BLOOD SAFETY

BIBLIOGRAPHY

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

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2018
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 plasma, platelets and red cells. 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, Springer. 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 produced with the THERAFLEX MB-Plasma procedure is in clinical use in 20 countries worldwide and more than 6.5 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, and commercialisation of THERAFLEX UV-Platelets is expected in 2019/2020.

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 recommended replacement is up to 80% SSP+ in platelet concentrates. 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 9 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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Plasma temperature during methylene blue/light treatment influences virus inactivation capacity and product quality.

Gravemann U, Handke W, Sumian C, Alvarez I, Reichenberg S, Müller TH & Seltsam A.

Vox Sang 2018; 10.1111/vox.12643.

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, Muller TH, Seltsam A.

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.

Babigumira JB, Lubinga SJ, Castro E, Custer B.

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.

Noens L, Vilarino MD, Megalou A, Qureshi H.

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.

Fryk JJ, Marks DC, Hobson-Peters J, Watterson D, Hall RA, Young PR, Reichenberg S, Sunlan C, Faddy HM.
Pathology 2017; 49: S115.

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.

Bachholer L, Wiltshire M, Pottlt S, Cookson P, Cardigan R.

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.

Fryk JJ, Marks DC, Hobson-Peters J, Prow NA, Waterson D, Hall RA, Young PR, Reichenberg S, Sumian C, Faddy HM.

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®GlobalNR, 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.


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, 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 (1:24593) 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 (1:24593), 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.
Paired analysis of plasma proteins and coagulant capacity after treatment with three methods of pathogen reduction.

Coene J, Devreeze K, Sabot B, Feys HB, Vandekerckhove P, Compansolle 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 II, FV, FVIII, FX, FVII), 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, FX, FVII, 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.

CONCLUSION:
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.
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, Benamar A, Fabrigli F & Garraud O.

BACKGROUND AND OBJECTIVES:
Our objective was to compare the frequency of adverse events (AEs) due to any of the 4 types of fresh-frozen plasma (FFP) prepared and delivered by the French Blood Establishment (EFS) over a 10-year period. Surveillance of AEs and vigilance was performed according to a homogeneous policy. The four types of FFP comprised of one type (methylene blue [MB]) that was stopped since then and of another type (amotosalen [AI]) that was recently introduced, along with two conventional products (quarantine [Q] and solvent-detergent [SD]).

MATERIALS AND METHODS:
This is a retrospective study based on the national AE reporting database and on the regional database system for deliveries. AEs recorded after the delivery of 1 of the 4 types of FFP were pairwise compared, with appropriate statistical corrections.

RESULTS:
105 964 FFP units were delivered (38.4% Q, 17.9% SD, 9.7% MB and 34% AI). Statistical comparisons of AEs identified only a difference in AE rates between quarantine and solvent-detergent plasma.

CONCLUSIONS:
FFP was confirmed to be extremely safe in general, especially if one considers ‘severe’ AEs. All types of FFP were associated with extremely low occurrences of AEs. Q, SD, MB and AI led, respectively, to 7.14, 4.86, 1.05 and 4.16 AEs per 10 000 deliveries.

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

Seltsam A, Müller TH.

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Storage of thawed plasma for a liquid plasma bank: impact of temperature and methylene blue pathogen inactivation.

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 (FVII, 69% [42%-125%]; Protein S, 20% [10%-35%]). MB/light treatment of thawed FFP resulted in minor decreases for FVIII (47% [12%-91%]) and Protein S (49% [18%-96%]) 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.

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


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.
### THERAFLEX UV-Platelets scientific publications

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Pathogen Inactivation of Cellular Blood Products—An Additional Safety Layer in Transfusion Medicine

Andr Sallust

German Red Cross Blood Service NRW, Mönchengladbach, Germany

In line with current microbial risk reduction efforts, pathogen inactivation (PI) technologies for blood components promise to reduce the residual risk of screening and emerging infectious agents. The implementation of PI for all blood components is slowly but steadily increasing. This review discusses the relevance of PI for the field of transfusion medicine and describes the available and emerging PI technologies that can be used to treat cellular blood products such as platelets and red blood cell units. In collaboration with the French medical device manufacturer Macopharma, the German Red Cross Blood Services developed a new UVC light-based PI method for platelet units, which is currently being investigated in clinical trials.

