BLOOD SAFETY

BIBLIOGRAPHY

• THERAFLEX MB-Plasma
• THERAFLEX UV-Platelets
• SSP+
Macopharma is an innovative Company in global healthcare with expertise in the fields of Transfusion, Infusion and Biotherapy. One of Macopharma’s aims is to provide a comprehensive range of products for the pathogen reduction of infectious agents in the different blood components. This is aligned with Macopharma’s product development strategy of the continuous quest, through partnerships, for improved safety, efficacy, and quality of transfusion, infusion and cellular therapy.

The THERAFLEX MB-Plasma system has been designed to inactivate both, recognized and emerging pathogens in plasma. The Pathogen Reduction technology for plasma has been developed in partnership with the Blood Centre of the German Red Cross Chapters of NSTOB, Springe. It is a user-friendly in-house treatment for single units of plasma adapted for the inactivation of pathogens in Fresh Frozen Plasma from apheresis or whole blood. MB-treated plasma transfused with the THERAFLEX MB-Plasma procedure is in clinical use in 20 countries worldwide and more than 7 million MB-plasma units have been treated and subsequently transfused to date.

The THERAFLEX UV-Platelets system is a joint development by the German Red Cross Blood Services and Macopharma, aiming at the inactivation of known and emerging pathogens in platelet products. The technology is based on the exposure of plasma-reduced platelet concentrates to UV-C light only, requiring no additional photoactive substance. It is a simple and fast, one-step inactivation process using SSP+ as platelet additive solution, and substitute for plasma. Clinical trials are in progress.

The Platelet Additive Solution SSP+ (“PAS-E”) is the most suitable PAS on the market. It is designed to partially replace plasma in the preparation and storage of buffy-coat derived platelet concentrates or apheresis platelet units. The solution enables platelets to be stored at 22°C ± 2°C, under gentle agitation, for up to 7 days following collection and according to local regulations.

Since 2002, more than 11 million units of Macopharma Platelet Additive Solution have been distributed in 55 countries worldwide. Macopharma is proud to share with you the most relevant articles showing the benefits of these blood safety technologies.

We wish you an enjoyable and fruitful reading.

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**THERAFLEX MB-Plasma scientific publications**

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<tr>
<td>2017</td>
<td>Fryk JJ, Marks DC, Haba-Peters J, Watterson D, Hall RA, Young PR, Reichenberg S, Surnian C. SSP+ (PAS-E) is the most suitable PAS on the market. <em>Vox Sang</em> 2017; 112:352-359.</td>
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Inactivation of yellow fever virus in plasma after treatment with methylene blue and visible light and in platelet concentrates following treatment with ultraviolet C light.


**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. Transfusion transmission of the yellow fever vaccine virus has been demonstrated, which is indicative of the potential for viral transfusion transmission. An approach to manage the potential YFV transfusion transmission risk is the use of pathogen inactivation (PI) technology systems, such as THERAFLEX MB-Plasma and THERAFLEX UV-Platelets (Macopharma). We aimed to investigate the efficacy of these PI technology systems to inactivate YFV in plasma or platelet concentrates (PCs).

**STUDY DESIGN AND METHODS:**
YFV 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 (approx. 0.8 μmol/L) and spiked PCs were treated using THERAFLEX UV-Platelets system (ultraviolet C doses: 0.05, 0.10, 0.15, and 0.20 [standard] J/cm²). Samples were taken before the first and after each illumination dose and tested for residual virus using a modified plaque assay.

**RESULTS:**
YFV infectivity was reduced by an average of 4.77 log or greater in plasma treated with the THERAFLEX MB-Plasma system and by 4.8 log or greater in PCs treated with the THERAFLEX UV-Platelets system.

**CONCLUSIONS:**
Our study suggests the THERAFLEX MB-Plasma and the THERAFLEX UV-Platelets systems can efficiently inactivate YFV in plasma or PCs to a similar degree as that for other arboviruses. Given the reduction levels observed in this study, these PI technology systems could be an effective option for managing YFV transfusion-transmission risk in plasma and PCs.
Plasma temperature during methylene blue/light treatment influences virus inactivation capacity and product quality.

Gravemann U, Handke W, Sunjic C, Alvarez I, Reichenberg S, Müller TH & Seltsam A.
Vox Sang 2018; 113:368-77.

BACKGROUND:
Photodynamic treatment using methylene blue (MB) and visible light is in routine use for pathogen inactivation of human plasma in different countries. Ambient and product temperature conditions for human plasma during production may vary between production sites. The influence of different temperature conditions on virus inactivation capacity and plasma quality of the THERAFLEX MB-Plasma procedure was investigated in this study.

METHODS:
Plasma units equilibrated to 5 – 2°C, room temperature (22 – 2°C) or 30 – 2°C were treated with MB/light and comparatively assessed for the inactivation capacity for three different viruses, concentrations of MB and its photoproducts, activity of various plasma coagulation factors and clotting time.

RESULTS:
Reduced solubility of the MB pill was observed at 5 – 2°C. Photocatalytic degradation of MB increased with increasing temperature, and the greatest formation of photoproducts (mainly azure B) occurred at 30 – 2°C. Inactivation of suid herpesvirus, bovine viral diarrhea virus and vesicular stomatitis virus was significantly lower at 5 – 2°C than at higher temperatures. MB/light treatment affected clotting times and the activity of almost all investigated plasma proteins. Factor VIII (-17.7 +/- 8.3%, 22 – 2°C) and fibrinogen (-14.4 +/- 16.4%, 22 – 2°C) showed the highest decreases in activity. Increasing plasma temperatures resulted in greater changes in clotting time and higher losses of plasma coagulation factor activity.

CONCLUSIONS:
Temperature conditions for THERAFLEX MB-Plasma treatment must be carefully controlled to assure uniform quality of pathogen-reduced plasma in routine production. Inactivation of cooled plasma is not recommended.

Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively.

Eickmann M, Gravemann U, Handke W, Tolksdorf F, Reichenberg S, Müller TH, Seltsam AA.
Transfusion 2018; 2018; 58:2202-7.

BACKGROUND:
Ebola virus (EBOV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have been identified as potential threats to blood safety. This study investigated the efficacy of the THERAFLEX UV-Platelets and THERAFLEX MB-Plasma pathogen inactivation systems to inactivate EBOV and MERS-CoV in platelet concentrates (PCs) and plasma, respectively.

STUDY DESIGN AND METHODS:
PCs and plasma were spiked with high titers of cell culture-derived EBOV and MERS-CoV, treated with various light doses of ultraviolet C (UVC; THERAFLEX UV-Platelets) or methylene blue (MB) plus visible light (MB/light; THERAFLEX MB-Plasma), and assessed for residual viral infectivity.

RESULTS:
UVC reduced EBOV (≥ 4.5 log) and MERS-CoV (≥ 3.7 log) infectivity in PCs to the limit of detection, and MB/light decreased EBOV (≥ 4.6 log) and MERS-CoV (≥ 3.3 log) titers in plasma to nondetectable levels.

CONCLUSIONS:
Both THERAFLEX UV-Platelets (UVC) and THERAFLEX MB-Plasma (MB/light) effectively reduce EBOV and MERS-CoV infectivity in platelets and plasma, respectively.
Cost-utility and budget impact of methylene blue-treated plasma compared to quarantine plasma.


BACKGROUND:
Methylene blue and visible light treatment and quarantine are two methods used to reduce adverse events, mostly infections, associated with the transfusion of fresh-frozen plasma. The objective of this study was to estimate and compare the budget impact and cost-utility of these two methods from a payer’s perspective.

MATERIALS AND METHODS:
A budget impact and cost-utility model simulating the risks of hepatitis B virus, hepatitis C virus, cytomegalovirus, a West Nile virus-like infection, allergic reactions and febrile non-haemolytic transfusion reactions achieved using plasma treated with methylene blue and visible light (MBP) and quarantine plasma (QP) was constructed for Spain. QP costs were estimated using data from one blood centre in Spain and published literature. The costs of producing fresh-frozen plasma from whole blood, apheresis plasma, and multicomponent apheresis, and separately for passive and active methods of donor recall for QP were included. Costs and outcomes over a 5-year and lifetime time horizon were estimated.

RESULTS:
Compared to passive QP, MBP led to a net increase of €850,352, and compared to active QP, MBP led to a net saving of €5,890,425 over a 5-year period. Compared to passive QP, MBP increased the cost of fresh-frozen plasma per patient by €7.21 and had an incremental cost-utility ratio of €705,126 per quality-adjusted life-year. Compared to active QP, MBP reduced cost by €50.46 per patient and was more effective.

DISCUSSION:
Plasma collection method and quarantine approach had the strongest influence on the budget impact and cost-utility of MBP. If QP relies on plasma from whole blood collection and passive quarantine, it is less costly than MBP. However, MBP was estimated to be more effective than QP in all analyses.

International, Prospective Haemovigilance Study on Methylene Blue-Treated Plasma.


BACKGROUND AND OBJECTIVES:
Methylene Blue is a phenothiazine dye, which in combination with visible light has virucidal and bactericidal properties, disrupting the replication of a broad range of enveloped viruses and some non-enveloped viruses. The study objective was to collect data on adverse reactions occurring with Methylene Blue plasma administered in a routine clinical practice environment and document their characteristics and severity.

MATERIALS AND METHODS:
This was an open label, multi-centre, non-controlled, non-randomized, non-interventional study. Patients who receive a Methylene Blue plasma transfusion were observed for any signs and symptoms (adverse reactions) within 24 hours after the start of the transfusion, in different hospitals for a study duration of at least one year.

RESULTS:
19,315 Methylene Blue plasma units were transfused. There were 8 patients with adverse reactions recorded during the study, one of them serious. Two had more than one reaction (2 and 4, respectively). Three patients had previous transfusions with Methylene Blue plasma only.

CONCLUSION:
Methylene Blue Plasma has a very acceptable safety profile with a rate of Serious Adverse Reactions of 0.5/10,000 units.
ZIKV virus in plasma is inactivated after treatment with methylene blue and light illumination.


AIM:
The emergence of Zika virus (ZIKV) in the Americas has resulted in a public health emergency. Three documented cases of ZIKV transfusion-transmission highlights that this virus is a potential threat to blood transfusion safety. An approach to manage this risk is pathogen inactivation, such as the THERAFLEX MB-PLASMA system. We examined the effectiveness of this system to inactivate ZIKV in plasma at different visible-light doses.

METHODS:
ZIKV was spiked into pooled plasma (n=3), then treated with the THERAFLEX MB-Plasma system. Pre- and post-treatment samples were taken at each illumination dose (0, 20, 40, 60, 120 J/cm²) and viral infectivity determined by plaque assay. The reduction in viral infectivity was calculated.

RESULTS:
Treatment of plasma with the THERAFLEX MB-Plasma system resulted in ≥5.68 log₁₀ reduction in ZIKV infectivity at 120 J/cm², with residual viral infectivity reaching the limit of detection of the assay with treatment at 40 J/cm².

DISCUSSION:
Our study has shown the THERAFLEX MB-PLASMA system can reduce the infectivity of ZIKV to the limit of detection of the assay used at one third of the standard illumination dose. Our data suggest this system may be an effective option for managing ZIKV transfusion-transmission risk in plasma.

