BLOOD SAFETY

BIBLIOGRAPHY

• THERAFLEX MB-Plasma
• THERAFLEX UV-Platelets
• SSP+
2017
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, Springe. It is a user-friendly in-house treatment for single units of plasma adapted for the inactivation of pathogens in Fresh Frozen Plasma from aphaeresis 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 development is a joint project between Forschungsgemeinschaft, the German Red Cross Blood Services, and Macopharma to inactivate recognized and emerging pathogens in platelets. 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 2018.

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 7 million units of Macopharma Platelet Additive Solution have been distributed in 43 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.
### THERAFLEX MB-Plasma scientific publications

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors, title and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Fryk JJ, Marks DC, Hobson-Peters J, Prow NA, Watterson D, Hall RA, Young PR, Reichenberg S, Surnian C, Faddy HM. <strong>Dengue and chikungunya viruses in plasma are effectively inactivated after treatment with methylene blue and visible light.</strong> Transfusion 2016; 56;2278–2285.</td>
</tr>
<tr>
<td>Year</td>
<td>Authors, title and references</td>
</tr>
<tr>
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</tr>
<tr>
<td>Year</td>
<td>Authors, title and references</td>
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</tbody>
</table>
Cost-utility and budget impact of methylene blue-treated plasma compared to quarantine plasma

Joseph B. Babigumira1,2, Solomon J. Lubinga1,2, Emma Castro3, Brian Custer4,5

1Global Medicines Program, Department of Global Health, University of Washington, Seattle, United States of America; 2Pharmaceutical Outcomes Research and Policy Program, Department of Pharmacy, University of Washington, Seattle, United States of America; 3Community Blood Transfusion Centre, Valencia, Spain; 4Blood Systems Research Institute, San Francisco, United States of America; 5Department of Laboratory Medicine, UCSF, San Francisco, United States of America

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, MPB was estimated to be more effective than QP in all analyses.

Keywords: plasma, pathogens, adverse events, costs and cost analysis.

Introduction

There is a range of pathogen inactivation technologies which are approved and used to treat blood components in Europe1. Methylene blue and visible light treatment and quarantine are two common methods used to reduce the risk of adverse events associated with plasma transfusion in Spain and in other countries. Each method has attendant costs and possibly different adverse event implications for recipients2,3. Fresh-frozen plasma (FFP) can be derived from whole blood (WB) or apheresis plasma collections. The available methods for improving plasma safety also have consequences for secondary use of FFP. For example, methylene blue and visible light-treated plasma (MBP) cannot be used for recovered plasma fractionation. In addition, each inactivation method may lead to alterations in the relative activity of therapeutic plasma proteins2, although evidence of increased plasma transfusion with the use of MBP compared to FFP has not been documented. On the other hand, quarantine plasma (QP) involves establishing the procedures for quarantine, the physical capability to store FFP for longer terms while in quarantine and the processes for donor recall that permit the release of FFP for transfusion. Blood centres in Spain use different approaches to collect plasma, but the majority of FFP is obtained from WB donations.

Similarly, blood centres may use different security measures with respect to quarantine. Quarantine relies on donors coming back to make a new blood donation or to provide a sample for testing before the stored FFP can be cleared for release. Passive quarantine relies on donors returning for the subsequent donation with
no mechanism, such as calling the donors, to seek the donors’ return. Active quarantine is where donors are contacted and asked to return as early as allowed in the quarantine period; maintaining such a programme requires human and other resources.

The risk of adverse outcomes for recipients of MBP or QP is not the same. MBP may have advantages in two areas. First, in the area of any type of infection for which donor screening is not or is only partially in place, and particularly for emerging infections that may have asymptomatic phases of infection such as West Nile, dengue and Zika viruses. Second, the available evidence demonstrates that the risk of allergic and other non-infectious reactions is lower for MBP than for QP.

The Alliance of Blood Operators Risk-Based Decision-Making initiative recently published recommendations for health economics and outcomes analysis of blood safety technologies. While these are consensus recommendations, which may not be applicable to all settings, two evaluation methods were recommended because of the complimentary health economic information they contribute to decision-making in the field of blood safety. The first method is used to assess the costs that accrue or are expected to accrue when an intervention is implemented; budget impact analysis (BIA) measures resource use and provides results in terms of the costs incurred or saved by adopting an intervention from the standpoint of the budgeting authority or health care decision-maker. The second methodology is used to assess value gained for resources spent; cost-utility analysis (CUA) where value for money is assessed in terms of cost per quality-adjusted life-years (QALY) gained. The objective of our study was to estimate the budget impact and cost-utility (sometimes known as cost-effectiveness), from a payer’s perspective, of using MBP compared to QP for the reduction of pathogens and adverse events related to transfused plasma in Spain considering different approaches to plasma collection and the costs of MBP and QP.