Keywords: transfusion, platelets, pathogen inactivation, ultraviolet light, red blood cells

INTRODUCTION

From the late 1970s to the mid-1980s, contaminated hemophilia blood products were a serious public health problem resulting in the infection of large numbers of hemophiliacs with the human immunodeficiency virus (HIV). If safety measures had been implemented in a timely and consistent manner after identification of the acquired immune deficiency syndrome (AIDS) epidemic in 1981 and isolation of the HIV in 1983, the transmission of HIV infection by blood products could have been prevented in most cases. This contaminated blood scandal sealed the community aware that new pathogens may emerge and threaten blood safety at any moment. However, there was a significant delay in the introduction of HIV detection systems in some countries and in some cases, the detection tests that were implemented proved to be unreliable. In addition, the plasma products for therapies were not evenly treated by heat inactivation—a pathogen inactivation (PI) method that was readily available and approved at that time. Consequently, blood and blood components became the focus of drug manufacturers (1, 2).

Increasingly stringent donor eligibility criteria and more sensitive virus detection methods have reduced the risk of transfusion-transmitted infection (TTI) by blood products significantly, but a residual risk of TTI with viruses, bacteria, protozoa, and prions remains. False-negative test results due to test failures, very low-pitched concentrations in the peripheral blood or escaped mutants can result in TTI in spite of negative screening tests (e.g., for Hepatitis B virus, Hepatitis C, HIV). In addition, transfusion recipients may be infected by pathogens not targeted in regular blood donor screening programs (e.g., hepatitis A and bacteria). Transfusion safety is particularly susceptible to pathogens that enter regions in which they are not yet endemic. The fact that viruses which are usually endemic in tropical regions have recently caused outbreaks in Western countries demonstrates that these pathogens can arise and threaten transfusion safety at any time (1, 2).

Blood safety is still mainly based on the reactive principle of introducing new test systems or new donor selection criteria after a threat to transfusion recipients has been identified. In other words, infections by contaminated blood products must first occur before appropriate countermeasures are established. At the beginning of the 1990s, an estimated number of cases of West Nile virus occurred in the USA through the transmission of blood components before the first detection system for donor testing was implemented (3). The recent Zika virus outbreak on the American continent has heightened concerns over this reactive approach to blood supply safety (4, 5).

Before an international consensus conference, transfusion experts and other stakeholders in the field of transfusion medicine recommended a change from the inherent reactive strategy toward a proactive, preventive approach to blood safety (7). Recently, developed and approved PI technologies for cellular blood products, such as red blood cell (RBC) and platelet units, are considered key measures for closing or at least reducing the safety gap in emerging pathogens. While virus reduction procedures are an integral part of the manufacturing processes for red blood cell plasma products, and aiming to remove the major viral risk, the UV-C sterilization system has been used for PI of single donor plasma units for nearly two decades (8). A new generation of PI methods for platelet units has recently become available (9-11). PI technologies for the treatment of RBC units are still in development and have not received market authorization yet.

TECHNOLOGIES

The use of PI technologies for blood products has a number of advantages. Because they inactivate clinically relevant viruses, bacteria, and protozoa, they can help to eliminate the residual risk of infection during the “window period” when transfusion-relevant pathogens (e.g., HIV) cannot be detected by donor screening tests. Their blood activity against pathogens also helps to reduce the risk of recognizable infectious agents (e.g., bacterial) which still cannot be prevented completely. In contrast to screening tests for transfusion-borne pathogens, PI proactively protects against emerging infectious agents entering the blood supply as a given commodity.