Paired comparison of methylene blue- and amotosalen-treated plasma and cryoprecipitate.


BACKGROUND AND OBJECTIVES:
Cryoprecipitate is used in the treatment of patients with acquired hypofibrinogenaemia. Studies have not directly compared cryoprecipitate produced following pathogen inactivation (PI) of fresh-frozen plasma (FFP) using different systems. The effects of methylene blue (MB) and amotosalen (AS) PI systems on the quality of FFP and cryoprecipitate were investigated in a paired study.

MATERIALS AND METHODS:
Seven group A and 7 group O pools of plasma were prepared and split into individual units and rapidly frozen to produce FFP. Units of FFP were thawed and either PI treated with MB or amotosalen, or left untreated (control). Samples of FFP along with the corresponding cryoprecipitate were tested for a range of coagulation factors, thrombin generation (TGT) and rotational thromboelastometry (ROTEM).

RESULTS:
AS-FFP showed a smaller decrease following treatment for most coagulation factors analysed than MB-FFP, except fibrinogen (antigen) and factor VII, partly due to lower volume losses. There was no significant difference between treatment methods for fibrinogen content of cryoprecipitate with treated units meeting current UK specification, or TGT and ROTEM parameters studied.

CONCLUSIONS:
MB-cryo contained a significantly higher content of FVIII and lower content of FXIII when compared to AS-cryo, with no difference in fibrinogen activity.
Dengue and chikungunya viruses in plasma are effectively inactivated after treatment with methylene blue and visible light.


BACKGROUND:
Arboviruses, such as dengue viruses (DENV) and chikungunya virus (CHIKV), pose a risk to the safe transfusion of blood components, including plasma. Pathogen inactivation is an approach to manage this transfusion transmission risk, with a number of techniques being used worldwide for the treatment of plasma. In this study, the efficacy of the THERAFLEX MB-Plasma system to inactivate all DENV serotypes (DENV-1 through DENV-4) or CHIKV in plasma, using methylene blue and light illumination at 630 nm, was investigated.

STUDY DESIGN AND METHODS:
Pooled plasma units were spiked with DENV-1, DENV-2, DENV-3 DENV-4 or CHIKV and treated with the THERAFLEX MB-Plasma system at four light illumination doses: 20, 40, 60 and 120 (standard dose) J/cm². Pre- and post-treatment samples were collected and viral infectivity determined. The reduction in viral infectivity was calculated for each dose.

RESULTS:
Treatment of plasma with the THERAFLEX MB-Plasma system resulted in a ≥4.46 log reduction in all DENV serotypes and CHIKV infectious virus. The residual infectivity for each was at the detection limit of the assay used at 60 J/cm², with dose-dependency also observed.

CONCLUSIONS:
Our study demonstrated the THERAFLEX MB-Plasma system can reduce the infectivity of all DENV serotypes and CHIKV spiked into plasma to the detection limit of the assay used at half of the standard illumination dose. This suggests this system has the capacity to be an effective option for managing the risk of DENV or CHIKV transfusion transmission in plasma.

Thrombin generation, ProC®Global, prothrombin time and activated partial thromboplastin time in thawed plasma stored for seven days and after methylene blue/ light pathogen inactivation.


BACKGROUND:
Methylene blue pathogen inactivation and storage of thawed plasma both lead to changes in the activity of several clotting factors. We investigated how this translates into a global loss of thrombin generation potential and alterations in the protein C pathway.

METHODS AND MATERIALS:
Fifty apheresis plasma samples were thawed and each divided into three subunits. One subunit was stored for 7 days at 4 °C, one was stored for 7 days at 22 °C and one was stored at 4 °C after methylene blue/light treatment. Thrombin generation parameters, ProC®Global, prothrombin time and activated partial thromboplastin time were assessed on days 0 and 7.

RESULTS:
The velocity of thrombin generation increased significantly after methylene blue treatment (increased thrombin generation rate; time to peak decreased) and decreased after storage (decreased thrombin generation rate and peak thrombin; increased lag time and time to peak). The endogenous thrombin generation potential remained stable after methylene blue treatment and storage at 4 °C. Methylene blue treatment and 7 days of storage at 4 °C activated the protein C pathway whereas storage at room temperature and storage after methylene blue treatment decreased the functional capacity of the protein C pathway. Prothrombin time and activated partial thromboplastin time showed only modest alterations.

CONCLUSION:
The global clotting capacity of thawed plasma is maintained at 4 °C for 7 days and directly after methylene blue treatment of thawed plasma. Thrombin generation and ProC®Global are useful tools for investigating the impact of pathogen inactivation and storage on the clotting capacity of therapeutic plasma preparations.
Quantitative analysis of plasma proteins in whole blood-derived fresh frozen plasma prepared with three pathogen reduction technologies.

Larrea L, Ortiz-de-Salazar MI, Martinez P, Roig R.

Several plasma pathogen reduction technologies (PRT) are currently available. We evaluated three plasma PRT processes: Cerus Amotosalen (AM), Terumo BCT riboflavin (RB) and Macopharma methylene blue (MB). RB treatment resulted in the shortest overall processing time and in the smallest volume loss (1%), and MB treatment in the largest volume loss (8%). MB treatment retained the highest concentrations of factors II, VII, X, IX, Protein C, and Antithrombin and the AM products of factor V and XI. Each PRT process evaluated offered distinct advantages such as procedural simplicity and volume retention (RB) and overall plasma protein retention (MB).

Challenge study of the pathogen reduction capacity of the THERAFLEX MB-Plasma technology.

Reichenberg S, Gravemann U, Sumian C, Seltsam A.

BACKGROUND AND OBJECTIVES:
Although most pathogen reduction systems for plasma primarily target viruses, bacterial contamination may also occur. This study aimed to investigate the bacterial reduction capacity of a methylene blue (MB) treatment process and its virus inactivation capacity in lipaemic plasma.

MATERIALS AND METHODS:
Bacterial concentrations in plasma units spiked with different bacterial strains were measured before and after the following steps of the THERAFLEX MB-Plasma procedure: leucocyte filtration, MB/light treatment and MB filtration. Virus inactivation was investigated for three virus types in non-lipaemic, borderline lipaemic and highly lipaemic plasma.

RESULTS:
Leucocyte filtration alone efficiently eliminated most of the tested bacteria by more than 4 logs (Staphylococcus epidermidis and Staphylococcus aureus) or to the limit of detection (LOD) (≥ 4.8 logs; Escherichia coli, Bacillus cereus and Klebsiella pneumoniae). MB/light and MB filtration further reduced Staphylococcus epidermidis and Staphylococcus aureus to below the LOD. The small bacterium Brevundimonas diminuta was reduced by 1.7 logs by leucocyte filtration alone, and to below the LOD by additional MB/light treatment and MB filtration (≥ 3.7 logs). Suid herpesvirus 1, bovine viral diarrhoea virus and human immunodeficiency virus 1 were efficiently inactivated by THERAFLEX MB-Plasma, independent of the degree of lipaemia.

CONCLUSION:
THERAFLEX MB-Plasma efficiently reduces bacteria, mainly via the integrated filtration system. Its virus inactivation capacity is sufficient to compensate for reduced light transparency due to lipaemia.
Haemovigilance data on the use of methylene blue virally inactivated fresh frozen plasma with the THERAFLEX MB-Plasma System in comparison to quarantine plasma: 11 years’ experience.


BACKGROUND:
Haemovigilance is an effective tool for identifying adverse effects of blood components. We analyse cumulative haemovigilance data in order to compare the two secured therapeutic plasmas that have been in use for more than 11 years in Greece - methylene blue-treated fresh frozen plasma (MB-FFP) and quarantine fresh frozen plasma (Q-FFP) - regarding safety and adverse events.

MATERIALS AND METHODS:
Data from the centralised active haemovigilance system of Greece for the period 2001-2011 were used to examine the association between FFP types and adverse events. Post-transfusion information on infectious and non-infectious adverse events was analysed. Events were examined by reaction type, severity and imputability to transfusion.

RESULTS:
The incidence of adverse events was higher with Q-FFP (1:3620) than MB-FFP (1:24 593) by a factor of 6.79 [95% confidence interval (CI) 2.52-27.8]. Allergic adverse events were also commoner with Q-FFP (1:7489) than with MB-FFP (1:24 593), by a factor of 3.28 (95% CI 1.17-13.7). All adverse reactions experienced by the MB plasma recipients were considered to be mild.

CONCLUSION:
Haemovigilance over 11 years has demonstrated the long-term safety of MB-FFP in comparison to untreated quarantine FFP. In addition to lowering the adverse event rate, implementing the system on a national scale in at-risk countries would presumably reduce the transmission of severe viral infections including emerging infectious diseases by transfusion.

FVIII and fibrinogen recovery after THERAFLEX MB-Plasma procedure following plasma source and treatment time.

Rapaille A, Reichenberg S, Najdovski T, Cellier N, de Valensart N, Denveys V.

BACKGROUND:
The quality of fresh-frozen plasma is affected by different factors. Factor VIII is sensitive to blood component storage processes and storage as well as pathogen-reduction technologies. The level of fibrinogen in plasma is not affected by the collection processes but it is affected by preparation and pathogen-reduction technologies.

MATERIALS AND METHODS:
The quality of plasma from whole blood and apheresis donations harvested at different times and treated with a pathogen-reduction technique, methylene blue/light, was investigated, considering, in particular, fibrinogen and factor VIII levels and recovery.

RESULTS:
The mean factor VIII level after methylene blue treatment exceeded 0.5 IU/mL in all series. Factor VIII recovery varied between 78% and 89% in different series. The recovery of factor VIII was dependent on plasma source as opposed to treatment time. The interaction between the two factors was statistically significant. Mean levels of fibrinogen after methylene blue/light treatment exceeded 200 mg/dL in all arms. The level of fibrinogen after treatment correlated strongly with the level before treatment. There was a negative correlation between fibrinogen level before treatment and recovery. Pearson’s correlation coefficient between factor VIII recovery and fibrinogen recovery was 0.58.

CONCLUSION:
These results show a difference in recovery of factor VIII and fibrinogen correlated with plasma source. The recovery of both factor VIII and fibrinogen was higher in whole blood plasma than in apheresis plasma. Factor VIII and fibrinogen recovery did not appear to be correlated.

Haemovigilance data on the use of methylene blue virally inactivated fresh frozen plasma with the THERAFLEX MB-Plasma System in comparison to quarantine plasma: 11 years’ experience.

BACKGROUND:
Haemovigilance is an effective tool for identifying adverse effects of blood components. We analyse cumulative haemovigilance data in order to compare the two secured therapeutic plasmas that have been in use for more than 11 years in Greece - methylene blue-treated fresh frozen plasma (MB-FFP) and quarantine fresh frozen plasma (Q-FFP) - regarding safety and adverse events.

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Haemovigilance over 11 years has demonstrated the long-term safety of MB-FFP in comparison to untreated quarantine FFP. In addition to lowering the adverse event rate, implementing the system on a national scale in at-risk countries would presumably reduce the transmission of severe viral infections including emerging infectious diseases by transfusion.
Paired analysis of plasma proteins and coagulant capacity after treatment with three methods of pathogen reduction.

