THE THERAFLEX UV-PLATELETS SYSTEM

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2Macopharma, Langen, Germany

Background:
Hepatitis E is a viral hepatitis caused by infection with a small, non-enveloped, single-stranded RNA virus called hepatitis E virus (HEV). It was shown in the past that HEV is transfusion transmissible. Although most cases of HEV genotype 3 infection are asymptomatic or mild and self-limiting, severe cases of hepatitis and chronic liver disease were reported in immunosuppressed patients.

Aims:
Up to now, a reliable in-vitro infectivity system for HEV has not yet been established. In this study, we used the feline calicivirus (FCV), a non-enveloped single-stranded RNA virus, as model for inactivation of non-enveloped viruses, in order to assess the inactivation capacity of the UVC-based THERAFLEX UV-Platelets pathogen inactivation system for HEV-like viruses in platelet concentrates (PCs).

Methods:
Plasma reduced PCs from buffy coats (35% plasma in additive solution SSP+, Macopharma) were spiked with virus suspension (10% v/v). PCs [n=6, 350 mL] were then UVC-irradiated on the Macotronic UV machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm²). The titer of the FCV (strain FCV-2280, ATCC VR-2057) was determined as tissue culture infective dose (TCID50) by endpoint titration in microtitre plate assays on feline kidney cell line CRFK (ATCC CCL-94).

Results:
FCV was dose-dependently inactivated by the THERAFLEX-UV Platelets system. After spiking a titer of 5.5 ± 0.5 log10 TCID50/mL was received in the PCs. At a UVC dose of 0.2 J/cm² the titer was reduced to 2.5 ± 0.5 log10 TCID50/mL, resulting in a log10 reduction factor of 3.0 ± 0.2.

Summary / Conclusions:
Several non-enveloped viruses are not inactivated by currently available pathogen-inactivation systems for PCs. It was shown in this investigation that the THERAFLEX-UV Platelets system has the potential to inactivate non-enveloped viruses like FCV.
Background: Middle East Respiratory Syndrome coronavirus (MERS-CoV) was first identified in 2012 and was most likely a zoonotic transmission event from camels. MERS-CoV causes severe lower respiratory tract infections that can result in pneumonia and multiorgan failure, particularly in patients with underlying comorbidities and the elderly. Most cases of MERS-CoV infections have arisen in the Middle East, particularly the Kingdom of Saudi Arabia and in the Republic of Korea but two documented cases in travelers have been reported in the United States. Thus, it can be expected that MERS-CoV will continue to emerge in Western countries being imported by travelers entering the countries or returning home.

Aims: This study aimed to investigate the efficacy of the THERAFLEX UV-Platelets system to inactivate MERS-CoV in platelet concentrates (PCs). The THERAFLEX UV-Platelets system (MacoPharma) uses UVC light only without the need of any additional photocative compound.

Methods: Plasma reduced PCs from 5 BCs (35% plasma in additive solution SSP+) were spiked with virus suspension (10% v/v). PCs (n=2; 375 mL) were then UVC-irradiated on the Macotronic UV machine (Macopharma, Langen, Germany). UVCwas used at a UVC dose of 0.15 J/cm² and higher MERS-CoV was inactivated down to the detection limit of the assay (0.10 J/cm²). The titer of the MERS-CoV (strain HCoV-EMC, Ron A. Fouchier) was determined as tissue culture infective dose (TCID₅₀) by endpoint titration in microtitre plate plating methods. For each PI system the level of viral reduction was determined.

Results: The results showed that viral infectivity at the standard visible light dose. Residual viral infectivity reached the detection limit of the assay at 0.10 J/cm².

Summary / Conclusions: Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate MERS-CoV in contaminated PCs.

MERS CORONAVIRUS IS EFFICIENTLY INACTIVATED IN PLATELET CONCENTRATES BY UVC LIGHT USING THE THERAFLEX UV-PLATELETS TECHNOLOGY

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ISBT Congress 2018, Toronto, P-323.

INACTIVATION OF YELLOW FEVER VIRUS IN PLASMA AND IN PLATELET CONCENTRATES FOLLOWING TREATMENT WITH THE THERAFLEX PATHOGEN INACTIVATION (PI) TECHNOLOGIES


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ISBT 2018 Congress, Toronto, P-440.

Background: Yellow fever virus (YFV) is endemic to tropical and subtropical areas in South America and Africa, and is currently a major public health threat in Brazil. Although a live, attenuated vaccine is available, for some individuals residing in endemic areas and for many travelers to countries endemic for YFV, affected areas still experience significant morbidity and mortality. Most YFV infections are asymptomatic, but when symptoms occur, they can include fever, muscle pain with prominent backache, headache, loss of appetite, and nausea or vomiting. Transfusion-transmission of the yellow fever virus vaccine has been demonstrated, indicative of potential for viral transfusion transmission. The Australian Red Cross Blood Service (Blood Service) receives donations from individuals who have been vaccinated against YFV for five weeks post vaccination and in individuals diagnosed with YFV infection for three months post-complete recovery. An alternative approach to manage the potential YFV transfusion-transmission risk is the use of pathogen inactivation (PI) systems, such as THERAFLEX MB-Plasma and THERAFLEX UV-Platelets.