All PI methods used to treat cellular blood products work by impairing the target pathogens ability to replicate. When used alone or in combination, ultraviolet (UV) light and inactivating agents cause irreversible damage to the nucleic acids of pathogens. Therefore, they effectively eliminate classical pathogens such as viruses, bacteria, fungi, and protozoa, but are ineffective against prions. The latter proteinaceous-pathogenic agents can cause sporadic and variant Creutzfeldt-Jakob disease in humans (12). The following PI technologies for cellular products are currently available in the pipeline.

INTERCEPT Blood System for Platelets and Plasma

The INTERCEPT Blood System for platelets and plasma is manufactured by Cerus Corporation (Concord, CA, USA). The mechanism of action of this PI technology is based on the properties of amotosalen HCl (S-59), a photosensitive compound which penetrates cellular and nuclear membranes and binds to the double-stranded regions of DNA and RNA. When activated by low-energy UVA light (320-400 nm), amotosalen cross-links nucleic acids and thus irreversibly blocks the replication of DNA and RNA (13). After illumination, the photosensitizer and its photodegradation products are non-toxic and non-immunogenic, they do not need to be removed prior to transfusion. In addition to plasma and platelets, protocols for elimination of the INTERCEPT system to whole blood are now in development.

THERAFLEX System for Platelets and Plasma

The THERAFLEX system was developed by Theratech (Lakewood, CO, USA). This photosensitization procedure employs riboflavin (vitamin B2) and broad spectrum UVC light (mainly UVA and UVB, 280-365 nm). On exposure to UVA and UVB light, riboflavin associates with nucleic acids and mediates oxygen-dependent electron transfer, causing irreversible damage to the nucleic acids (14). Decoloration of their photodegradation products is non-toxic and non-immunogenic. The clinical studies of the product in non-toxic and non-immunogenic, they do not need to be removed prior to transfusion. In addition to plasma and platelets, protocols for elimination of the INTERCEPT system to whole blood are now in development.

S-303 PI System for RBCs

The S-303 PI system (INTERCEPT RBC system, Cerus Corporation, Concord, CA, USA) was specifically developed for RBC units. S-303 is a modular component that prevents nucleic acid replication by targeting and cross-linking nucleic acids. Once added to the RBC unit, this amphiphatic compound rapidly passes through cell and viral envelope membranes and intercalates into the helical regions of nucleic acids. S-303, the non-reactive byproduct of the reactions, is subsequently removed by incubation and centrifugation, which can take up to 20 h (4). In contrast to the other PI technologies described above, the S-303 system does not require UVC light. However, glutathione (GSH), a naturally occurring antioxidant, must be used to prevent non-specific interactions between S-303 and other nucleophiles present in the RBC unit. This may result in small
and recovery rates were consistently lower in patients receiving platelets treated with PI technology than in those transfused with untreated platelets (16-19). Accordingly, the transfusion of pathogen-reduced platelets resulted in lower platelet count increments (CI), lower corrected count increments, shorter intervals between platelet transfusions, and a higher number of platelet transfusions per patient. However, observational studies showed no evidence of increased product consumption rates when pathogen-reduced platelet units were used as a routine setting (20).

Interestingly, the rate of acute transfusion reactions may be lower after the transfusion of pathogen-reduced versus untreated platelets. However, there have been concerns over acute respiratory distress associated with amniotic/aqua-treated platelets (13). While the results of animal studies suggest that UV light-treated platelets mediate a higher risk of pulmonary toxicity (21), an analysis of clinical data by an expert panel does not confirm significant differences in the rate of acute lung disorders between PI-treated and untreated platelets (12). The results of ongoing large-scale phase III and hemovigilance studies will help to further clarify the incidence of this side with respect to therapeutic efficacy and potential side effects of pathogen-reduced platelets (23).

