Lead the way in blood safety

BLOOD SAFETY

BIBLIOGRAPHY

• THERAFLEX UV-Platelets
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 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.

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.
<table>
<thead>
<tr>
<th>Year</th>
<th>Authors, title and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>Authors, title and references</td>
</tr>
<tr>
<td>------</td>
<td>-------------------------------</td>
</tr>
</tbody>
</table>
Pathogen Inactivation of Cellular Blood Products—An Additional Safety Layer in Transfusion Medicine

Axel Seltsam*

German Red Cross Blood Service NSTOB, Institute Springs, Springs, Germany

In line with current microbial risk reduction efforts, pathogen inactivation (PI) technologies for blood components promise to reduce the residual risk of known and emerging infectious agents. The implementation of PI of labile 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 platelet 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 these blood products could have been prevented in most cases. This contaminated blood scandal made the community aware that new pathogens may emerge and threaten blood safety at any time. 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 used for therapy were not even treated by heat inactivation—a pathogen inactivation (PI) method that was readily available and approved at that time. Consequently, blood and blood components became subject to drug law in some countries (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-pathogen concentrations in the peripheral blood or escaped mutants can result in TTI in spite of negative screening tests (e.g., for Treponema pallidum, hepatitis B, hepatitis C, and 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 that 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 (3, 4).
Blood safety is still mainly based on the reactive principle of introducing new test systems or new donor election criteria after a threat to transfusion recipients has been identified. In other words, infections by contaminated blood products must first occur before appropriate counter-measures are established. At the beginning of the last decade, a 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 (5, 6).

During an international consensus conference, transfusion experts and other stakeholders in the field of transfusion medicine recommended a change from the hitherto 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 caused by emerging pathogens. While virus reduction procedures are an integral part of the process of manufacturing plasma derivatives from plasma pools, and although the methylene blue 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 have recently become available (9, 10). 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 most 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 broad activity against pathogens also helps to reduce the risk of recognizable infectious agents (e.g., bacteria), 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 in a given community.

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 alkylating 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 protein-based pathogenic agents can cause sporadic and variant Creutzfeldt–Jakob disease in humans.

The following PI technologies for cellular products are currently available or 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 photoactive 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 (11). After illumination, residual amotosalen and its photoproducts must be removed during an incubation step lasting up to 16 h. The amotosalen/UVA procedure is not suitable for RBCs because of UVA light absorption by hemoglobin.

MIRASOL PRT System for Platelets and Plasma

The MIRASOL system was developed by TerumoBCT (Lakewood, CO, USA). This photodynamic procedure employs riboflavin (vitamin B2) and broad spectrum UV light (mainly UVA and UVB, 285–365 nm). On exposure to UVA and UVB light, riboflavin associates with nucleic acids and mediates oxygen-independent electron transfer, causing irreversible damage to the nucleic acids (12). Because naturally occurring vitamin B2 and its photodegradation products are non-toxic and non-mutagenic, they do not need to be removed prior to transfusion. In addition to plasma and platelets, protocols for extension of the MIRASOL system to whole blood are now in development.

THERAFLEX System for Platelets

THERAFLEX UV-Platelets is a novel UVC-based PI technology that works without photoactive substances. It is the product of a joint venture between Macopharma (Mouvaux, France) and the German Red Cross Blood Service NSTOB in Springe, Germany. Shortwave UVC light (254 nm) directly interacts with nucleic acids to form pyrimidine dimers that block the elongation of nucleic acid transcripts (13). UVC irradiation mainly affects the nucleic acids of pathogens and leukocytes and does not impair plasma and platelet quality. As no photoactive substances are involved, UVC treatment is just as simple but faster (takes less than 1 min) than gamma irradiation, and can easily be integrated into the manufacturing processes at blood banks (Figure 1). The THERAFLEX system was originally developed for platelets but is also suitable for plasma and RBC units.

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 compound 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-300, the non-reactive byproduct of this reaction, is subsequently removed by incubation and centrifugation, which can take up to 20 h (14). In contrast to the other PI technologies described here, the S-303 system does not require UV light. However, glutathione (GSH), a naturally occurring antioxidant, must be used to prevent non-specific reactions between S-303 and other nucleophiles present in the RBC unit. These may include small
molecules, such as phosphate and water, and macromolecules, such as proteins.

The INTERCEPT and MIRASOL systems for platelets and plasma have already been approved in the USA and some European and Asian countries, while both the THERAFLEX system and the S-303 system are still in clinical development. The UVC-based THERAFLEX system is expected to receive marketing authorization within the next few years (Table 1).