Materials and Methods

Model structure

We developed a decision analytic model in Microsoft Excel (2011) to estimate the costs, outcomes, and budget impact of transfusing patients with MBP and QP in Spain. The model combined a “frontend” decision tree and two “backend” Markov models. The decision tree (Figure 1) was used to model: (i) hepatitis B or C virus infection (HBV, HCV) with risk of rapid liver failure, (ii) human immunodeficiency virus (HIV) infection, (iii) cytomegalovirus (CMV) infection (asymptomatic CMV, CMV retinitis, and CMV mononucleosis), (iv) a West Nile virus (WNV)-like emerging infection (asymptomatic, WNV fever and chronic neuro-invasive disease), (v) severe and non-severe allergic reactions, and (vi) febrile non-haemolytic transfusion reactions (FNHTR). We assumed that patients would not simultaneously experience more than one adverse event. Markov models (Online Supplementary Figure S1) were used to simulate the costs and outcomes of chronic hepatitis B and C, and HIV infection. The hepatitis Markov model (Online Supplementary Figure S1A) had ten states (chronic hepatitis, compensated cirrhosis, hepatocellular carcinoma, oesophageal varices, hepatic encephalopathy, ascites, three liver transplant states, and death). The HIV Markov model (Online Supplementary Figure S1B) had four states (HIV, chronic HIV, AIDS, and death). The cycle length for Markov modelling was 1 year and the time horizon of the analysis was lifetime. For all other adverse events we assumed that the consequences would occur within the first year except for the sequelae of WNV neuro-invasive disease, which could last a lifetime.

![Figure 1 - Decision tree of adverse events and outcomes of plasma transfusion.](image-url)
Event probabilities

To estimate probabilities of adverse events, we used haemovigilance data on MBP and QP collected over 11 years in Greece except for the annual probability of WNV for transfused QP (assumed to be 0.0005) and HIV for both transfused MBP and QP. For the annual probability of HIV, we used a recent publication from Spain which reported the first case of breakthrough HIV infection using MBP and the estimated number of MBP transfusions during the same time period based on MBP kit distribution data. Adverse event probabilities are summarised in Table I.

We used publically available literature to estimate transition probabilities for the Markov models. These probabilities are summarised in the Online Supplementary Table SI. We estimated background post-transfusion mortality from population-based studies of survival. Post-transfusions survival data are from sources outside Spain.

Outcomes

To estimate QALY, we obtained health state utilities for adverse events, infections, and their sequelae from the published literature, supported by assumptions where estimates were unavailable. Health state preference weight utility estimates are summarised in the Online Supplementary Table SII.

Costs

We estimated costs from the payer’s perspective and included costs of production of MBP and QP, and the management of adverse events. All costs were adjusted to the year 2014 using the Consumer Price Index for healthcare in Spain. Future costs were discounted at 3% per annum. The estimate of the mean costs of MBP and QP per patient assumes a single transfusion episode using four FFP units.

We estimated the costs of producing FFP from WB, by apheresis plasma, and by multicomponent apheresis, and separately for the passive and active methods of donor recall. To estimate the costs of FFP production, we used the approach described by Eandi and colleagues. According to their method, the cost per unit of transfusable plasma is calculated by adjusting the cost of obtaining a litre of plasma by the mean plasma yield and the mean number of 200 mL-containing units that can be obtained using WB, apheresis plasma, and multicomponent apheresis.

To estimate the costs of QP, we used volume and costs (handling and storage) estimates from a single blood centre in Spain. This centre processes 65,000 WB donations plus 2,500 apheresis collections per year. Approximately 8,000 units of plasma (280 mL each) are released to hospitals each year. We adjusted costs to account for the proportion of QP FFP units from non-returning donors that are sold for fractionation. For QP, we accounted for the costs of donor recall and retesting in the active donor recall scenario, the loss rates from donors who do not return for testing, and loss rates due to handling. For MBP, data were based on the Macopharma system. We assumed no additional storage and handling costs for MBP beyond FFP storage. The parameters for the estimation of the costs of MBP and QP are summarised in Table II.