Red Blood Cells

The S-305 system, which is still in clinical development, is the only PI technology available for RBCs. Current studies are investigating the second-generation S-305 PI process. The first-generation S-305 procedure only marginally affected RBC quality and function, but after reports of immunization against pathogen-reduced RBCs in transfused patients emerged, a new generation of the S-305 system had to be developed. In the second-generation S-305 system, the quencher concentration of GSH was increased from 2 to 20 mM/liter in order to decrease the ability of S-305 for proteins and thus to avoid the formation of neoantigens on the surface of erythrocytes (24). However, recent studies show that immunization against S-305-coated RBCs still occurs after modification of the S-305 system (25). In particular, the fact that antibodies against S-305-coated cells were also detected in healthy donors who had never been transfused with pathogen-reduced RBCs suggests that some individuals may be immunized by S-305-like substances in the environment (e.g., food or air) or may have already occurred antibodies against epitopes on the S-305 molecule. These data clearly show that the use of chemical agents for PI of cellular products increases the risk of immune responses against blood components in transfusion recipients. Various phase II clinical trials tested the second-generation S-305 PI system for RBCs in autologous and allogeneic patients, and the results indicated that S-305 PI may be effective in preventing immune responses against blood components.

EVALUATION OF TECHNOLOGIES FOR PLATELETS IN UNDERGROUND SITES

In 2011, the Swiss national authority for blood transfusion (Swissmedic) ordered the nationwide implementation of PI of platelet units. This measure was mainly aimed at preventing or at least minimizing the risk of fatal transfusion reactions caused by bacterially contaminated platelet units. Analysis of hemovigilance data revealed that without PI, one fatal case of transfusion-transmitted sepsis by contaminated platelet units would occur in Switzerland every 2 years. The Swiss Food and Drug Administration (FDA) recently recommended the use of approved PI technologies as an alternative to bacterial detection methods in order to adequately control the risk of bacterial contamination of platelet units (26, 27).

The preventative potential of PI of cellular blood components first became apparent during a chikungunya virus epidemic on the French island of La Réunion in the Indian Ocean in 2006 (28). Because more than 90% of the inhabitants were infected, local blood donation was suspended in premature to sustain the availability of platelet components, the French national blood service (Établissement Français du Sang) implemented universal PI of platelet components. The results of these studies demonstrate that PI can effectively support the availability of safe blood components during an epidemic.

The West Nile virus epidemic in the USA was the first example of a large-scale arterial blood to the blood supply of a Western country that required think response across government agencies and non-governmental organizations. The dramatic spread of Zika virus in the Americas since 2015 has generated a sense of public health urgency akin to AIDS, along with immediate concerns over blood safety. In areas of active transmission, TIVA guidance for PI of blood components to prevent potential transmission of the virus in the most affected areas, unless there are measures to screen donations using a laboratory test, or unless the blood components are collected from donors treated with an approved method (29). The INTERCEPT system was approved by the FDA in 2014 and has already been implemented at a number of US blood centers.

OUTLOOK

Despite the increasing prevalence and safety efficacy record of pathogen-reduced blood cellular products, there are still concerns that may impede the implementation of PI technology in hematopoietic processes. The INTERCEPT protocol includes incubation and adsorption steps that result in a significant loss of platelets (apart from RBCs) and platelets. The therapeutic potential of PI of platelet units is currently being investigated in the phase III clinical trials with the INTERCEPT platelet system, which is expected to have a significant impact on the availability of platelet units for patients. All PI technologies mentioned in this review exhibit gains in terms of PI efficacy. The automated USA-based system (INTERCEPT) is effective for non-enveloped viruses such as hepatitis A and hepatitis B, and parovirus B19 (25). The rIFNs (recombinant IFN)-based system (MIRASOL) has only weak effects against

**TABLE 1**: Pathogen-inactivation technologies.

<table>
<thead>
<tr>
<th>Technology</th>
<th>INTERCEPT blood system</th>
<th>MIRASOL PR system</th>
<th>INTERCEPT Platelets</th>
<th>S-305 system</th>
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<tbody>
<tr>
<td>Mechanism of action</td>
<td>Loss of nucleic acid by adsorption and filtration</td>
<td>Loss of nucleic acid by adsorption and filtration</td>
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<tr>
<td>Blood products</td>
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<tr>
<td>Plasma and platelets concentrates</td>
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**CLINICAL STUDIES**