**TABLE 1**: Pathogen inactivation technologies.

<table>
<thead>
<tr>
<th>Technology</th>
<th>INTERCEPT blood system</th>
<th>MIRASOL PRT system</th>
<th>THERAFLEX UV-Platelets</th>
<th>S-303 system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism of action</td>
<td>UVA plus amotosalen (alkylating agent)</td>
<td>UV plus riboflavin (vitamin B2, = photosensitizer)</td>
<td>UVC alone</td>
<td>Alkylating agent</td>
</tr>
<tr>
<td>Blood products</td>
<td>Plasma and platelets</td>
<td>Plasma and platelets (in development for whole blood)</td>
<td>Plasma and platelets (in development for RBCs)</td>
<td>RBCs</td>
</tr>
<tr>
<td>Status</td>
<td>Approved in some countries</td>
<td>Approved in some countries</td>
<td>In clinical development</td>
<td>In clinical development</td>
</tr>
</tbody>
</table>

_UV, ultraviolet light; UVA, wavelength A; UVC, wavelength C; RBC, red blood cell._

**CLINICAL STUDIES**

**Platelets**

Clinical studies show that platelets retain their hemostatic efficacy after PI treatment. Following prophylactic transfusion, there was no difference in the ability of pathogen-reduced and untreated platelet units to prevent severe bleeding (15). However, almost all clinical trials demonstrated that post-transfusion survival
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 (CIs), 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 in 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 amotosalen/UVA-treated platelets (15). While the results of animal studies suggest that UV light-treated platelets mediated a higher risk of pulmonary toxicity (21), an analysis of clinical data by an expert panel does not confirm significant differences in the rates of acute lung disorders between PI-treated and untreated platelets (22). The results of ongoing large-scale phase III and hemovigilance studies will help to further clarify open questions with respect to therapeutic efficacy and potential side effects of pathogen-reduced platelets (23).

Red Blood Cells
The S-303 system, which is in clinical development, is the only PI technology available for RBCs. Current studies are investigating the second-generation S-303 PI process. The first-generation S-303 procedure only marginally affected RBC quality and function, but after reports of immunization against pathogen-inactivated RBCs in transfused patients emerged, a new generation of the S-303 system had to be developed. In the second-generation S-303 system, the quencher concentration of GSH was increased from 2 to 20 mmol/l in order to decrease the affinity of S-303 for proteins and thus to avoid the formation of neoantigens on the surface of erythrocytes (24). However, recent studies show that immunization against S-303-coated RBCs still occurs after modification of the S-303 system (25). In particular, the fact that antibodies against S-303-treated cells were also detected in healthy blood donors who had never been transfused with pathogen-reduced RBCs suggests that some individuals may be immunized by S-303-like substances in the environment (e.g., food or air) or may have naturally occurring antibodies against epitopes on the S-303 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 III clinical trials to test the second-generation S-303 PI system for RBCs in acute and chronic anemia patients are currently ongoing or planned.

IMPLEMENTATION IN ROUTINE USE
The INTERCEPT and MIRASOL PI systems for platelets and plasma are used in some parts of Asia, Europe, and the USA. In Europe, the willingness to use pathogen-reduced platelet units varies between countries and regions. PI technologies are implemented nationwide in some countries (e.g., Switzerland and Belgium), but only regionally in others (e.g., Poland).

Evaluation of PI technologies for platelets is under way at some blood centers in Germany. In 2011, the Swiss national authority (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 Swiss 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 US 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 platelets (26, 27).

The preventive 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 30% of the inhabitants were infected, local blood donation was suspended to prevent TTI. To sustain the availability of platelet components, the French national blood service (Etablissement Français du Sang) implemented universal PI of platelet components on the island. The success of this measure demonstrated that PI can effectively support the availability of safe labile blood components during an epidemic.

The West Nile virus epidemic in the USA was the first example of a large-scale arboviral threat to the blood supply of a Western country that required an urgent 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, “FDA guidance recommends that blood be outsourced from unaffected areas, unless there are measures to screen donations using a laboratory test, or unless the blood components are subjected to PI technology” 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 and profound safety and efficacy record of pathogen-reduced blood cellular products, there are still concerns that impede the introduction of PI technology in hemotherapy. The INTERCEPT protocol includes incubation and adsorption steps that result in a significant loss of platelets (up to 15%) during preparation and PI treatment. However, this loss could be offset by performing PI with higher platelet counts in the starting products. The platelet yields could be increased by using more buffy coats (e.g., five instead of four) to manufacture pooled platelets, or by collecting higher numbers of platelets during the apheresis process. Moreover, this measure could compensate for reduced platelet CIs in transfusion recipients and thus lower the possible need for increased platelet unit utilization.

All PI technologies mentioned in this review exhibit gaps in their PI efficacy. The amotosalen/UVA-based system (INTERCEPT) is ineffective for non-enveloped viruses such as hepatitis A, hepatitis E, and parvovirus B19 (30). The riboflavin/UV-based system (MIRASOL) has only weakly effects against
bacteria and some viruses (31). The UVC light-based system (THERAFLEX) is highly effective against bacteria and most transfusion-relevant viruses, but only moderately effective against HIV (32). However, when highly sensitive screening tests for HIV are performed, UVC-based PI could further reduce the risk of virus transmission during the “window period” in which the pre-nucleic acid testing can be negative and in patients with occult infections. Despite these weaknesses, PI systems generally have the potential to significantly add an additional layer of safety to blood transfusion.