We used data from the published literature to estimate the costs of managing adverse events and treating breakthrough infections using country-specific data where possible. These data are summarised in Table II.

Budget impact

We estimated the budget impact of MBP and QP over a 5-year time horizon. We projected the number of plasma transfusions using the overall population of

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Value Source</th>
<th>Value Source</th>
<th>Value Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>0.00000001</td>
<td>Politis et al.</td>
<td>0.00000001</td>
</tr>
<tr>
<td>HCV</td>
<td>0.00000001</td>
<td>Politis et al.</td>
<td>0.00000001</td>
</tr>
<tr>
<td>HIV</td>
<td>0.00000001</td>
<td>Politis et al.</td>
<td>0.00000005</td>
</tr>
<tr>
<td>CMV</td>
<td>0.000001</td>
<td>Politis et al.</td>
<td>0.00000001</td>
</tr>
<tr>
<td>WNV-like</td>
<td>0.0005</td>
<td>Custer et al.</td>
<td>0.00000001</td>
</tr>
<tr>
<td>Non-severe allergic reaction</td>
<td>0.00495</td>
<td>Politis et al.</td>
<td>0.0000037</td>
</tr>
<tr>
<td>Severe allergic reaction</td>
<td>0.002127</td>
<td>Politis et al.</td>
<td>0.0001</td>
</tr>
<tr>
<td>FNHTR</td>
<td>0.0007</td>
<td>Politis et al.</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