**Platelets**

Clinical studies show that platelets retain their hemostatic efficacy after PI treatment. This was the case for both the INTERCEPT Platelets system and the S-305 system, which are still in clinical development. The UV-C-based INTERCEPT system is expected to receive marketing authorization within the next few years (Table 1).
bacteria and some viruses (31). The UV-light based system (THERAFLEX) is highly effective against bacteria and most transmissible agents, but only moderately effective against HIV (32). However, when highly sensitive screening tests for HIV are performed, UV-based PI could further reduce the risk of virus transmission during the “window period” in which the pre-prodromal stage begins and may be negative and in patients with occult infections. Despite these warnings, PI systems generally hold the potential to significantly add an additional layer of safety to blood transfusion.

Major concerns surrounding the implementation of PI have to due with its impact on the integrity of blood components and the toxicity of the chemicals used in these systems. In particular, acute and chronic toxicities may be caused by PI technologies that use active chemicals. Although only small quantities of photochemical compounds are used in PI technologies and they appear to provide sufficient safety margins, it cannot be excluded that altering agents such as antioxidants may be carcinogenic in the long term in a cohort of transfused patients. A major advantage of the THERAFLEX system is that it works without photosensitive substances that may cause allergenic or antigen-related adverse events (33, 34).

According to various stakeholders in the field of transfusion medicine, it is crucial to inactivate pathogens in all blood components in order to increase the safety margins of the entire blood supply. As long as PI is not rigorously implemented in the production of RBC units (the most commonly used blood products), PI cannot achieve its full potential to enhance blood safety. Experts and health authorities are increasingly recommending the implementation of PI systems for platelets and plasma as an important step toward improving blood safety. A Canadian risk analysis suggests that if a new patient received the blood supply, the use of pathogen-reduction techniques for platelets and plasma would reduce the risk of TTH by 60% (35).

The additional costs of PI implementation may be responsible for the hesitant acceptance of this technology by hospitals and funding agencies. Although based on assumptions and simplifications, the available cost-effectiveness analyses suggest that PI implementation, like other measures for the improvement of blood safety, has an acceptable cost-benefit ratio in this specific application (34, 35). The potential cost savings from PI implementation could offset some costs associated with the technology (e.g. production costs); however, the amount of potential offsetting cost reductions may vary considerably between different countries and regions and must be evaluated on an individual basis for blood centers and hospitals (35).

Finally, the available resources influence how politics and health authorities decide on how to meet public concerns for safety in transfusion medicine. If emerging evidence continues to demonstrate the efficacy of PI in reducing the risk of transfusion-transmitting diseases, new policies that encourage individual patients with severe transfusion-anxiety associated infections whom this readily available risk mitigation and safety measure was not implemented.

AUTHOR CONTRIBUTIONS

The authors confirm being the sole contributors of this work and approved it for publication.

REFERENCES

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-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.

Mitochondrial DNA multiplex real-time polymerase chain reaction inhibition assay for quality control of pathogen inactivation by ultraviolet C light in platelet concentrates.

Kim S, Handke W, Grovemann U, Döscher A, Brüner V, Müller TH, Seltsam A.
Transfusion 2018; 58(3):758-765.

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.

CONCLUSION:
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.


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).

CONCLUSIONS:
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.

CONCLUSION:
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, Tolksdorf 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.

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.


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.

Pohler P, Müller M, Winkler C, Schaudien D, Sewald K, Müller TH, & Seltsam A.

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, 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.

Tynngård N, Trinks M, Berlin G.

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. These 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 are 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 adaption 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 $10^7$ B. divergens–infected red blood cells. Infected BC-PCs were irradiated with 0.2 J/cm$^2$ 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 $0.1 \times 10^{-6}$% 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 visible 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 pathogenes 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.