Major concerns surrounding the implementation of PI have to do 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 alkylation agents such as amotosalen may be carcinogenic in the long term in a subset of transfused patients. A major advantage of the THERAFLEX system is that it works without phototoxic substances, thus eliminating the risk of photoreagent-related adverse events (10, 13).

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 margin of the entire blood supply. As long as PI is not routinely implemented in the production of RBC units (the most commonly used blood components), 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-benefit analysis suggests that if a new pathogen entered the blood supply, the use of pathogen-reduced plasma and platelets would reduce the risk of TTI by 40% (33).

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 (36). Finally, the available resources influence how politicians 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, it will be difficult to explain to individuals with severe transfusion-associated infections why this readily available risk mitigation and safety measure was not implemented.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

REFERENCES


Conflict of Interest Statement: The author works for German Red Cross Blood Service NSTO, a blood donation center that collaborates with Macopharma on the development of a pathogen inactivation system for platelets. The author did not receive any financial support relevant to this manuscript.
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.
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, Gravemann U, Döscher A, Brixner 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 shortamplicon (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.

Fryk JJ, Marks DC, Hobson-Peters J, Watterson D, Hall RA, Young PR, Reichenberg S, Tolksdorf F, Sumian C, Gravemann U, Seltsam A, Faddy HM.

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

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

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

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

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.

Van Aelst B, Devloo R, Vandekerckhove P, Compernolle V, Feys HB.


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

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

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

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


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

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

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

CONCLUSIONS:
The THERAFLEX UV-Platelets (MacoPharma) PR system can substitute gamma irradiation for TA-GVHD prophylaxis in platelet (PLT) transfusion. Moreover, UVC treatment achieves suppression of antigen presentation and inhibition of cytokine accumulation during storage of PCs, which has potential benefits for transfusion recipients.
In vitro function of platelets treated with ultraviolet C light for pathogen inactivation: a comparative study with non-irradiated and gamma-irradiated platelets.

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. Three PCs were pooled and divided into 3 units. One unit was used as a non-irradiated control, the second was a gamma-irradiated control, and the third unit was treated with UVC light. In vitro variables including analysis of coagulation by free oscillation rheometry were analysed on Days 1, 5, and 7 of storage. Ten units in each group were investigated.

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

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

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


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

Schlenke, P.


Nowadays patients receiving blood components are exposed to much less transfusion-transmitted infectious diseases than three decades before when among others HIV was identified as causative agent for the acquired immunodeficiency syndrome and the transmission by blood or coagulation factors became evident. Since that time the implementation of measures for risk prevention and safety precaution was socially and politically accepted. Currently emerging pathogens like arboviruses and the well-known bacterial contamination of platelet concentrates still remain major concerns of blood safety with important clinical consequences, but very rarely with fatal outcome for the blood recipient. In contrast to the well-established pathogen inactivation strategies for fresh frozen plasma using the solvent-detergent procedure or methylene blue and visible light, the bench-to-bedside translation of novel pathogen inactivation technologies for cell-containing blood components such as platelets and red blood cells 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 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.

Castro E, González LM, Rubio JM, Ramiro R, Gironés N, Montero E.
Transfusion 2014; 54(9):2207-2216.

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/cm² UVC light using the THERAFLEX UV-Platelets method (Macopharma). Viability and parasite growth were evaluated before and after inactivation. Culture growth kinetics were monitored by DNA incorporation of [³H] thymidine. The ability of B. divergens to survive in PLT concentrates was also analysed.

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

CONCLUSION:
Since B. divergens can survive in PLT concentrates and given the performance of UVC, this system could be considered as an alternative to prevent B. divergens and other Babesia species from being transmitted through PLT transfusions.
Two pathogen reduction technologies—methylene blue plus light and shortwave ultraviolet light—effectively inactivate hepatitis C virus in blood products.


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

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

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

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

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, Tolksdorf 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/Caridian BCT, Lakewood, CO, USA) are based on adding a photosensitive compound to PC. The mixture is then activated by UV light in the UVB and/or UVA spectral regions. A novel procedure, THERAFLEX UV-Platelets (MacoPharma, Mouvaux, France), was recently developed that uses short-wave ultraviolet light (UVC), without addition of any photoactive agent. This technology has proven to be highly effective in sterilising bacteria (the major cause of morbidity/mortality after platelet transfusion) as well as inactivating other transfusion transmitted DNA/RNA containing pathogens and residual leucocytes. Any PIT reflects a balance between the efficacy of pathogen inactivation and preservation of platelet quality and function. A broad spectrum of in vitro tests have become available for the assessment of platelet storage lesion (PSL), aiming to better predict clinical outcome and untoward effects of platelet therapy. Recent paired studies on the release of platelet-derived cytokines, as 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.
Lead the way in blood safety

www.bloodsafety.macopharma.com