Coene J, Devreese K, Sabot B, Feys HB, Vandekerckhove P, Compereolle V.

BACKGROUND:
The effect of photochemical pathogen reduction (PR) methods on plasma quality has been the subject of several reports but solid comparative data for the different technologies are lacking.

STUDY DESIGN AND METHODS:
Plasma (n = 24) photoinactivated with methylene blue (MB), riboflavin (RF), or amotosalen (AS) was compared using a pool-and-split design. Samples were taken before and after treatment with each method and tested for coagulation factors (fibrinogen, Factor [F] II, FV, FVIII, FIX, FXII), natural coagulation inhibitors (Protein C [PC], Protein S [PS], antithrombin III [AT]), prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin generation (TG). The three methods were mutually compared by repeated-measures analysis of variance.

RESULTS:
All three PR methods cause significant reduction (p < 0.01) of activity of the procoagulant proteins fibrinogen, FII, FV, FVIII, FIX, and FXII. Coagulation is also affected, with significant changes in PT, APTT, and TG. RF treatment causes a significantly higher decrease in concentration of coagulation factors, PS, and AT than the other methods (p < 0.01). PT, APTT, and TG are also affected most by RF treatment. FII, FVIII, FIX, PC, AT, and PT are best preserved with the MB method and FV, FXII, and TG after AS treatment (p < 0.01). Coagulation factor loss due to the volume loss during PR treatment is more important for MB and AS than for RF.

CONCLUSION:
PR treatment of plasma affects coagulation proteins and coagulant capacity. For the RF method this effect is most pronounced, although to some extent compensated by a smaller volume loss.

Update on the use of pathogen-reduced human plasma and platelet concentrates.

Seltsam A, Müller TH.

The use of pathogen reduction technologies (PRTs) for labile blood components is slowly but steadily increasing. While pathogen-reduced plasma is already used routinely, efficacy and safety concerns impede the widespread use of pathogen-reduced platelets. The supportive and often prophylactic nature of blood component therapy in a variety of clinical situations complicates the clinical evaluation of these novel blood products. However, an increasing body of evidence on the clinical efficacy, safety, cost-benefit ratio and development of novel technologies suggests that pathogen reduction has entered a stage of maturity that could further increase the safety margin in haemotherapy. This review summarizes the clinical evidence on PRTs for plasma and platelet products that are currently licensed or under development.
A regional haemovigilance retrospective study of four types of therapeutic plasma in a ten-year survey period in France.

Bost V, Odent-Malaure H, Chavarin P, Benamara H, Fabrigli F & Garraud O.

Two pathogen reduction technologies-methylene blue plus light and shortwave ultraviolet light-effectively inactivate hepatitis.

Steinmann E, Gravemann U, Friesland M, Doerbeck J, Müller TH, Pietschmann T, Seltsam A.

BACKGROUND:
Contamination of blood products with hepatitis C virus (HCV) can cause infections resulting in acute and chronic liver diseases. Pathogen reduction methods such as photodynamic treatment with methylene blue (MB) plus visible light as well as irradiation with shortwave ultraviolet (UVC) light were developed to inactivate viruses and other pathogens in plasma and platelet concentrates (PCs), respectively. So far, their inactivation capacities for HCV have only been tested in inactivation studies using model viruses for HCV. Recently, a HCV infection system for the propagation of infectious HCV in cell culture was developed. Contamination of blood products with hepatitis.

STUDY DESIGN AND METHODS:
Inactivation studies were performed with cell culture-derived HCV and bovine viral diarrhea virus (BVDV), a model for HCV. Plasma units or PCs were spiked with high titers of cell culture-grown viruses. After treatment of the blood units with MB plus light (Theraflex MB-Plasma system, MacoPharma) or UVC (Theraflex UV-Platelets system, MacoPharma), residual viral infectivity was assessed using sensitive cell culture systems.

RESULTS:
HCV was sensitive to inactivation by both pathogen reduction procedures. HCV in plasma was efficiently inactivated by MB plus light below the detection limit already by 1/12 of the full light dose. HCV in PCs was inactivated by UVC irradiation with a reduction factor of more than 5 log. BVDV was less sensitive to the two pathogen reduction methods.

CONCLUSIONS:
Functional assays with human HCV offer an efficient tool to directly assess the inactivation capacity of pathogen reduction procedures. Pathogen reduction technologies such as MB plus light treatment and UVC irradiation have the potential to significantly reduce transfusion-transmitted HCV infections.
Storage of thawed plasma for a liquid plasma bank: impact of temperature and methylene blue pathogen inactivation.


BACKGROUND:
Rapid transfusion of fresh-frozen plasma (FFP) is desired for treating coagulopathies, but thawing and issuing of FFP takes more than 40 minutes. Liquid storage of plasma is a potential solution but uncertainties exist regarding clotting factor stability. We assessed different storage conditions of thawed FFP and plasma treated by methylene blue plus light (MB/light) for pathogen inactivation.

STUDY DESIGN AND METHODS:
Fifty thawed apheresis plasma samples (approx. 750 mL) were divided into three subunits and either stored for 7 days at 4°C, at room temperature (RT), and at 4°C after MB/light treatment. Clotting factor activities (Factor [F] II, FV, FVII through FXIII, fibrinogen, antithrombin, von Willebrand factor antigen, Protein C and S) were assessed after thawing and on Days 3, 5, and 7. Changes were classified as “minor” (activities within the reference range) and “major” (activities outside the reference range).

RESULTS:
FFP storage at 4°C revealed major changes for FVIII (median [range], 56% [33%-114%]) and Protein S (51% [20%-88%]). Changes were more pronounced when plasma was stored at RT (FVIII, 59% [37%-123%]; FV, 69% [42%-125%]; Protein S, 20% [10%-35%]). MB/light treatment of thawed FFP resulted in minor changes. However, further storage for 7 days at 4°C revealed major decreases for FVIII (47% [12%-91%]) and Protein S (49% [18%-95%]) and increases for FVII (150% [48%-285%]) and FX (126% [62%-206%]).

CONCLUSION:
Storage of liquid plasma at 4°C for 7 days is feasible for FFP as is MB/light treatment of thawed plasma. In contrast, storage of thawed plasma for 7 days at RT or after MB/light treatment at 4°C affects clotting factor stability substantially and is not recommended.

THERAFLEX UV-Platelets scientific publications

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**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. Transfusion transmission of the yellow fever vaccine virus has been demonstrated, which is indicative of the potential for viral transfusion transmission. An approach to manage the potential YFV transmission risk is the use of pathogen inactivation (PI) technology systems, such as THERAFLEX MB-Plasma and THERAFLEX UV-Platelets (Macopharma). We aimed to investigate the efficacy of these PI technology systems to inactivate YFV in plasma or platelet concentrates (PCs).

**STUDY DESIGN AND METHODS:**

YFV 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 (approx. 0.8 μmol/L) and spiked PCs were treated using THERAFLEX UV-Platelets system (ultraviolet C doses: 0.05, 0.10, 0.15, and 0.20 [standard] J/cm²). Samples were taken before the first and after each illumination dose and tested for residual virus using a modified plaque assay.

**RESULTS:**

YFV infectivity was reduced by an average of 4.77 log or greater in plasma treated with ultraviolet C light. Inactivated YFV was also efficiently inactivated in platelet concentrates following treatment with ultraviolet C light.

**CONCLUSIONS:**

Our study suggests the THERAFLEX MB-Plasma and the THERAFLEX UV-Platelets systems can efficiently inactivate YFV in plasma or PCs to a similar degree as that for other arboviruses. Given the reduction levels observed in this study, these PI technology systems could be an effective option for managing YFV transfusion-transmission risk in plasma and PCs.
BACKGROUND AND OBJECTIVES:
Refrigeration (cold-storage) of pathogen inactivated (PI) platelet components may increase the shelf-life and safety profile of platelet components, compared to conventional room-temperature (RT) storage. Whilst there is substantial knowledge regarding the impact of these individual treatments on platelets, the combined effect has not been assessed.

MATERIALS AND METHODS:
Using a pool-and-split study design, paired buffy-coat derived platelets in 70% platelet additive solution (SSP+; MacoPharma) were left untreated or PI-treated using the THERAFLEX UV-Platelets System (UVC; MacoPharma). Units from each pair were split and stored at room temperature (20-24°C) or cold-stored (2-6°C) to yield RT, cold, RT-UVC and cold-UVC study groups (n = 8 in each group). In vitro quality and function was tested over 9 days.

RESULTS:
Cold-storage of UVC-treated platelets reduced glycolytic metabolism (glucose consumption and lactate production) compared to RT-UVC units. Cold-UVC platelets demonstrated complete abrogation of HSR by day 5, increased externalisation of phosphatidylserine (annexin-V binding) and activation of the GPIIb/IIIa receptor (PAC-1 binding) above the levels observed with the individual treatments. Aggregation responses (ADP and collagen) were enhanced in the cold-UVC platelets compared to both RT groups, but this was primarily mediated by cold-storage. Haemostatic function, as measured using TEG, was similar between the groups.

CONCLUSIONS:
Cold-storage of UVC-treated platelets reduced PI-induced acceleration of glycolytic metabolism. However, combining cold-storage and UVC-treatment resulted in additional phenotypic changes compared to each treatment individually. Further work is required to understand the impact of these changes in clinical efficacy.

Bacterial inactivation of platelet concentrates with the THERAFLEX UV-Platelets pathogen inactivation system.

Gravemann U, Handke W, Müller TM, Seilsam A.

BACKGROUND:
The THERAFLEX UV-Platelets system (Maco Pharma) uses ultraviolet C (UVC) light for pathogen inactivation (PI) of platelet concentrates (PCs) without any additional photoactive compound. The aim of the study was to systematically investigate bacterial inactivation with this system under conditions of intended use.

STUDY DESIGN AND METHODS:
The robustness of the system was evaluated by assessing its capacity to inactivate high concentrations of different bacterial species in accordance with World Health Organization guidelines. The optimal use of the PI system was explored in time-to-treatment experiments by testing its ability to sterilize PCs contaminated with low levels of bacteria on the day of manufacture (target concentration, 100 colony-forming units/unit). The bacteria panel used for spiking experiments in this study included the World Health Organization International Repository Platelet Transfusion Relevant Reference Strains (n = 14), commercially available strains (n = 13), and in-house clinical isolates (n = 2).

RESULTS:
Mean log reduction factors after UVC treatment ranged from 3.1 to 7.5 and varied between different strains of the same species. All PCs (n = 12/species) spiked with up to 200 colony-forming units/bag remained sterile until the end of storage when UVC treated 6 hours after spiking. UVC treatment 8 hours after spiking resulted in single breakthrough contaminations with the fast-growing species Escherichia coli and Streptococcus pyogenes.

CONCLUSION:
The UVC-based THERAFLEX UV-Platelets system efficiently inactivates transfusion-relevant bacterial species in PCs. The comprehensive data from this study may provide a valuable basis for the optimal use of this UVC-based PI system.
Ultraviolet C light efficiently inactivates nonenveloped hepatitis A virus and feline calicivirus in platelet concentrates.