All probabilities were varied by ±20% for sensitivity analyses. QP: quarantine plasma; MBP: methylene blue- and visible light-treated plasma; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; CMV: cytomegalovirus; WNV: West Nile virus; FNHTR: febrile non-haemolytic transfusion reaction.
### Table II - Parameters used to estimate plasma production and storage costs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (range)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cost of production for 1 litre of plasma, €</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>113 (90, 135)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td>Plasma apheresis</td>
<td>284 (227, 341)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td>Multicomponent apheresis</td>
<td>183 (146, 220)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td><strong>Mean plasma yield per donation, mL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>270 (216, 324)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td>Plasma apheresis</td>
<td>600 (480, 720)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td>Multicomponent apheresis</td>
<td>399 (320, 478)</td>
<td>Eandi et al.12</td>
</tr>
<tr>
<td><strong>Units of transfusable plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>1</td>
<td>Assumption</td>
</tr>
<tr>
<td>Plasma apheresis</td>
<td>3</td>
<td>Assumption</td>
</tr>
<tr>
<td>Multicomponent apheresis</td>
<td>2</td>
<td>Assumption</td>
</tr>
<tr>
<td><strong>Plasma storage and handling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area occupied by freezer, €</td>
<td>3,500 (2,800, 4,200)</td>
<td>PC</td>
</tr>
<tr>
<td>Amortization of freezer, €</td>
<td>10,315 (8,252, 12,378)</td>
<td>PC</td>
</tr>
<tr>
<td>Freezer maintenance, €</td>
<td>6,444 (5,155, 7,732)</td>
<td>PC</td>
</tr>
<tr>
<td>Alarm maintenance, €</td>
<td>2,100 (1,680, 2,520)</td>
<td>PC</td>
</tr>
<tr>
<td>Alarm calibration, €</td>
<td>457 (365, 548)</td>
<td>PC</td>
</tr>
<tr>
<td>Electricity, €</td>
<td>25,200 (20,160, 30,240)</td>
<td>PC</td>
</tr>
<tr>
<td>Storage canisters, €</td>
<td>800 (640, 960)</td>
<td>PC</td>
</tr>
<tr>
<td>Information system adaptation, €</td>
<td>600 (480, 720)</td>
<td>PC</td>
</tr>
<tr>
<td>Technical personnel, €</td>
<td>28,000 (22,400, 33,600)</td>
<td>PC</td>
</tr>
<tr>
<td>Calling donors, €</td>
<td>1.5 (1.20, 1.80)</td>
<td>PC</td>
</tr>
<tr>
<td>Retesting donors, €</td>
<td>16.5 (13.20, 19.80)</td>
<td>PC</td>
</tr>
<tr>
<td>Loss rate, handling MBP</td>
<td>0.010 (0.008, 0.012)</td>
<td>PC</td>
</tr>
<tr>
<td>Loss rate, handling QP</td>
<td>0.035 (0.028, 0.042)</td>
<td>PC</td>
</tr>
<tr>
<td>Passive quarantine, loss rate because donors do not return</td>
<td>0.450 (0.360, 0.540)</td>
<td>PC</td>
</tr>
<tr>
<td>Active quarantine, loss rate because donors do not return</td>
<td>0.300 (0.240, 0.360)</td>
<td>PC</td>
</tr>
<tr>
<td>Plasmapheresis loss rate because donors do not return</td>
<td>0.200 (0.160, 0.240)</td>
<td>PC</td>
</tr>
<tr>
<td>Loss rate because donors test positive for infectious agent</td>
<td>0.0005 (0.0004, 0.0006)</td>
<td>PC</td>
</tr>
<tr>
<td>Per litre value of quarantine plasma for fractionation, €</td>
<td>43 (22, 65)</td>
<td>PC</td>
</tr>
<tr>
<td>Per unit cost of MBP treatment, €</td>
<td>22 (17, 26)</td>
<td>PC</td>
</tr>
<tr>
<td><strong>Adverse event and breakthrough infection costs, €</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic WNV, febrile</td>
<td>7,577 (6,100, 9,100)</td>
<td>Staples et al. 25</td>
</tr>
<tr>
<td>Symptomatic WNV, neuroinvasive disease</td>
<td>52,934 (42,300, 63,500)</td>
<td>Staples et al. 25</td>
</tr>
<tr>
<td>WNV, sequelae of neuroinvasive disease</td>
<td>23,672 (18,937, 28,400)</td>
<td>Staples et al. 25</td>
</tr>
<tr>
<td>CMV retinitis</td>
<td>4,908 (3,874, 5,961)</td>
<td>Keilberger et al. 26</td>
</tr>
<tr>
<td>CMV infectious mononucleosis</td>
<td>4,908 (3,874, 5,961)</td>
<td>Keilberger et al. 26</td>
</tr>
<tr>
<td>Severe allergy</td>
<td>4,910 (3,928, 5,892)</td>
<td>Kacker et al. 27</td>
</tr>
<tr>
<td>Non-severe allergy (utility decrement)</td>
<td>179 (143, 215)</td>
<td>Kacker et al. 27</td>
</tr>
<tr>
<td>HCV, acute</td>
<td>4,864 (3,891, 5,836)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>HCV, chronic</td>
<td>243 (194, 291)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>HCV, compensated cirrhosis</td>
<td>435 (348, 523)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>HCV, hepatocellular carcinoma</td>
<td>6,811 (5,449, 8,173)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>HBV, acute</td>
<td>871 (697, 1045)</td>
<td>Idris et al. 29</td>
</tr>
<tr>
<td>HBV, chronic</td>
<td>259 (208, 311)</td>
<td>Idris et al. 29</td>
</tr>
<tr>
<td>HBV, compensated cirrhosis</td>
<td>465 (372, 558)</td>
<td>Idris et al. 29</td>
</tr>
<tr>
<td>HBV, hepatocellular carcinoma</td>
<td>7,267 (5,813, 8,720)</td>
<td>Idris et al. 29</td>
</tr>
<tr>
<td>Variceal bleeding, year 1</td>
<td>4,967 (3,973, 5,960)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Variceal bleeding, subsequent years</td>
<td>1,511 (1,209, 1,813)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Hepatic encephalopathy, year 1</td>
<td>6,035 (4,827, 7,242)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Hepatic encephalopathy, subsequent years</td>
<td>1,540 (1,232, 1,848)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Ascites, year 1</td>
<td>1,424 (1,139, 1,799)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Ascites, subsequent years</td>
<td>10,854 (8,683, 13,024)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Liver transplant</td>
<td>139,400 (111,500, 167,300)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>Post-liver transplant</td>
<td>15,394 (12,315, 18,473)</td>
<td>Buti et al. 28</td>
</tr>
<tr>
<td>HIV, acute</td>
<td>-</td>
<td>Assumed untreated</td>
</tr>
<tr>
<td>HIV, chronic</td>
<td>9,877 (7,902, 11,853)</td>
<td>López-Bastida et al. 29</td>
</tr>
<tr>
<td>AIDS</td>
<td>12,765 (10,212, 15,318)</td>
<td>López-Bastida et al. 29</td>
</tr>
<tr>
<td>FNHTR</td>
<td>90.78 (72.62, 108.94)</td>
<td>Kacker et al. 27</td>
</tr>
</tbody>
</table>

*For 8,000 units of plasma. PC: personal communication; QP: quarantine plasma; MBP: methylene blue- and visible light-treated plasma; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; CMV: cytomegalovirus; WNV: West Nile virus; AIDS: acquired immunodeficiency syndrome; FNHTR: febrile non-haemolytic transfusion reaction.*
Spain and an annual incidence of plasma transfusion of 0.0039\(^4\). We assumed 50-50% market share for MBP and QP.