Aims: To investigate the efficacy of the THERAFLEX MB-Plasma and THERAFLEX UV-Platelets systems to inactivate YFV spiked into plasma or buffy-coat-derived platelet concentrates (PCs).

Methods: YFV was spiked into plasma or PCs units (n=3 per blood component). Spiked plasma units were treated using THERAFLEX MB-Plasma system (visible light doses: 20, 40, 60 and 120 (standard) J/cm²) in the presence of methylene blue (MB; approximately 0.8 μmol/L). Spiked PCs were treated using THERAFLEX UV-Platelets system (UVC doses: 0.05, 0.10, 0.15 and 0.20 (standard) J/cm²). Samples were taken prior to the first and after each illumination dose and tested for residual virus using a modified plaque assay (normal and large-volume plating methods). For each PI system the level of viral reduction was determined.

Results: Treatment of plasma with THERAFLEX MB-Plasma system resulted in an average of 4.77 log₁₀ reduction in YFV infectivity at the standard visible light dose. Residual viral infectivity reached the detection limit of the assay at 40 J/cm². Similarly, for PCs treated with THERAFLEX UV-Platelets system, an average of 4.8 log₁₀ reduction in YFV infectivity was observed at the standard UVC dose, with residual viral infectivity at the limit of detection of the assay at 0.10 J/cm².

Summary / Conclusions: Our study suggests the THERAFLEX MB-Plasma and the THERAFLEX UV-Platelets systems can efficiently inactivate YFV in plasma or PCs. The observed reduction in viral infectivity after treatment with these PI systems was similar to that for other arboviruses, including dengue, chikungunya, Zika and West Nile viruses. Studies examining the threshold concentration to elicit disease are needed in order to determine whether the level of reduction in viral infectivity by these PI systems in plasma and PCs is sufficient to prevent transfusion-transmission. Nonetheless, given the reduction levels observed in this study, these PI systems could be an effective option for managing YFV transfusion-transmission risk in plasma and PCs.
EBOLAVIRUS IS EFFICIENTLY INACTIVATED IN PLATELET CONCENTRATES BY UVC LIGHT USING THE THERAFLEX UV-PLATELETS TECHNOLOGY

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ISBT Congress 2018, Toronto, P-326.

Background:
Ebola virus (EBOV) disease is a severe, often fatal illness, with a death rate of up to 90% caused by EBOV, a member of the filovirus family. The recent outbreaks of EBOV disease in West Africa had devastated Guinea, Liberia, and Sierra Leone, causing almost 30,000 human infections with over 11,000 fatalities.

Aims:
This study aimed to investigate the efficacy of the THERAFLEX UV-Platelets system to inactivate EBOV in platelet concentrates (PCs). The THERAFLEX UV-Platelets system (Macopharma) uses UVC light only without the need of any additional photoactive compound.

Methods:
Plasma reduced PCs from 5 BCs (35% plasma in additive solution SSP+) were spiked with virus suspension (10% v/v). PCs (n=2, 375 mL) were then UVC-irradiated on the Macotronic UV machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm²). The titre of the Zaire EBOV (strain Mayinga-76) was determined as tissue culture infective dose (TCID50) by endpoint titration in microtitre plate assays on Vero E6 cells (ATCC CCL-22).

Results:
The results of the infectivity assay demonstrated that UVC irradiation dose-dependently inactivated EBOV. After spiking an EBOV titer of 6.84 (bag no. 1) and 6.96 (bag no. 2) log10 TCID50/mL was received in the PCs. At a UVC dose of 0.15 J/cm² and higher EBOV was inactivated down to the detection limit of the system (2.37 log10 TCID50/mL), resulting in log10 reduction factors of ≥4.5 (bag no. 1) and ≥4.6 (bag no. 2).

Summary / Conclusions:
Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate EBOV in contaminated PCs.
CAN PLATELETS WITH PAS (SSP+) BE CONSIDERED SAFE FOR TRANSFUSION TO RECIPIENT OF ANY BLOOD GROUP?

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Platelets with high antibody titers have been reported to cause adverse reactions in recipients. Our PAS-platelets protocol involves removal of 80% of plasma from the platelet unit and its replacement by PAS. We have performed IgM and IgG titers of such platelet units with PAS to ensure the safety of these platelets with reference to antibody titers.

To evolve a protocol for universal use of PAS added platelets across all blood groups.

Platelet concentrates (N=167) comprising (N=79) pooled platelets and (N=88) apheresis platelets were studied. The IgM and IgG titers of the platelet units were performed prior to PAS addition and after PAS addition for comparison. The volume of plasma removed and platelet content of the unit was also studied. The results of the above parameters before and after PAS addition were compared. Platelets of blood group A (N=40), B (N=75) and O (N=52) were studied.

The mean volume of platelet units before PAS addition was 379.9ml and volume of the plasma removal prior to PAS addition was 303.7ml (79%).