BACKGROUND:
Nonenveloped transfusion-transmissible viruses such as hepatitis A virus (HAV) and hepatitis E virus (HEV) are resistant to many of the common virus inactivation procedures for blood products. This study investigated the pathogen inactivation (PI) efficacy of the THERAFLEX UV-Platelets system against two nonenveloped viruses: HAV and feline calicivirus (FCV), in platelet concentrates (PCs).

STUDY DESIGN AND METHODS:
PCs in additive solution were spiked with high titers of cell culture-derived HAV and FCV, and treated with ultraviolet C at various doses. Pre- and posttreatment samples were taken and the level of viral infectivity determined at each dose. For some samples, large-volume plating was performed to improve the detection limit of the virus assay.

RESULTS:
THERAFLEX UV-Platelets reduced HAV titers in PCs to the limit of detection, resulting in a virus reduction factor of greater than 4.2 log steps, and reduced FCV infectivity in PCs by 3.0 ± 0.2 log steps.

CONCLUSIONS:
THERAFLEX UV-Platelets effectively inactivates HAV and FCV in platelet units.

Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively.


BACKGROUND:
Ebola virus (EBOV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have been identified as potential threats to blood safety. This study investigated the efficacy of the THERAFLEX UV-Platelets and THERAFLEX MB-Plasma pathogen inactivation systems to inactivate EBOV and MERS-CoV in platelet concentrates (PCs) and plasma, respectively.

STUDY DESIGN AND METHODS:
PCs and plasma were spiked with high titers of cell culture-derived EBOV and MERS-CoV, treated with various light doses of ultraviolet C (UVC; THERAFLEX UV-Platelets) or methylene blue (MB) plus visible light (MB/light; THERAFLEX MB-Plasmas), and assessed for residual viral infectivity.

RESULTS:
UVC reduced EBOV (≥ 4.5 log) and MERS-CoV (≥ 3.7 log) infectivity in PCs to the limit of detection, and MB/light decreased EBOV (≥ 4.6 log) and MERS-CoV (≥ 3.3 log) titers in plasma to nondetectable levels.

CONCLUSION:
Both THERAFLEX UV-Platelets (UVC) and THERAFLEX MB-Plasma (MB/light) effectively reduce EBOV and MERS-CoV infectivity in platelets and plasma, respectively.
Mitochondrial DNA multiplex real-time polymerase chain reaction inhibition assay for quality control of pathogen inactivation by ultraviolet C light in platelet concentrates.


BACKGROUND:
Several ultraviolet (UV) light-based pathogen inactivation (PI) technologies for platelet (PLT) products have been developed or are under development. Upon implementation of PI technologies, quality control measures are required to ensure consistent efficiency of the treatment process. Previous reports showed that amotosalen/UVA and riboflavin/UV based PI technologies induce modifications of the PLT derived mitochondrial DNA (mtDNA) that can be detected by polymerase chain reaction (PCR) inhibition assays. In this study, we sought to establish a PCR inhibition assay to document the impact of ultraviolet C (UVC) treatment with the THERAFLEX UV-Platelets system on the mitochondrial genome in PLT concentrates (PCs).

STUDY DESIGN AND METHODS:
A multiplex realtime PCR inhibition assay with simultaneous short-amplicon (143 bp) and long-amplicon (794 bp) amplification was developed to detect mtDNA modifications in PLTs after UVC treatment. Assay performance was tested in UVC-treated and untreated, plasma-reduced pooled PCs, and apheresis PCs and challenged using PCs manufactured for a clinical trial under routine-like conditions.

RESULTS:
UVC illumination of PLTs resulted in dose dependent inhibition of mtDNA amplification for the larger amplicon. Amplification of the shorter amplicon was not affected by UVC treatment. Evaluation of 283 blinded apheresis and pooled PLT samples from routine-like PC production resulted in prediction of UVC treatment status with 100% accuracy.

CONCLUSIONS:
The proposed dual-amplicon size real-time mtDNA PCR assay effectively detects nucleic acid damage induced by UVC illumination of PLTs and could be useful as an informative indicator of PI quality of the THERAFLEX UV-Platelets system.
Reduction of Zika virus infectivity in platelet concentrates after treatment with ultraviolet C light and in plasma after treatment with methylene blue and visible light.


Transfusion 2017; 57(11):2677-2682.

BACKGROUND:
Zika virus (ZIKV) has emerged as a potential threat to transfusion safety worldwide. Pathogen inactivation is one approach to manage this risk. In this study, the efficacy of the THERAFLEX UV-Platelets system and THERAFLEX MB-Plasma system to inactivate ZIKV in platelet concentrates (PCs) and plasma was investigated.

STUDY DESIGN AND METHODS:
PCs spiked with ZIKV were treated with the THERAFLEX UV-Platelets system at 0.05, 0.10, 0.15, and 0.20 J/cm² UVC. Plasma spiked with ZIKV was treated with the THERAFLEX MB-Plasma system at 20, 40, 60, and 120 J/cm² light at 630 nm with at least 0.8 mmol/L methylene blue (MB). Samples were taken before the first and after each illumination dose and tested for residual virus. For each system the level of viral reduction was determined.

RESULTS:
Treatment of PCs with THERAFLEX UV-Platelets system resulted in a mean of 5 log reduction in ZIKV infectivity at the standard UVC dose (0.20 J/cm²), with dose dependency observed with increasing UVC dose. For plasma treated with MB and visible light, ZIKV infectivity was reduced by a mean of at least 5.68 log, with residual viral infectivity reaching the detection limit of the assay at 40 J/cm² (one-third the standard dose).

CONCLUSION:
Our study demonstrates that the THERAFLEX UV-Platelets system and THERAFLEX MB-Plasma system can reduce ZIKV infectivity in PCs and pooled plasma to the detection limit of the assays used. These findings suggest both systems have the capacity to be an effective option to manage potential ZIKV transfusion transmission risk.

In vitro Quality of Platelets with Low Plasma Carryover Treated with Ultraviolet C Light for Pathogen Inactivation.


BACKGROUND:
The THERAFLEX UV-Platelets system uses shortwave ultraviolet C light (UVC, 254 nm) to inactivate pathogens in platelet components. Plasma carryover influences pathogen inactivation and platelet quality following treatment. The plasma carryover in the standard platelets produced by our institution are below the intended specification (<30%).

METHODS:
A pool and split study was carried out comparing untreated and UVC-treated platelets with <30% plasma carryover (n = 10 pairs). This data was compared to components that met specifications (>30% plasma). The platelets were tested over storage for in vitro quality.

RESULTS:
Platelet metabolism was accelerated following UVC treatment, as demonstrated by increased glucose consumption and lactate production. UVC treatment caused increased externalization of phosphatidylserine on platelets and microparticles, activation of the GPIIb/IIIa receptor (PAC-1 binding), and reduced hypotonic shock response. Platelet function, as measured with thrombelastogram, was not affected by UVC treatment. Components with <30% plasma were similar to those meeting specification with the exception of enhanced glycolytic metabolism.

CONCLUSIONS:
This in vitro analysis demonstrates that treatment of platelets with <30% plasma carryover with the THERAFLEX UV-Platelets system affects some aspects of platelet metabolism and activation, although in vitro platelet function was not negatively impacted. This study also provides evidence that the treatment specifications of plasma carryover could be extended to below 30%.
Effect of increased agitation speed on pathogen inactivation efficacy and *in vitro* quality in UVC-treated platelet concentrates.

Van der Meer PF, Gravemann U, de Korte D, Sumian C, Toksdorf F, Muller TH and Seltsam A.

Vox Sang 2016; 111(2):127-34.

**BACKGROUND:**
Pathogen inactivation technologies require continuous development for adjustment to different blood components and products. With Theraflex UV-Platelets, a system using shortwave ultraviolet C (UVC) light (254 nm), efficient mixing of platelet concentrates (PCs) during UVC treatment is essential to ensure homogeneous illumination of the blood components. In this study, we investigated the impact of increasing the agitation speed during UVC treatment on pathogen inactivation capacity and platelet quality.

**MATERIAL AND METHODS:**
The pathogen inactivation efficacy of UVC treatment was evaluated at two agitation speeds (110 vs. 180 rpm) using four different transfusion-relevant bacteria strains and three model viruses. Using a pool-and-split design, the *in vitro* quality of buffy coat-derived PCs stored in SSP+ additive solution for up to 7 days was assessed in UVC-treated PCs agitated at either 110 rpm (standard speed) or 180 rpm (increased speed) and in untreated controls.

**RESULTS:**
The higher agitation speed improved bacterial inactivation but did not influence viral inactivation. Metabolic activity (glucose consumption and lactate accumulation) in UVC-treated platelets was slightly higher than in untreated controls. Increases in parameters such as CD62P expression and annexin A5 binding indicated moderate activation of UVC-treated platelets. Quality variables for UVC treated platelets agitated at standard vs. increased agitation speed were comparable.

**CONCLUSION:**
The mixing rate during illumination may be a process parameter for further development of UVC-based pathogen inactivation procedures for PLT concentrates.

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Inactivation of dengue, chikungunya, and Ross River viruses in platelet concentrates after treatment with ultraviolet C light.


**BACKGROUND:**
Arboviruses, including dengue (DENV 1-4), chikungunya (CHIKV), and Ross River (RRV), are emerging viruses that are a risk for transfusion safety globally. An approach for managing this risk is pathogen inactivation, such as the THERAFLEX UV-Platelets system. We investigated the ability of this system to inactivate the above mentioned arboviruses.

**STUDY DESIGN AND METHODS:**
DENV 1-4, CHIKV, or RRV were spiked into buffy coat (BC)-derived platelet (PLT) concentrates in additive solution and treated with the THERAFLEX UV-Platelets system at the following doses: 0.05, 0.1, 0.15, and 0.2 J/cm² (standard dose). Pre- and posttreatment samples were taken for each dose, and the level of viral infectivity was determined.

**RESULTS:**
At the standard ultraviolet C (UVC) dose (0.2 J/cm²), viral inactivation of at least 4.43, 6.34, and 5.13 log or more, was observed for DENV 1-4, CHIKV, and RRV, respectively. A dose dependency in viral inactivation was observed with increasing UVC doses.

**CONCLUSIONS:**
Our study has shown that DENV, CHIKV, and RRV, spiked into BC-derived PLT concentrates, were inactivated by the THERAFLEX UV-Platelets system to the limit of detection of our assay, suggesting that this system could contribute to the safety of PLT concentrates with respect to these emerging arboviruses.
Tolerance of platelet concentrates treated with UVC-light only for pathogen reduction - a phase I clinical trial.


BACKGROUND:
The THERAFLEX UV-Platelets pathogen reduction system for platelet concentrates (PCs) operates with ultraviolet C light (UVC; 254 nm) only without addition of photosensitizers. This phase I study evaluated safety and tolerability of autologous UVC-irradiated PCs in healthy volunteers.

METHODS:
Eleven volunteers underwent two single (series 1 and 2) and one double apheresis (series 3). PCs were treated with UVC, stored for 48 h and retransfused in a dose-escalation scheme: 12.5, 25% and 50% of a PC (series 1); one complete PC (series 2); two PCs (series 3). Platelet counts, fibrinogen, activated partial thromboplastin time, prothrombin time, D-dimer, standard haematology, temperature, heart rate, blood pressure and clinical chemistry parameters were measured. One- and 24-h corrected count increments were determined in series 2 and 3. Platelet-specific antibodies were assessed before and at the end of the study.