**Cost-utility**

Our base case analysis was a comparison of MBP and QP FFP produced from WB with passive and active donor recall. We used cost and QALY estimates to calculate incremental costs and QALY and the incremental cost-effectiveness ratio (ICER) presented as €/QALY gained.

**Sensitivity analysis**

Univariate sensitivity analyses were conducted for both the budget impact and cost-utility models. We performed univariate sensitivity analyses to determine the impact of all individual model parameters on results. We derived sensitivity ranges from 95% confidence intervals for parameters when available. When these were not available we used ±20% for probabilities and ±50% for costs. When the parameter was assumed or based on experts’ opinion, we also used ±50% to represent greater uncertainty, capping probability estimates whose ranges exceeded 0 or 1 at these respective values.

We conducted probabilistic sensitivity analysis to evaluate the overall uncertainty by assigning probability distributions to all parameters in the model. We performed 5,000 second order Monte Carlo simulations and used the net-benefit framework (a linearisation of the ICER based on varying willingness to pay per QALY gained) to compute the probability of cost effectiveness and to construct a cost-effectiveness acceptability curve.

**Results**

**Budget impact**

In the passive quarantine scenario, MBP led to a net increase of € 850,352 compared to QP over 5 years, or a net cost increase of approximately € 170,000 per year. In the active quarantine scenario MBP led to net savings of € 5,890,425 compared to QP over 5 years, or approximately € 1,178,000 per year. If the cost of QP is not recovered by selling non-usable FFP for fractionation, MBP is cost saving for all scenarios considered, including WB collections with passive quarantine. For this scenario MBP led to net savings of € 1,503,235 compared to QP over 5 years.

**Cost-utility**

Results of the baseline cost-utility analysis are summarised in Table III. In both the passive and active quarantine scenarios, on average MBP increased QALY by 0.000010225 compared to QP. Under the passive quarantine scenario, MBP increased mean cost by € 7.21 per patient compared to QP for ICER of € 705,126/QALY gained. In the active quarantine scenario, MBP reduced mean cost by € 50.46 per patient compared to QP and dominated QP i.e., MBP was both more effective and less costly.

**Sensitivity analyses**

Sensitivity analysis results presented here are restricted to the cost-utility analysis. A tornado diagram of the univariate sensitivity analysis for the passive quarantine scenario is shown in Figure 2 (panel A). The ICER was most sensitive to the cost of MBP processing, the mean yield per donation from WB, the cost per unit of plasma derived from WB collections, the cost per litre of plasma obtained for fractionation, and the estimated number of units produced in a year. Panels B and C of Figure 2 are tornado diagrams of the univariate sensitivity analysis for the active quarantine scenario. Because we estimated that MBP FFP was dominant over QP FFP, we present separately the impact of varying individual parameters on incremental QALY (panel B) and incremental costs (panel C). The incremental QALY were most sensitive to the time in days a patient would have a severe allergic reaction and the incremental cost was most sensitive to the cost of MBP processing.

The results of the probabilistic sensitivity analyses for the passive quarantine scenario are presented in Figure 3 as a scatter plot and cost-effectiveness acceptability curve. As shown in the scatter plot, there is uncertainty as to whether MBP increases costs compared to QP (some simulations indicate increased incremental costs whereas others indicate decreased incremental.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Passive quarantine</th>
<th>Active quarantine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quarantine FFP</td>
<td>MB treated FFP</td>
</tr>
<tr>
<td>Cost</td>
<td>€ 201.85</td>
<td>€ 209.06</td>
</tr>
<tr>
<td>Life years</td>
<td>3.347591921</td>
<td>3.347592727</td>
</tr>
<tr>
<td>QALY</td>
<td>3.012823085</td>
<td>3.012833310</td>
</tr>
<tr>
<td>Cost per QALY gained</td>
<td>€ 705 126</td>
<td>Dominant*</td>
</tr>
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</table>

*Dominant means MBP is more effective and less costly compared to QP. MBP: methylene blue- and visible light-treated plasma; QP: quarantine plasma; FFP: fresh frozen plasma; QALY: quality-adjusted life years.