The platelet content prior to PAS addition was mean 4.9 x 10¹¹ per unit whereas the mean value after PAS addition was 4.8 x 10¹¹. The baseline Anti-A titers were 32 for IgM and 128 for IgG and the same were reduced to 4 for IgM and 16 for IgG after plasma depletion and PAS addition.

ROLE OF PLATELET ADDITIVE SOLUTION (PAS) in ABO-INCOMPATIBLE SINGLE DONOR PLATELETS TRANSFUSION - A RETROSPECTIVE ANALYSIS OF 126 CASES

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Platelet apheresis is used to obtain platelets from volunteer donors, patients’ family members, or donors with HLA or platelet-antigen-compatible phenotypes. By design, apheresis procedures are intended to collect large numbers of platelets from an individual, thereby providing a more potent product with fewer donor exposures for the patient. Although platelets are often transfused without regard to ABO compatibility, the use of mismatched platelets frequently results in lower post-transfusion recovery rates.

In some cases, high-titer immunoglobulin G (IgG) A,B antibodies in blood group O recipients are reactive with transfused platelets carrying large amounts of A or B antigens, resulting in platelet transfusion refractoriness. Recovery of transfused platelets can also be influenced by the transfusion of group O platelets to group A recipients. Anti-A and/or anti-B in the donor plasma might be reactive with soluble A or B in the recipient plasma to form immune complexes that bind to transfused platelets decreasing the survival of the transfused platelets. Clinical trials comparing ABO-identical to unmatched platelets in patients with cancer who require multiple platelet transfusions have suggested that rates of refractoriness are significantly higher when unmatched components are used.

The main aim of this case study is to highlight the importance of Platelet Additive Solution (PAS) in ABO incompatible Single Donor Platelets (SDP) Transfusions.

The study was carried out at Department of Transfusion Medicine over a period of 1 year from January 2017 to December 2017. A total number of 419 SDP Procedures were done during this period. Out of 419, 126 ABO incompatible SDP Procedures were done with PAS SSP+. A ratio of 80% SSP+/20% plasma was used.
USE OF PLATELET ADDITIVE SOLUTION (PAS), IS THIS BLESSING IN DISGUISE FOR TRANSFUSION MEDICINE?

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India is among medium developing country with population over 1.25 billion with areas of difficult terrains, landslides and seasonal outbreaks of Dengue. Benefits of apheresis platelets (SDP) V/S random donor platelets (RDP) are well documented. But the challenge that comes is requirement of group specific donor. Arranging group specific donor is really a challenge especially in cases of international patients, outstation patients and patients that require numerous platelet transfusions in cases like dengue etc. and especially in corporate hospitals that have limited voluntary apheresis blood donor registry.

Advantages associated with use of PAS are: Greater removal of ABO-incompatible plasma, thus reducing the risk of hemolysis, product can be issued across all group patients, lesser allergic reaction to patients, lesser vasovagal reaction to donors, smooth inventory, wastage prevention.

Demonstrate clinical equivalence of PAS-SDP with group specific SDP if any.

This study was conducted in multispecialty tertiary health care, corporate hospital of Delhi/NCR where major patient client age is from International patients and Pan-India. Use of PAS (Platelet Additive solution) is approved by DCGI, India and Central Hospital Transfusion Committee of Group Hospital. Use of PAS was implemented on all apheresis prepared using storage solution for platelets using Apheresis platform. PAS is added later on after collection of hyper concentrated PLT units.

Contaminating white blood cells were removed from all units by the leukoreduction system of the cell separator.

Study Duration: 6 Months
All donors participating in this study met the Guidelines for the Selection of Blood Donors.

All platelets collected are stored at 22°C under constant agitation, undergo quality control check at Day 1 till Day 5 from platelet pouch even after product issue.

Study markers: Volume of the final product, platelet counts and mean platelet volume (MPV), pH, swirling movement by visual inspection, patient platelet count pre-transfusion, patient platelet count on next day after transfusion (Day 1,3) if no fresh transfusion.

Totally, 130 apheresis units were prepared on PAS during the study. 126 units were issued to 77 patients. Four units were discarded being date expired. 99.2% of donors under the study were male and 47.7% in age group 18–30 years and 31.5% of blood Group O positive. Final platelet volume prepared was 300 ± 20 ml. MPV was within normal range 7.4–10.4 Fl. Swirling was fully maintained during the study (graded by visual inspection) in all units. Product pH was in the range of 7.1 ± 0.1, platelet count 1538 ± 17 × 10^3/μL. Patient platelet count was assessed before apheresis transfusion and then 24 h after transfusion. Mean percentage increment (MPI) was 232% and range 64.71%–650%. Mean calculated was 2.82 ± 1.42. No adverse transfusion reactions were noted in any case.

Use of PAS for apheresis platelets eliminates the need for group specific platelets converting all SDPs to universal platelets, thereby helping in the better management of available groups without compromise in patient safety. PAS–SDP were comparable to normal SDP both in terms of Platelet count in the blood bag and platelet increment obtained in patients, thereby demonstrating clinical equivalence. No adverse reactions were reported with any of the PAS-SDP group indicating safety. Papers on this subject, especially in the Indian subcontinent are few and larger studies are needed before PAS-SDPs become the norm.
Lead the way in blood safety

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