RESULTS:
Neither adverse reactions related to transfusions nor antibodies against UVC-treated platelets were observed. Corrected count increments did not differ between series 2 and 3.

CONCLUSIONS:
Repeated transfusions of autologous UVC-treated PCs were well tolerated and did not induce antibody responses in all volunteers studied. EudraCT No. 2010-023404-26.

Ultraviolet c light pathogen inactivation treatment of platelet concentrates preserves integrin activation but affects thrombus formation kinetics on collagen in vitro.

Van Aelst B, Devloo B, Vandekerckhove P, Compernolle V, Fays HB.

BACKGROUND:
Ultraviolet (UV) light illumination in the presence of exogenously added photosensitizers has been used to inactivate pathogens in platelet (PLT) concentrates for some time. The THERAFLEX UV-C system, however, illuminates PLT concentrates with UV-C light without additional photoactive compounds. In this study residual PLT function is measured in a comprehensive paired analysis of UV-C-treated, gamma-irradiated, and untreated control PLT concentrates.

STUDY DESIGN AND METHODS:
A pool-and-split design was used with buffy coat-derived PLT concentrates in 65% SSP+ additive solution. Thrombus formation kinetics in microfluidic flow chambers onto immobilized collagen was investigated with real-time video microscopy. PLT aggregation, membrane markers, and cellular metabolism were determined concurrently.

RESULTS:
Compared to gamma-treated and untreated controls, UV-C treatment significantly affected thrombus formation rates on Days 5 and 7, not Day 2. PLT degranulation (P-selectin) and PLT apoptosis (annexin V binding) was slightly but significantly increased from Day 2 on. UV-C treatment moreover induced integrin alpha-IIb beta3 conformational changes reminiscent of activation. However, subsequent integrin activation by either PAR1-activating hexapeptide (PAR1AP) or convulxin was unaffected. This was confirmed by PLT aggregation studies induced with collagen, PAR1AP, and ristocetin at two different agonist concentrations. Finally, UV-C slightly increased lactic acid production rates, resulting in significantly decreased pH on Days 5 and 7, but never dropped below 7.2.

CONCLUSION:
UV-C pathogen inactivation treatment slightly but significantly increases PLT activation markers but does not profoundly influence activability nor aggregation. The treatment does, however, attenuate thrombus formation kinetics in vitro in microfluidic flow chambers, especially after storage.
Pathogen reduction by ultraviolet C light effectively inactivates human white blood cells in platelet products.


BACKGROUND:
Residual white blood cells (WBCs) in cellular blood components induce a variety of adverse immune events, including nonhemolytic febrile transfusion reactions, alloimmunization to HLA antigens, and transfusion-associated graft-versus-host disease (TA-GVHD). Pathogen reduction (PR) methods such as the ultraviolet C (UVC) light-based THERAFLEX UV-Platelets system were developed to reduce the risk of transfusion-transmitted infection. As UVC light targets nucleic acids, it interferes with the replication of both pathogens and WBCs. This preclinical study aimed to evaluate the ability of UVC light to inactivate contaminating WBCs in platelet concentrates (PCs).

STUDY DESIGN AND METHODS:
The in vitro and in vivo function of WBCs from UVC-treated PCs was compared to that of WBCs from gamma-irradiated and untreated PCs by measuring cell viability, proliferation, cytokine secretion, antigen presentation in vitro, and xenogeneic GVHD responses in a humanized mouse model.

RESULTS:
UVC light was at least as effective as gamma irradiation in preventing GVHD in the mouse model. It was more effective in suppressing T-cell proliferation (>5-log reduction in the limiting dilution assay), cytokine secretion, and antigen presentation than gamma irradiation.

CONCLUSIONS:
The THERAFLEX UV-Platelets (MacoPharma) PR system can substitute gamma irradiation for TA-GVHD prophylaxis in platelet (PLT) transfusion. Moreover, UVC treatment achieves suppression of antigen presentation and inhibition of cytokine accumulation during storage of PCs, which has potential benefits for transfusion recipients.

In vitro function of platelets treated with ultraviolet C light for pathogen inactivation: a comparative study with non-irradiated and gamma-irradiated platelets.


BACKGROUND:
During storage of platelet concentrates (PCs) replication of contaminating pathogens might occur, which can be prevented by various pathogen inactivation (PI) methods using photoactive substances in combination with ultraviolet (UV) light. A new method uses only UVC light for PI without photoactive substances. This study evaluates the in vitro function, including hemostatic properties (clot formation and elasticity), of platelets (PLTs) treated with UVC light.

STUDY DESIGN AND METHODS:
A PC with 35% plasma and 65% PLT additive solution (SSP+) was prepared from five buffy coats. Three PCs were pooled and divided into 3 units. One unit was used as a non-irradiated control, the second was a gamma-irradiated control, and the third unit was treated with UVC light. In vitro variables including analysis of coagulation by free oscillation rheometry were analysed on Days 1, 5, and 7 of storage. Ten units in each group were investigated.

RESULTS:
Swirling was well preserved, and the pH level was higher than the reference limit (6.4) during storage of PLTs in all groups. Glycolysis and PLT activation were higher for UVC-treated PLTs but the clot-forming capacity was unaffected. However, immediately after UVC treatment, the clot elastic properties were slightly affected. Hypotonic shock response decreased immediately after UVC treatment but recovered partly during the storage period.

CONCLUSION:
UVC treatment affected the in vitro properties, but PLT quality and storage stability were well preserved for up to 7 days, and the in vitro hemostatic capacity of UVC-treated PLTs was only minimally altered. The clinical relevance of these changes needs to be evaluated in controlled trials.
Proteome changes in platelets after pathogen inactivation--an interlaboratory consensus.

Prudent, M, D’Alessandro, A, Cazenave, JP, Devine, DV, Gachet, C, Greinacher, A, Zolla L.

Pathogen inactivation (PI) of platelet concentrates (PCs) reduces the proliferation/replication of a large range of bacteria, viruses, and parasites as well as residual leucocytes. Pathogen-inactivated PCs were evaluated in various clinical trials showing their efficacy and safety. Today, there is some debate over the hemostatic activity of treated PCs as the overall survival of PI platelets seems to be somewhat reduced, and in vitro measurements have identified some alterations in platelet function. Although the specific lesions resulting from PI of PCs are still not fully understood, proteomic studies have revealed potential damages at the protein level. This review merges the key findings of the proteomic analyses of PCs treated by the Mirasol Pathogen Reduction Technology, the Intercept Blood System, and the Theraflex UV-C system, respectively, and discusses the potential impact on the biological functions of platelets. The complementarities of the applied proteomic approaches allow the coverage of a wide range of proteins and provide a comprehensive overview of PI-mediated protein damage. It emerges that there is a relatively weak impact of PI on the overall proteome of platelets. However, some data show that the different PI treatments lead to an acceleration of platelet storage lesions, which is in agreement with the current model of platelet storage lesion in pathogen-inactivated PCs. Overall, the impact of the PI treatment on the proteome appears to be different among the PI systems. Mirasol impacts adhesion and platelet shape change, whereas Intercept seems to impact proteins of intracellular platelet activation pathways. Theraflex influences platelet shape change and aggregation, but the data reported to date are limited. This information provides the basis to understand the impact of different PI on the molecular mechanisms of platelet function. Moreover, these data may serve as basis for future developments of PI technologies for PCs. Further studies should address the impact of both the PI and the storage duration on platelets in PCs because PI may enable the extension of the shelf life of PCs by reducing the bacterial contamination risk.

Pathogen inactivation technologies for cellular blood components: an update.

Schlenke, P.

Nowadays patients receiving blood components are exposed to much less transfusion-transmitted infectious diseases than three decades before when among others HIV was identified as causative agent for the acquired immunodeficiency syndrome and the transmission by blood or coagulation factors became evident. Since that time the implementation of measures for risk prevention and safety precaution was socially and politically accepted. Currently emerging pathogens like arboviruses and the well-known bacterial contamination of platelet concentrates still remain major concerns of blood safety with important clinical consequences, but very rarely with fatal outcome for the blood recipient. In contrast to the well-established pathogen inactivation strategies for fresh frozen plasma using the solvent-detergent procedure or methylene blue and visible light, the bench-to-bedside translation of novel pathogen inactivation technologies for cell-containing blood components such as platelets and red blood cells is still underway. This review summarizes the pharmacological/toxicological assessment and the inactivation efficacy against viruses, bacteria, and protozoa of each of the currently available pathogen inactivation technologies and highlights the impact of the results obtained from several randomized clinical trials and hemovigilance data. Until now in some European countries pathogen inactivation technologies are in routine use for single-donor plasma and platelets. The invention and adaptation of pathogen inactivation technologies for red blood cell units and whole blood donations suggest the universal applicability of these technologies and foster a paradigm shift in the manufacturing of safe blood.
The efficacy of the ultraviolet C pathogen inactivation system in the reduction of Babesia divergens in pooled buffy coat platelets.


BACKGROUND:
Babesia spp. is an intraerythrocytic parasite that causes human babesiosis and its transmission by transfusion has been extensively demonstrated. The aim of this study was to ascertain the efficacy of an ultraviolet C (UVC)-based pathogen inactivation system in the reduction of Babesia divergens–infected platelet (PLT) concentrates and to determine the parasite’s ability to survive in PLT concentrates stored under blood bank conditions.

STUDY DESIGN AND METHODS:
This study was conducted using in vitro cultures of B. divergens. The detection limit of the culture assay was established and, subsequently, 15 buffy coat–derived PLT concentrates (BC-PCs) were inoculated with 1.07 B. divergens–infected red blood cells. Infected BC-PCs were irradiated with 0.2 J/cm² UVC light using the THERAFLEX UV-Platelets method (Macopharma). Viability and parasite growth were evaluated before and after inactivation. Culture growth kinetics were monitored by DNA incorporation of [3H] thymidine. The ability of B. divergens to survive in PLT concentrates was also analysed.

RESULTS:
The limit of detection in cultures was established at 0.1 × 10−6% parasites. The THERAFLEX UV-Platelets system inactivated B. divergens to below the limit of detection in 12 of 15 BC-PCs (log reduction, >6.0) and to the limit of detection (log reduction, 5.0) in three of 15. It was also demonstrated that B. divergens remains viable in BC-PCs stored up to 7 days.

CONCLUSION:
Since B. divergens can survive in PLT concentrates and given the performance of UVC, this system could be considered as an alternative to prevent B. divergens and other Babesia species from being transmitted through PLT transfusions.

Two pathogen reduction technologies-methylene blue plus light and shortwave ultraviolet light-effectively inactivate hepatitis C virus in blood products.


BACKGROUND:
Contamination of blood products with hepatitis C virus (HCV) can cause infections resulting in acute and chronic liver diseases. Pathogen reduction methods such as photodynamic treatment with methylene blue (MB) plus visible light as well as irradiation with shortwave ultraviolet (UVC) light were developed to inactivate viruses and other pathogens in plasma and platelet concentrates (PCs), respectively. So far, their inactivation capacities for HCV have only been tested in inactivation studies using model viruses for HCV. Recently, a HCV infection system for the propagation of infectious HCV in cell culture was developed. Contamination of blood products with hepatitis.