**Table III - Baseline cost, outcomes and cost-utility analysis comparing MBP to QP from whole blood-derived plasma.**
Figure 2 - Tornado diagrams of univariate sensitivity analyses showing the impact of varying parameters through their ranges (i) on the ICER comparing MBP to QP in the passive quarantine scenario (panel A), (ii) on the increase in QALY comparing MBP to QP prepared from whole blood in the active quarantine scenario (panel B) and on the decrease in costs comparing MBP to QP prepared from whole blood in the active quarantine scenario (panel C). The ten most sensitive parameters are shown.

MBP: methylene blue- and visible light-treated plasma; WB: whole blood; QP: quarantine plasma; QALY: quality-adjusted life years; WNV: West Nile virus.

**Panel A: Incremental costs (€) per QALY gained**

- Unit cost for MBP processing, € (17.2, 25.8)
- Mean yield/donation (WB) (216, 324)
- WB production cost per litre of plasma, € (90.2, 135.3)
- Unit (per litre) cost of QP for fractionation (21.5, 64.5)
- Valid units produced per year (6000, 9000)
- Passive system loss rate (donors do not return) (0.36, 0.54)
- Technical personnel annual cost, € (22400, 33600)
- Annual cost of electricity, € (20160, 30240)
- Units (of plasma) per patient (3, 5)
- Time (days) with non-severe allergic reaction (3, 7)

**Panel B: QALYs gained**

- Time (days) with non-severe allergic reaction (3, 7)
- Non-severe allergic reaction (utility decrement) (0.05, 0.1)
- QALY weight for person getting transfusion (0.72, 1)
- Severe allergic reaction (utility decrement) (0.1, 0.3)
- Annual probability of non-severe allergic reactions (QP) (0.00396, 0.00594)
- WNV symptomatic, neuroinvasive disease but recover, with sequelae (utility) (0.6333, 0.7740333)
- Annual probability of WNV-like infection (QP) (0.00004, 0.00006)
- Annual probability of symptomatic WNV-like virus (0.2, 0.3)
- Annual probability of WNV-related neuroinvasive disease (0.16, 0.24)
- Annual probability of severe allergic reactions (QP) (0.00017022, 0.00025532)

**Panel C: Incremental costs (€)**

- Unit cost for MBP processing, € (17.2, 25.8)
- Donor retesting costs, € (per unit) (13.2, 19.8)
- Units (of plasma) per patient (3, 5)
- Valid units produced per year (6000, 9000)
- Mean yield/donation (WB) (216, 324)
- WB production cost per litre of plasma, € (90.2, 135.3)
- Unit (per litre) cost of QP for fractionation (21.5, 64.5)
- Technical personnel annual cost, € (22400, 33600)
- Active system loss rate (donors do not return) (0.24, 0.36)
- Annual cost of electricity, € (20160, 30240)
Health economics of methylene blue-treated plasma

In this analysis comparing MBP to QP we found that the type of plasma collection approach and quarantine system will influence the budget impact and cost-utility of MBP. If QP is as simple as possible relying on plasma from WB collection and passive quarantine, QP is less costly than MBP, but only if recovered plasma that cannot be transfused from QP is sold for fractionation. If recovered QP is not sold for fractionation the budget impact favours MBP in all scenarios. Without the recovered plasma option for QP, MBP would be more financially favourable, more effective, and represent a dominant strategy over QP. Several other analyses were developed including costs and consequences of collecting apheresis plasma and multicomponent apheresis coupled with passive or active donor recall, but these approaches are used less commonly in Spain and so the results are not reported here. In these analyses, the patterns observed for WB were largely replicated (data not shown).

In terms of cost-utility, MBP was estimated to be more effective than QP in all of our analyses, although the gain in QALY was small. As a result, in the WB collection and passive quarantine scenario, the ICER for MBP was high relative to typical acceptable thresholds in health and medicine when recovered QP is sold for fractionation. In the context of blood safety, the ICER result of just over € 700,000/QALY is consistent with that of several other interventions which have been adopted in countries with high development indices15, including Spain. The cost-utility findings of the use of MBP compared to QP including recovered plasma sold for fractionation are similar to those for other pathogen inactivation technologies focused on the treatment of plasma16,17. Nucleic acid testing is commonplace in most countries with high development indices and its cost-utility has been estimated to range between € 1,500,000-€ 6,000,000 per QALY depending on whether mini-pool or individual donation testing is adopted18,19. In Spain, individual donation nucleic acid testing is used to screen donations for HIV, HBV, and HCV.