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.

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, Tokisdorf F.

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/ Cerus Corporation, Concord, CA, USA; MIRASOL/Cardian 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, Mouvaz, France), was recently developed that uses short-wave ultraviolet light (UVC), without addition of any photosactive 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 new platelet performance indicators, revealed a parallel increase in both THERAFLEX UV-treated and control PC throughout storage, supporting the notion that the bioavailability of platelet function is not grossly affected by UVC treatment. This is corroborated by some newer technologies for proteomic analysis, showing that the THERAFLEX UV-Platelets system results in limited disruption of integrin-regulating extracellular disulphide bonds and minimal protein alterations when compared to UVB and gamma irradiation. Moreover, standard in vitro parameters reflecting activation, metabolic activity and function of platelets are useful indicators of the overall performance of processing and storage and may be used as surrogate markers of platelet quality in vivo. However, there is some doubt as to what degree each marker alone or in combination reflects the true clinical outcome of transfused platelets. Therefore, an appropriate clinical programme has been initiated. The preclinical evaluation demonstrated tolerability and immunological safety of THERAFLEX UV-Platelets using an animal model. Additionally, the system has successfully completed two autologous Phase I trials on recovery and survival. Preliminary results suggest that the recovery and survival rates are consistent with other pathogen reduced platelet products that are licensed and in use. The method is currently under evaluation for safety and tolerability of UVC-treated platelets in healthy volunteers. Presently the THERAFLEX UV-Platelets system is the simplest and purest PIT easily adaptable to the existing blood bank setting. In the future, extension of the application range of the THERAFLEX UV-Platelets system is expected, in order to make this new technology compatible with a broad spectrum of collection and processing platforms, and with other blood products.

Evaluation of the tolerability and immunogenicity of ultraviolet C-irradiated autologous platelets in a dog model.


BACKGROUND:
The THERAFLEX ultraviolet (UV) platelets (PLTs) pathogen reduction system for PLT concentrates (PCs) operates using ultraviolet C (UVC) light at a wavelength of 254 nm. UVC treatment can potentially alter proteins, which may affect drug tolerance in humans and influence the immunogenicity of blood products. This preclinical study in beagle dogs was designed to evaluate the safety pharmacology of UVC-irradiated PCs after intravenous administration and to determine whether they are capable of eliciting humoral responses to PLTs and plasma proteins.

STUDY DESIGN AND METHODS:
Six beagle dogs each were transfused once every other week for 10 weeks with UVC-irradiated or non-irradiated PCs. All PCs were autologous canine single-donor products prepared from whole blood. Safety pharmacology variables were regularly assessed. The impact of UVC irradiation on PLT and plasma proteomes was analysed by one- and two-dimensional gel electrophoresis. Serum samples were tested for UVC-induced antibodies by Western blot and flow cytometry.

RESULTS:
Dogs transfused with UVC-irradiated PCs showed no signs of local or systemic intolerance. Few but significant changes in PLT protein 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.

CONCLUSIONS:
Repeated transfusions of autologous UVC-treated PCs were well tolerated in all dogs studied. UVC irradiation did not cause significant plasma or PLT protein modifications capable of inducing specific antibody responses in the dogs. High-resolution proteomics combined with antibody analysis introduces a comprehensive and sensitive method for screening of protein modifications and antibodies specific for pathogen reduction treatment.
## SSP+ scientific publications

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Quality of irradiated and non-irradiated plateletpheresis concentrates stored in platelet additive solution.

Moog R, Rothe R, Aft 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, swirl, 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 Hakimi 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 PF.

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 oxidised 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.


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.

Platelet storage media.

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-5, 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, Kalab M, Yi QL, Ramirez-Arcos SM, Gyongyossy-Issa ML.

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 quality markers by 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 those 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 asuffy-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-II) 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.

De Wildt-Eggen J, Nauta S, Schrijver JG, van Marwijk Kooy M, Bins M, van Pooijen KC.


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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