STUDY DESIGN AND METHODS:
Inactivation studies were performed with cell culture-derived HCV and bovine viral diarrhea virus (BVDV), a model for HCV. Plasma units or PCs were spiked with high titers of cell culture-grown viruses. After treatment of the blood units with MB plus light (Theraflex MB-Plasma system, MacoPharma) or UVC (Theraflex UV-Platelets system, MacoPharma), residual viral infectivity was assessed using sensitive cell culture systems.

RESULTS:
HCV was sensitive to inactivation by both pathogen reduction procedures. HCV in plasma was efficiently inactivated by MB plus light below the detection limit already by 1/12 of the full light dose. HCV in PCs was inactivated by UVC irradiation with a reduction factor of more than 5 log. BVDV was less sensitive to the two pathogen reduction methods.

CONCLUSIONS:
Functional assays with human HCV offer an efficient tool to directly assess the inactivation capacity of pathogen reduction procedures. Pathogen reduction technologies such as MB plus light treatment and UVC irradiation have the potential to significantly reduce transfusion-transmitted HCV infections.
Update on the use of pathogen-reduced human plasma and platelets concentrates.


The use of pathogen reduction technologies (PRTs) for labile blood components is slowly but steadily increasing. While pathogen-reduced plasma is already used routinely, efficacy and safety concerns impede the widespread use of pathogen-reduced platelets. The supportive and often prophylactic nature of blood component therapy in a variety of clinical situations complicates the clinical evaluation of these novel blood products. However, an increasing body of evidence on the clinical efficacy, safety, cost-benefit ratio and development of novel technologies suggests that pathogen reduction has entered a stage of maturity that could further increase the safety margin in haemotherapy. This review summarizes the clinical evidence on PRTs for plasma and platelet products that are currently licensed or under development.

Pathogen inactivation of platelets using ultraviolet C light: effect on in vitro function and recovery and survival of platelets.


BACKGROUND:
We evaluated the effect of treating platelets (PLTs) using ultraviolet (UV)C light without the addition of any photosensitizing chemicals on PLT function in vitro and PLT recovery and survival in an autologous radiolabelled volunteer study.

STUDY DESIGN AND METHODS:
For in vitro studies, pooled or single buffy coat-derived PLT concentrates (PCs) were pooled and split to obtain identical PCs that were either treated with UVC or untreated (n = 6 each) and stored for 7 days. PLT recovery and survival were determined in a two-arm parallel autologous study in healthy volunteers performed according to BEST guidelines. UVC-treated or untreated PCs (n = 6 each) were stored for 5 days and were compared to fresh PLTs from the same donor.

RESULTS:
There were no significant differences on Day 7 of storage between paired UVC-treated and control PC units for pH, adenosine triphosphate, lactate dehydrogenase, CD62P, CD63, PLT microparticles, and JC-1 binding, but annexin V binding, lactate accumulation, and expression of CD41/61 were significantly higher in treated units (p < 0.05). Compared with control units, the recovery and survival of UVC-treated PC were reduced after 5 days of storage (p < 0.05) and when expressed as a percentage of fresh values, survival was reduced by 20% (p = 0.005) and recovery by 17% (p = 0.088).

CONCLUSION:
UVC-treated PLTs stored for 5 days showed marginal changes in PLT metabolism and activation in vitro and were associated with a degree of reduction in recovery and survival similar to other pathogen inactivation systems that are licensed and in use.
Characteristics of the THERAFLEX UV-Platelets pathogen inactivation system - An update.

Seghatchian J, Tolkendorff R

Considerable progress has been made in the last decade in producing purer, safer, leucocyte and plasma reduced platelet concentrates (PC) with an extended shelf life. The development of different pathogen inactivation technologies (PIT) has made a substantial contribution to this trend. Preceding platelet PIT (INTERCEPT Blood System/ Ceras Corporation, Concord, CA, USA; MIRASOL/Caridian BCT, Lakewood, CO, USA) are based on adding a photosensitive compound to PC. The mixture is then activated by UV light in the UVB and/or UVA spectral regions. A novel procedure, THERAFLEX UV-Platelets (MacoPharma, Mouvaux, France), was recently developed that uses short-wave ultraviolet light (UVC), without addition of any photoactive agent. This technology has proven to be highly effective in sterilising bacteria (the major cause of morbidity/mortality after platelet transfusion) as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT反映平衡之间的效果和耐久性，以及存储血浆中的蛋白质含量。此外，还有一些新方法，如基于免疫印迹的蛋白质分析，表明该技术在预clinical研究中，验证了其在临床上的可行性和安全性。这为更好地预测临床结果和预测结果提供了支持。

CONCLUSIONS: Repeated transfusions of autologous UVC-treated PCs were well tolerated in all dogs studied. UVC irradiation did not cause systemic or local intolerance. Few but significant changes in PLT integrity were observed after UVC irradiation. Even after repeated administration of UVC-irradiated PCs, no antibodies against UVC-exposed plasma or PLT proteins were detected.
**Cold storage of platelets in additive solution: the impact of residual plasma in apheresis platelet concentrates**


**ABSTRACT:** Platelet transfusion became an indispensable therapy in the modern medicine. Although the clinical advantage of platelet transfusion is well established, adverse reactions upon transfusion, especially transmission of bacterial infection, still represent a major challenge. While bacterial contamination is favored by the storage of platelets at room temperature, cold storage may represent a solution for this clinically relevant issue. In this study, we aimed to elucidate whether plasma has protective or detrimental effects on cold-stored platelets. We investigated the impact of different residual plasma contents in apheresis-derived platelet concentrates, stored at 4°C or room temperature, on platelet function and survival. We found that platelets stored at 4°C have higher expression of apoptosis marker compared to room temperature-stored platelets leading to an accelerated clearance from the circulation in a humanized animal model. While cold-induced apoptosis was independent of the residual plasma concentration, cold storage was associated with better adhesive properties and higher response to activators. Of note, delta granule-related functions, such as ADP-mediated aggregation and CD63 release, were better preserved at 4°C especially in 100% plasma. An extended study to assess cold-stored platelet concentrates produced under standard care GMP conditions showed that platelet function, metabolism and integrity were better compared to those stored at room temperature. Taken together, our results show that residual plasma concentration does not have a cardinal impact on the cold storage responses of apheresis-derived platelet concentrates and indicates that the current generation of additive solutions is suitable substitutes for plasma to store platelets at 4°C.
Platelet Additive Solutions: A review of the latest developments and their Clinical implications
Van der Meer, P. F. and D. de Korte.

SUMMARY:
Platelet additive solutions (PASs) have undergone many reformulations in order to further improve platelet storage. Studies of platelets stored in PAS-F (containing acetate, magnesium and potassium as key constituents) showed that platelets may be stored for 13 days with recovery and survival outcomes that are equal or even superior to 7-day stored platelets in plasma. Clinically, patients transfused with platelets in PAS have fewer allergic reactions, while for febrile reactions data are conflicting. Transfusion-related acute lung injury (TRALI) occurs less frequently if PAS is used for buffy coat-derived platelets, but for apheresis platelets there is no difference. For PAS-B and PAS-C, corrected count increments (CCIs) are lower than for platelets stored in plasma, but for PAS-E (like PAS-F also with acetate, magnesium and potassium but with additional phosphate), though limited data is available in the literature, the CCIs seem to be comparable to those observed for platelets in plasma. With platelets in PAS, there is an accumulated dilution effect of anticoagulant and PAS as well as a loss of number and function (due to storage and/or pathogen inactivation treatment) of platelets, of which it is not clear how this impacts clinical outcomes of patients undergoing massive transfusion. Worst-case in vitro studies, where the entire plasma fraction is replaced by supernatant of platelets in PAS, do show an effect on the ability of reconstituted whole blood to clot, but in a more realistic scenario, functional clotting parameters are not different. In this review, recent laboratory and clinical data are discussed, focusing on studies published after 2010.

Maximising platelet availability by delaying cold storage
Wood B, Johnson L, Hyland R. A. & Marks D. C.
Vox Sanguinis 2018. doi: 10.1111/vox.12649

BACKGROUND:
Cold-stored platelets may be an alternative to conventional room temperature (RT) storage. However, cold-stored platelets are cleared more rapidly from circulation, reducing their suitability for prophylactic transfusion. To minimise wastage, it may be beneficial to store platelets conventionally until near expiry (4 days) for prophylactic use, transferring them to refrigerated storage to facilitate an extended shelf life, reserving the platelets for the treatment of acute bleeding.

MATERIALS AND METHODS:
Two ABO-matched buffy-coat-derived platelets (30% plasma/70% SSP+) were pooled and split to produce matched pairs (n = 8 pairs). One unit was stored at 2-6°C without agitation (day 1 postcollection; cold); the second unit was stored at 20-24°C with constant agitation until day 4 then stored at 2–6°C thereafter (delayed-cold). All units were tested for in vitro quality periodically over 21 days.

RESULTS:
During storage, cold and delayed-cold platelets maintained a similar platelet count. While pH and HSR were significantly higher in delayed-cold platelets, other metabolic markers, including lactate production and glucose consumption, did not differ significantly. Furthermore, surface expression of phosphatidylserine and CD62P, release of soluble CD62P and microparticles were not significantly different, suggesting similar activation profiles. Aggregation responses of delayed-cold platelets followed the same trend as cold platelets once transferred to cold storage, gradually declining over the storage period.

CONCLUSION:
The metabolic and activation profile of delayed-cold platelets was similar to cold-stored platelets. These data suggest that transferring platelets to refrigerated storage when near expiry may be a viable option for maximising platelet inventories.
Adding to platelet safety and life: Platelet additive solutions.
Mathur A, Swamy N, Thapa S, Chakraborthy S, Jagannathan L.

BACKGROUND:
Platelet additive solutions (PAS) are crystalloid nutrient media used in place of plasma for platelet storage. They replace 60%–70% of plasma in platelet components, so the amount of storage plasma can be decreased. Platelets in PAS have lower risk for allergic transfusion reactions with equivalent clinical efficacy for controlling bleeding.

AIM:
The aim of this study is to evaluate the clinical and laboratory efficacy of PAS-platelets.

MATERIALS AND METHODS:
A total of 1674 single donor platelet (SDP) were collected in PAS in the month of June to September 2016 by different apheresis systems. The quality control tests were done on 356 units in 4 months. Total number of SDP were processed with Amicus device (n = 232), Trima Accel (n = 84), and MCS+ (n = 40). The parameters analyzed were antibody titer of anti-A and anti-B, volume, platelet count, pH, bacterial contamination, and reporting of adverse transfusion reaction. Antibody titers were checked by tube technique, and platelet counts were checked by hematology analyzer Sysmex poch 100i. The swirling was checked manually, and pH was checked with pH strips.

RESULTS:
Out of 356, 164 units were O group, 113 units were B group, 68 units were of A group, and the remaining 11 units were of AB Group. Anti-A and anti-B titer was significantly reduced in PAS-SDP and found 1:32 or less for all the units. All the units found negative for bacterial contamination. No transfusion reaction was reported of the units transfused. All other quality parameters for platelets also found satisfactory after implementing the additive solution.