**Figure 3** - Probabilistic sensitivity analysis comparing MBP to QP prepared from whole blood under the passive and active quarantine scenarios.

The figure on the left is a scatter plot of incremental cost and QALY gained pairs, and the figure on the right shows a cost-effectiveness acceptability curve. For the passive quarantine scenario, the scatter plot shows that MBP is more effective in all 5,000 Monte Carlo simulations, but approximately half of the time the total incremental cost is higher for MBP. For the active quarantine scenario, MBP is more effective and has lower incremental costs in all 5,000 Monte Carlo simulations. The cost-effectiveness acceptability curve indicates the probability of cost-effectiveness at different thresholds of willingness to pay (WTP) for a QALY gained. MBP: methylene blue- and visible light-treated plasma; QP: quarantine plasma; QALY: quality-adjusted life years.

*Blood Transfus* DOI 10.2450/2016.0130-16

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The cost of treating HCV infection with new drug therapies was not included in this analysis. With individual donation HCV NAT testing in place the residual risk of transmission is very low. If we were to include the cost of new drug therapies in the analysis, the effect would be to improve the relative costs and cost-utility of MBP compared to QP albeit to a small degree because the risk of transfusion-transmitted HCV is very low.

There are limitations to our analyses. Data for Spain were not available for us to use for adverse events after plasma transfusion. For example, Spanish haemovigilance data on adverse events following the transfusion of plasma do not differentiate between QP and MBP. Better-differentiated outcome data could alter our results. We used data from other Mediterranean countries in Europe, which show higher adverse events for QP than for MBP6-20. The majority of these events are allergic reactions or FNHTR. The patterns observed in Greece and other countries have been observed in Spain1, but have not yet been reported in sufficient detail such that Spanish data could be used in our evaluation.

Another limitation is that the QP costs are from one blood centre. Different centres in Spain use different approaches to QP, including passive and active quarantine, and also different approaches to inventory control, such as manual and automated storage and retrieval of plasma units. Each of these approaches to QP will influence the budget impact and cost-effectiveness of the approaches used to increase the safety of plasma transfusions.

A further limitation is that the adverse events associated with plasma transfusion, which have been included in this analysis, do not represent an exhaustive list of all infectious and non-infectious threats. For the majority of known transfusion-transmissible viruses, plasma is the component with the highest risk of transmission while platelets have the highest risk of bacterial contamination and red cells the highest risk of transmission of cell-associated pathogens such as

Plasmodium (malaria) and Babesia (babesiosis). A further aspect of this limitation with respect to the available haemovigilance data is the inclusion of CMV as one of the adverse events associated with FFP. While CMV is a cell-associated virus and, therefore, unlikely to be primarily transmitted by plasma21, the haemovigilance data from Greece did report the occurrence of plasma-associated CMV transmission. This risk is low and CMV outcomes or costs were not influential parameters in any of our analyses. Even so, the inclusion of CMV in our analysis serves as a surrogate for other viral infections which might have serious consequences on specific populations of patients and for which testing may not be in place, thus establishing a differential risk of transmission between QP and MBP. Any plasma intervention that uses an active reduction or inactivation technology, such as MBP, solvent/detergent treatment, riboflavin plus ultraviolet light, or amotosalen plus ultraviolet light treatment, has increased potential to reduce other viral infections, which QP alone could not prevent. However, the low absolute risk of adverse events, both infectious and immunological, associated with plasma infusion, may explain the lack of randomised controlled trials directly comparing the safety of FFP prepared using different technologies22. If additional but other uncommon infections were included, the overall estimate of better effectiveness for using MBP compared to QP would be expected to increase. Finally, the reduction of infectious risk is counter-balanced by the risks related to the inactivation technology or specific reactants, which are inherent in the process of each inactivation procedure23. On balance, these non-infectious immunological adverse effects are minimal and have been shown to be lower for MBP than for QP.