CONCLUSION:
The ABO antibody titers were significantly reduced after addition of PAS. This facilitates the ABO incompatible SDP transfusion and helps in inventory management. The risk of allergic transfusion reaction decreases after reducing the amount of plasma from SDP units. Using PAS-SDP certainly improve the inventory management for platelets with no compromise on clinical and laboratory efficacy.

Quality of irradiated and non-irradiated plateletpheresis concentrates stored in platelet additive solution.
Moog R, Rothe R, Arlt N, Burkhardt T.

INTRODUCTION:
Platelet additive solutions (PAS) allow to maintain platelet storage properties in platelet concentrates (PCs). The aim of the present study was to evaluate the in-vitro quality of irradiated and non-irradiated PCs, suspended in PAS, over a storage period of 6 days.

METHODS:
Plateletpheresis donors fulfilling current eligibility criteria underwent plateletpheresis with the MCS+ blood cell separator. The PAS SSP+ was used to store platelets (PLT) for up to 6 days. Aliquots were drawn from the PCs after collection, at day 4, 5 and 6 of storage. A battery of tests was performed to analyse the quality of the PCs: PLT count, mean PLT volume (MPV), PLT activation marker CD 62, switl, RBC and WBC contamination, pH, citrate, glucose, lactate and lactate dehydrogenase.

RESULTS:
An average of 2.53 ± 0.21 × 1011 PLT were collected in a product volume of 231 ± 5 mL in irradiated and 233 ± 6 mL in non-irradiated PCs, respectively. RBC- and WBC-contamination were within the allowed ranges. Δ CD62 steadily decreased in irradiated and non-irradiated PCs while the pH was well maintained over storage time. Glucose and lactate levels of irradiated and non-irradiated PCs showed characteristic pattern of PC storage within acceptable ranges.

CONCLUSION:
Our data demonstrate that parameters of PC quality were well maintained over a storage period of 6 days using PAS. Irradiation had no impact on the quality of PCs. The product quality of irradiated and non-irradiated PCs met national and European guidelines.
First Indian initiative for preparation of low-titer group «O» single-donor platelets with platelet additive solution.

Jain P, Tendulkar A, Gupta A.

BACKGROUND:
Guidelines recommend ABO-identical platelet (PLT) transfusions. Hemolytic reactions after a minor ABO-incompatible PLT transfusion have escalated due to single-donor platelets (SDP) containing ABO-incompatible plasma. Avoiding such events by examining titers or performing plasma reduction is cumbersome. The introduction of platelet additive solutions (PAS) has enabled to reduce these reactions by avoiding passive transfer of isoagglutinin. Our aim was to study antibody titers (anti-A, anti-B) in «O» SDP by adding PAS at source and the quality parameters with reference to viability, morphology, and metabolism.

MATERIALS AND METHODS:
Group «O» SDP (n = 50) were prepared on a standard cell separator. PAS in a ratio of 70:30 (PAS: plasma) was added at source under sterile conditions (study arm). The units were studied on day of collection (day 0) and day 4 and compared with SDP containing 100% plasma (control arm). A titer study was performed after PAS addition.

RESULTS:
In the study group, the median antibody titers (anti-A, anti-B) reduced from 128 to 16, post-PAS addition (P < 0.001). Morphology scores were superior in PAS platelet concentrates (P < 0.001). Metabolic parameters pO2 and pCO2 were similar in the two arms signifying good unit storage and stable oxygen consumption (P > 0.05). Lactate levels, glucose consumption rate, and lactate production rates were significantly low in study arm showing the advantage of PAS.

CONCLUSION:
O group SDPs can be prepared with PAS and the beneficial effects were significant with respect to antibody titers. Quality parameters were well maintained. Availability of PAS units has benefitted patients.

Platelet storage lesion in interim platelet unit concentrates: A comparison with buffy-coat and apheresis concentrates.

Singh S, Shams Haikini C, Jeppsson A, Hesse C.

Platelet storage lesion is characterized by morphological changes and impaired platelet function. The collection method and storage medium may influence the magnitude of the storage lesion. The aim of this study was to compare the newly introduced interim platelet unit (IPU) platelet concentrates (PCs) (additive solution SSP+, 40% residual plasma content) with the more established buffy-coat PCs (SSP 20% residual plasma content) and apheresis PCs (autologous plasma) in terms of platelet storage lesions. Thirty PCs (n=10 for each type) were assessed by measuring metabolic parameters (lactate, glucose, and pH), platelet activation markers, and in vitro platelet aggregability on days 1, 4, and 7 after donation. The expression of platelet activation markers CD62p (P-selectin), CD63 (LAMP-3), and phosphatidylserine was measured using flow cytometry and in vitro aggregability was measured with multiple electrode aggregometry. Higher platelet activation and lower in vitro aggregability was observed in IPU than in buffy-coat PCs on day 1 after donation. In contrast, metabolic parameters, expression of platelet activation markers, and in vitro aggregability were better maintained in IPU than in buffy-coat PCs at the end of the storage period. Compared to apheresis PCs, IPU PCs had higher expression of activation markers and lower in vitro aggregability throughout storage. In conclusion, the results indicate that there are significant differences in platelet storage lesions between IPU, buffy-coat, and apheresis PCs. The quality of IPU PCs appears to be at least comparable to buffy-coat preparations. Further studies are required to distinguish the effect of the preparation methods from storage conditions.
PAS or plasma for storage of platelets? A concise review.

Van der Meer P.F.

Transfusion Medicine 2016; 26 (5):339-342

Platelet additive solutions (PASs) are becoming increasingly popular for storage of platelets, and PAS is steadily replacing plasma as the storage medium of platelets. PASs are electrolyte solutions intended for storage of platelets, and they are used to modulate the quality of the platelets by adding specific ingredients. All currently available PASs contain acetate. Acetate reduces the amount of glucose that is oxidized into lactic acid and thereby prevents the lowering of pH, which decreases platelet quality. Furthermore, the oxidation of acetate leads to the production of bicarbonate, which serves as buffer. The presence of potassium and magnesium in PAS prevents the lowering of pH and reduces the degree of spontaneous activation of the platelets during storage. In the hospital, platelets stored in PAS result in about half of the number of allergic transfusion reactions as compared with platelets in plasma. Recovery and survival after transfusion, as well as corrected count increments, are at least as good for platelets in PAS as for plasma, and recent data suggest they may even be better. Therefore, with the current generation of PASs, PAS should be preferred over the use of plasma for the storage of platelet concentrates.

In vitro evaluation of platelet concentrates suspended in additive solution and treated for pathogen reduction: effects of clumping formation.

Castillo A, Alvarez I, Tolksdorf F.


BACKGROUND:
Platelet concentrates may demonstrate visual, macroscopic clumps immediately after collection following aphaeresis or production from whole blood, independently of the preparation method or equipment used. The relationship between the occurrence of clumping and their effect on in vitro quality of platelets was investigated.

MATERIALS AND METHODS:
Platelet concentrates, suspended in SSP+ additive solution (Macopharma), were obtained by automated processing and also from routine processing. A total of twelve units were allocated to the test group (n=12) due to the presence of clumps. Platelet concentrates without clumps were used as controls (n=10). All platelet units were treated for pathogen reduction following storage under continuous agitation for in vitro testing over a 9-day storage period.

RESULTS:
No significant differences were found throughout storage between the groups. The lactate dehydrogenase levels increased in both groups; this increase was higher in the test group on the last day of testing, without there being a significant difference on day 2. In contrast, pH values on day 2 were significantly different between the test and control groups. Platelet-derived cytokines increased comparably during storage.

DISCUSSION:
The results confirm good in vitro quality and storage stability of platelets suspended in SSP+ and treated with the Intercept pathogen reduction system. The presence of «non-compacted» clumps in platelet concentrates does not appear to affect the in vitro quality of the platelets.

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Platelet storage media.
Gulliksson H.
Vox Sang 2014; 107(3):205-212

Present platelet storage media often designated platelet additive solutions (PAS) basically contain acetate, citrate and phosphate and recently also potassium and magnesium. However, there seems to be an increasing interest in developing PASs that can be used also after further reduction of residual plasma content below 15-20% plasma. Inclusion of glucose but also calcium and bicarbonate in such solutions have been suggested to improve platelet (PLT) storage, especially when plasma content is reduced to very low levels. Results from a limited number of studies using novel PAS alternatives have been presented during the last years, such as Intersol-G, PAS-S, M-sol, PAS-G and SAS. Most of them are experimental solutions. The combined results presented in those studies suggest that presence of glucose may be necessary during PLT storage, primarily to maintain ATP at acceptable levels. At plasma inclusion below 15-20%, the content of glucose will generally be too low to support PLT metabolism for more than a few days making glucose addition in PAS necessary. Significant effects associated with presence of calcium was observed in PLTs stored in PAS with 5% inclusion but not with 20-35% plasma inclusion, suggesting that the content of plasma could be of importance. Bicarbonate only seems to be of importance for pH regulation, primarily when plasma inclusion is reduced to about 5%. Reduction in rate of glycolysis was observed in some PAS alternatives containing potassium and magnesium but not in others. Differences in pH or in concentrations of the various compounds included in PAS may be possible explanations. Additionally, novel PAS containing glucose, calcium and bicarbonate does not seem to be associated with improved in vitro results as compared to SSP+ or Composol when PLTs are stored with 35% plasma inclusion. The results would then also suggest that excess of glucose in novel PAS environment may not be associated with additional positive effects on PLT metabolism. This review is based on the few publications on novel PAS available, and additional studies would be needed in the future.

Parallel comparison of apheresis-collected platelet concentrates stored in four different additive solutions.

BACKGROUND AND OBJECTIVES:
Partially replacing plasma with additive solutions in platelet (PLT) concentrates (PCs) may help to reduce transfusion reactions. Constituents of PLT additive solutions (PASs) have been revealed to affect the quality of PCs. Previous studies involved pairwise comparison of identical PLTs with two different PASs or multicomparison using random PLTs with three or more PASs. In this study, we performed parallel comparison using PCs from identical donors with four PASs. In addition to traditional parameters, the release of bioactive substances and plasma proteins was assessed.

MATERIALS AND METHODS:
Platelets collected four times by apheresis from three donors were suspended in Intersol, SSP+, Composol or M-sol with 35% autologous plasma. The PC parameters, including PLT activation markers, glucose consumption, chemokines and plasma proteins, were assessed during 5-day storage.

RESULTS:
Mean PLT volumes were decreased in SSP+, Composol and M-sol after 5-day storage, with significant differences, whereas the hypertonic shock response (HSR) was decreased only in Intersol. Glucose consumption was faster in Intersol and M-sol than in SSP+ or Composol. PLT activation, determined as CD62P, sCD62P, sCD40L and RANTES, was significantly higher in Intersol than the other three PASs. No marked change was observed in fibrinopeptide A and C3a in any PASs.

CONCLUSIONS:
M-sol, SSP+ and Composol effectively preserved the quality of PCs. PLT activation was significantly enhanced in Intersol compared with the other three PASs. These effects seem to depend on magnesium and potassium as a constituent. Parallel comparison further verified that the PC quality largely depended on PASs but not donors.
Current status of additive solutions for platelets.
Alhumaidan H, Sweeney J. 

The storage of platelets in additive solution (PAS) had lagged behind red cell concentrates, especially in North America. The partial or complete removal of anticoagulated plasma and storage of platelet concentrates in AS presents many advantages. The PAS can be formulated to optimize aerobic metabolism or decrease platelet activation, thus abrogating the platelet storage lesion and potentially improving in vivo viability. Plasma removal has been shown to reduce allergic reactions and the plasma harvested could contribute to the available plasma pool for transfusion or fractionation. PAS coupled to pathogen reduction technology results in a platelet product of equivalent hemostatic efficacy to conventionally stored platelets. Given the above, the likely future direction of platelet storage will be in new generation designer PAS with an extended shelf life and a superior safety profile to plasma stored platelets.

In vitro properties of platelets stored in three different additive solutions.
Tynngård N, Trinks M, Berlin G. 
Transfusion 2012; 52(5):1003-1009

BACKGROUND: New platelet (PLT) additive solutions (PASs) contain compounds that might improve the storage conditions for PLTs. This study compares the in vitro function, including hemostatic properties (clot formation and elasticity), of PLTs in T-Sol, Composol, or SSP+ during storage for 5 days.

STUDY DESIGN AND METHODS: Fifteen buffy coats were pooled and divided into three parts. PLT concentrates (PCs) with 30% plasma and 70% PAS (T-Sol, Composol, or SSP+) were prepared (n = 10). Swirling, PLT count, blood gases, metabolic variables, PLT activation markers, and coagulation by free oscillation rheometry (FOR) were analysed on Days 1 and 5.

RESULTS: Swirling was well preserved and pH acceptable (6.4-7.4) during storage for all PASs. Storage of PLTs in T-Sol led to a decrease in PLT count whereas the number of PLTs was unchanged in Composol or SSP+ PCs. PLTs in T-Sol showed higher glucose metabolism than PLTs in Composol or in SSP+. At the end of storage PLTs in T-Sol had higher spontaneous activation and lower ability to respond to an agonist than PLTs in Composol or SSP+. PLTs in all the PASs had a similar ability to promote clot formation and clot elasticity.

CONCLUSION: Storage of PLTs in Composol or in SSP+ improved the quality of PCs in terms of better maintained PLT count, lower glucose metabolism, lower spontaneous activation, and improved response to a PLT agonist compared to PLTs in T-Sol. PLTs stored in the various PASs had similar hemostatic properties. These findings make Composol and SSP+ interesting alternatives as PASs.
Evaluation of the automated collection and extended storage of apheresis platelets in additive solution.

Johnson L, Winter KM, Hartkopf-Theis T, Reid S, Kwok M, Marks DC.
Transfusion 2012; 52(3):503-509

BACKGROUND:
Collecting apheresis platelets (PLTs) into additive solution has many potential benefits. The new Trima software (Version 6.0, Caridian BCT) allows automated addition of PLT additive solution (PAS) after collection, compared to Trima Version 5.1, which only collects PLTs into plasma. The aim of this study was to compare PLT quality during extended storage, after collection with the different Trima systems.

STUDY DESIGN AND METHODS:
Apheresis PLTs were collected using both Trima Accel apheresis systems. The test PLT units (n = 12) were collected using the new Trima Version 6.0 into PLT AS (PAS-IIIM), while the control units (n = 8) were collected into autologous plasma using Trima Version 5.1. All units were stored for 9 days, and in vitro cell quality variables were evaluated during this time.

RESULTS:
PLTs collected in PAS-IIIM maintained a stable pH between 7.2 and 7.4, whereas plasma-stored apheresis units exhibited significantly increased acidity during storage, due to lactate accumulation and bicarbonate exhaustion. Plasma-stored PLTs also demonstrated a more rapid consumption of glucose. However, there was little difference in PLT activation or cytokine secretion between PAS-IIIM and control PLTs.

CONCLUSION:
These data indicate that apheresis PLT concentrates collected in PAS-IIIM, using Trima Version 6.0 software, maintained acceptable PLT metabolic and cellular characteristics until Day 9 of storage.

Effect of platelet additive solution on bacterial dynamics and their influence on platelet quality in stored platelet concentrates.

Greco CA, Zhang JG, Kikal M, Yi QL, Ramirez-Arcos SM, Gyongyassy-Issa MI.
Transfusion 2010; 50(11):2344-2352

BACKGROUND:
Platelet additive solutions (PASs) are an alternative to plasma for the storage of platelet concentrates (PCs). However, little is known about the effect of PAS on the growth dynamics of contaminant bacteria. Conversely, there have been no studies on the influence of bacteria on platelet (PLT) quality indicators when suspended in PAS.

STUDY DESIGN AND METHODS:
Eight buffy coats were pooled, split, and processed into PCs suspended in either plasma or PAS (SSP+, MacoPharma). PCs were inoculated with 10 and 100 colony-forming units (CFUs)/bag of either Serratia liquefaciens or Staphylococcus epidermidis. Bacterial growth was measured over 5 days by colony counts and bacterial biofilm formation was assayed by scanning electron microscopy and crystal violet staining. Concurrently, PLT markers were measured by an assay panel and flow cytometry.

RESULTS:
S. liquefaciens exhibited an apparent slower doubling time in plasma-suspended PCs (plasma-PCs). Biofilm formation by S. liquefaciens and S. epidermidis was significantly greater in PCs stored in plasma than in PAS. Although S. liquefaciens altered several PLT markers after Days 3 to 4 postinoculation in both PAS- and plasma-PCs, S. epidermidis contamination did not produce measurable PLT changes.

CONCLUSIONS:
S. liquefaciens can be detected more quickly in PAS-suspended PCs (PAS-PCs) than in plasma-PCs by colony counting. Furthermore, reduced biofilm formation by S. liquefaciens and S. epidermidis during storage in PAS-PCs increases bacteria availability for sampling detection. Culture-based detection remains the earliest indicator of bacterial presence in PAS-PCs, while changes of PLT quality can herald S. liquefaciens contamination when in excess of 10(8) CFUs/ml.
The new generation of platelet additive solution for storage at 22 degrees C: development and current experience.

Ringwald J, Zimmermann R, Eckstein R.


The storage of platelets (PLTs) in PLT additive solutions (PASs) might have several advantages. It can reduce allergic and febrile transfusion reactions, facilitate AB0-incompatible PLT transfusions, enable pathogen inactivation, and make more plasma available for other purposes (e.g., for fractionation). For this reason, there has been considerable focus on the development of new PASs that assure maintenance of good PLT quality throughout storage. Several compounds in PASs such as citrate, acetate, phosphate, potassium, and magnesium have all turned out to be important, and the same applies to the necessary amount of glucose as determined by the plasma carryover. The latest generation of PASs, the modified PAS-III and Composol-PS, contains most or all of these compounds. Recently published data on the in vitro quality of either buffy coat- or apheresis-derived PLT concentrates stored in 70% or even 80% of PAS might encourage transfusion specialists to consider using these PASs in routine blood banking. However, because in vitro tests do not adequately predict clinical effectiveness of PLTs after transfusion, in vivo studies are still needed to assess the quality of PAS-stored PLTs.

Storage of platelets in additive solutions: a multicentre study of the in vitro effects of potassium and magnesium.


Vox Sang 2003; 85(3):199-205

BACKGROUND AND OBJECTIVES:

In a preliminary study, the presence of potassium and magnesium in a modified synthetic medium (PAS-III) was found to have a significant influence on platelet metabolism (using apheresis-derived, as well as buffy-coat-derived platelets) when compared with standard PAS-III. The differences included reduced glycolysis, as evidenced by lower consumption of glucose and lower production of lactate, but also better preservation of pH and hypotonic shock response reactivity. The results suggested that storage in modified PAS-III containing 20% plasma was comparable to storage in standard PAS-III containing 30% plasma. To confirm the preliminary results and to evaluate the effects of different preparation protocols, an international multicentre study, which included 11 different sites, was conducted.

MATERIALS AND METHODS:

Platelets from 30 pools of approximately 20 buffy coat (BC) units each and 24 pooled apheresis platelet units were aliquoted for storage in plasma (reference) or synthetic medium using either a specific additive solution (PAS-III) containing 30% plasma or a modification of PAS-III containing 5.0 mm potassium and 1.5 mm magnesium (PAS-IIIM) and either 30% or 20% plasma. Units were stored at room temperature with agitation for 7 days during which in vitro testing was carried out for biochemical, haematological and functional parameters.

RESULTS:

Storage of platelets in PAS-IIIM resulted in a reduction in the rate of glycolysis and better retention of pH and hypotonic shock response reactivity. Storage in PAS-IIIM containing 20% plasma appeared to result in the retention of in vitro properties, similar to those observed during storage in standard PAS-III containing 30% plasma.

CONCLUSIONS:

The results of this study confirm the preliminary results. Similar results were seen with platelets prepared by BC and apheresis methods, despite differences in equipment, the preparation technique and in the final platelet contents achieved in the platelet units. Storage of platelets in PAS-IIIM should be considered to improve platelet function and allow plasma reduction to 20%.
Reactions and platelet increments after transfusion of platelet concentrates in plasma or an additive solution: a prospective, randomized study.


BACKGROUND:
Reactions after platelet transfusions are rather common and frequently are caused by plasma constituents. In recent developments, the preparation and storage of platelet concentrates (PCs) in a platelet additive solution (PAS-2) have been shown to result in acceptable storage conditions. A major drawback of the use of these PCs is the progressive increase of P-selectin-positive platelets during storage. The clinical benefit of transfusions of PCs in PAS-2 was studied.

STUDY DESIGN AND METHODS:
PCs prepared from buffy coats were suspended in either plasma or PAS-2 and stored for up to 5 days. Clinical responses were evaluated in a prospective study in 21 patients treated with intensive chemotherapy for hematologic malignancies. Eligible patients were randomly assigned to receive prophylactic transfusions of PCs prepared in either plasma or PAS-2. Reactions and CCIs were recorded after each transfusion.

RESULTS:
The incidence of reactions in 12 patients given PCs in plasma (n = 192) was 12 percent. Transfusions to 9 patients of PCs in PAS-2 (n = 132) showed a reduction in the incidence of reactions to 5.3 percent (p<0.05). The average 1-hour and 20-hour CCIs after transfusion of PCs in plasma were 20.7 +/- 8.5 and 11.5 +/- 8.0, respectively. CCIs after transfusion of PCs in PAS-2 were significantly lower: the average 1-hour CCI was 17.1 +/- 6.6 (p<0.001) and the average 20-hour CCI was 9.5 +/- 7.0 (p<0.05). Storage conditions of PCs were optimal: in each group, average 1-hour CCIs of both fresh and stored PCs were similar. The 20-hour CCIs after the transfusion of fresh and stored PCs in PAS-2 also were similar.

CONCLUSION:
Transfusion of PCs in PAS-2 significantly reduces the incidence of reactions. The 1-hour and 20-hour CCIs after transfusion of PCs in PAS-2 were significantly lower than the CCIs after transfusion of PCs in plasma. Because storage conditions of both PCs were found to be optimal, the decrease in CCIs after transfusion of PCs prepared in PAS-2 may be caused by rapid elimination of a subpopulation of P-selectin-positive platelets from the circulation.
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