Conclusions

In many countries in Europe the decision to use pathogen reduction technology has not been driven by the results of cost-utility analyses because the thresholds that are commonly regarded as cost-effective in clinical practice have not been met by most blood safety interventions, at least in countries in which as close to zero-risk for infectious threats has been perceived as the most appropriate blood safety policy24. Health economics involves two considerations: the overall impact on health care budgets and value for money spent. The budget impact for MBP varied according to the approach used to obtain FFP and the quarantine system in use for plasma. Although the full cost of QP is difficult to calculate and dependent on the structure of the QP system, when costs previously unaccounted for are included, MBP approaches cost neutrality for WB and is cost-saving and more cost-effective under any active QP and/or apheresis approach. Finally, the analysis of MBP shows that this technology is more effective than QP in terms of generating additional health benefit for plasma-transfused patients, regardless of the quarantine system in place.

Funding and resources

This study was funded by an unrestricted grant from Macopharma.

Authorship contributions

BC conceived the study. JBB, SJL, and BC designed the study, including the development of the economic models. JBB, SJL, EC, and BC obtained data and performed the analysis. JBB wrote the first draft of the manuscript. SJL, EC, and BC each wrote sections of the final manuscript. All Authors approved the final submission version of the manuscript.

Babigumira JB et al

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Health economics of methylene blue-treated plasma

The Authors declare no conflicts of interest.

References

14) Subdirección General de Promoción de la Salud y Epidemiología.
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.
Paired comparison of methylene blue- and amotosalen-treated plasma and cryoprecipitate.

Backholer L, Wiltshire M, Proffitt S, Cookson P, Cardigan R.

Vox Sang 2016; 110:352–361

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.

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


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

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

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

CONCLUSION:
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.


Blood Transfus 2016;14: 66-72

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

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

Vox Sang 2015 Aug;109(2) :129-37

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.

MATERIAL 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.


Transfus Med 2014;24: 316-320

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

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

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

CONCLUSION:
Haemovigilance over 11 years has demonstrated the long-term safety of MB-FFP in comparison to untreated quarantine FFP. In addition to lowering the adverse event rate, implementing the system on a national scale in at-risk countries would presumably reduce the transmission of severe viral infections including emerging infectious diseases by transfusion.
FVIII and fibrinogen recovery after THERAFLEX MB-Plasma procedure following plasma source and treatment time.

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

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

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

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

CONCLUSION:
These results show a difference in recovery of factor VIII and fibrinogen correlated with plasma source. The recovery of both factor VIII and fibrinogen was higher in whole blood plasma than in apheresis plasma. Factor VIII and fibrinogen recovery did not appear to be correlated.
Paired analysis of plasma proteins and coagulant capacity after treatment with three methods of pathogen reduction.

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

Transfusion. 2014;54: 1321-1231

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

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

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

CONCLUSION:
PR treatment of plasma affects coagulation proteins and coagulant capacity. For the RF method this effect is most pronounced, although to some extent compensated by a smaller volume loss.
Update on the use of pathogen-reduced human plasma and platelet concentrates.

Seltsam A, Müller TH.


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

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


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


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

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

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

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


**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 alphaIIb 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 activatability 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.  
Transfusion. 2015 Jun;55(6):1169-77

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 nonirradiated 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 analyzed 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 107 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 analyzed.

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 radiolabeled 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 disulfide 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 nonirradiated 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 analyzed 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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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.

Castrillo A, Alvarez I, Tolksdorf F.


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

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

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

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

Gulliksson H.


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.

Tyngård N, Trinks M, Berlin G.
Transfusion. 2012 May;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 analyzed 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 a PLT 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.


BACKGROUND:
Collecting apheresis platelets (PLTs) into additive solution has many potential benefits. The new Trima software (Version 6.0, CaridianBCT) 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.


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 (eg, 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 \textit{in vitro} effects of potassium and magnesium.


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

**MATERIALS AND METHODS:**
Platelets from 30 pools of approximately 20 buffy coat (BC) units each and 24 pooled apheresis platelet units were aliquoted for storage in plasma (reference) or synthetic medium using either a specific additive solution (PAS-III) containing 30% plasma or a modification of PAS-III containing 5.0 mm potassium and 1.5 mm magnesium (PAS-IIIM) and either 30% or 20% plasma. Units were stored at room temperature with agitation for 7 days during which \textit{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 \textit{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%. 

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Reactions and platelet increments after transfusion of platelet concentrates in plasma or an additive solution: a prospective, randomized study.


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

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

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

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