LEAD THE WAY IN BLOOD SAFETY

Blood Safety
Poster Compilation

- THERAFLEX MB-Plasma
- THERAFLEX UV-Platelets
- P-CAPT

ISBT Amsterdam 2013
# THERAFLEX MB-Plasma

<table>
<thead>
<tr>
<th>Year</th>
<th>Congress</th>
<th>Citation</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>IHS (+ oral presentation)</td>
<td>Blood Transfus 2013: 11 Suppl 1, P-01</td>
<td>Methylene Blue-treated fresh frozen plasma and related adverse reactions in France: the longer the experience the stronger the results</td>
<td>Alvarez I, Reichenberg S</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-634</td>
<td>The incidence of allergic reactions related to fresh frozen plasma in France</td>
<td>Alvarez I, Reichenberg S</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-201</td>
<td>The BLUEFLEX filter of the THERAFLEX MB-Plasma system (MACOPHARMA) efficiently eliminates different bacteria species from therapeutic plasma</td>
<td>Ute Gravemann, Stefan Reichenberg, Chryslain Sumian, Axel Seltsam</td>
</tr>
</tbody>
</table>

# THERAFLEX UV-Platelets

<table>
<thead>
<tr>
<th>Year</th>
<th>Congress</th>
<th>Citation</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-200</td>
<td>Influenza A virus H3N2 is efficiently inactivated by the THERAFLEX UV-Platelets system</td>
<td>Gravemann U, Schmidt J.P, Tolksdorf F, Sumian C, Müller T, Seltsam A, Bauer G, Bicysko S, Kepper O.T, Rabenau H.F</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-208</td>
<td>In vitro quality of platelets treated in THERAFLEX UV-Platelets system well preserved during storage</td>
<td>U. Gravemann, P. Pohler, T.H. Müller, A. Seltsam</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-199</td>
<td>The efficacy of UVC pathogen inactivation on the reduction of Babesia divergens in buffy coat derived platelets</td>
<td>Emma Castro, Luis Miguel González, Jose Miguel Rubio, Raquel Ramiro, Nuria Gironés and Estrella Monter</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-198</td>
<td>The efficacy of UVC pathogen inactivation on the reduction of Plasmodium falciparum in buffy coat derived platelets.</td>
<td>Emma Castro, Patricía Marín-Garcia, José Manuel Bautista and Jose Miguel Rubio</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-202</td>
<td>The effect of volume on platelet quality during storage after UV-C treatment</td>
<td>Peter Schubert, Brankica Culibrk, Tamiko Stewart, Frank Tolksdorf and Dana V. Devine</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-194</td>
<td>Clot forming capacity and storage stability of platelets after UVC pathogen inactivation</td>
<td>Nahreen Tynngård, Marie Trinks, Gösta Berlin</td>
</tr>
<tr>
<td>Year</td>
<td>Congress</td>
<td>Citation</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-382</td>
<td>Removal of exogenous prion infectivity in leucoreduced red blood cells unit by P-CAPT™ prion removal filter</td>
<td>N. Lescoutra-Etchegaray, C. Sumian, A. Culeux, V. Durand, J.P Deslys, E. Comoy</td>
</tr>
<tr>
<td>2013</td>
<td>ISBT</td>
<td>P-383</td>
<td>Update on the ability of the prion capture filter, P-CAPT™, to delay onset of an original myelopathic disease observed in primates exposed to prion infected blood</td>
<td>N. Lescoutra-Etchegaray, N. Jaffré, A. Culeux, C. Sumian, V. Durand, J. Mikol, S. Luccantoni-Freire, J.P Deslys, E. Comoy</td>
</tr>
</tbody>
</table>
Methylene Blue-treated fresh frozen plasma and related adverse reactions in France: the longer the experience the more robust the results

Dr. Alvarez, Reichenberg S.

Blood Transfus 2013; 11 Suppl 1, P-01

Background: From mid-2008 the THERAFLEX Methylene Blue (MB)-Plasma system for pathogen reduction (PR) was implemented in routine use for the production of MB-treated plasma in France, in parallel with other PR plasma types. All blood products are monitored by an active haemovigilance system. In 2011 the French Agency for Safety of Health Products (AFSSAPS, now ANSM) decided to withdraw MB-treated plasma for alleged excessive related allergic reactions based on the 2010 Haemovigilance report.

Objectives: Reassess the risk probability of the different virally secured fresh frozen plasma types used in France during the period 2005-2011 using the 2005-2011 Haemovigilance reports and to determine the consistency of AFSSAPS’ decision to withdraw MB-treated plasma when considering longer clinical experience and therefore obtaining a smaller error probability of results than was derived from AFSSAPS’ analysis.

Methods: Data regarding allergic/anaphylactic reactions (AR), that therapeutic plasma recipients experienced, was extracted from the 2005-2011 Official Haemovigilance Reports prepared and published by AFSSAPS. According to their relevance and guided by previous studies, we considered those reactions of severity grade 2-3 (moderate-severe) and imputability level above 1 (more than doubtful) according to the severity grading proposed by the AFSSAPS Working Party on Allergic Reactions (GT Allergie-CNHy). Unfortunately, imputability level rates between 2 and 4 could not be compared between plasma processing systems because this data was not individually detailed in the 2010 Haemovigilance Report. The potential relationship between the therapeutic plasma types and the frequency of reported AR was analyzed by two-sided Fisher’s exact test, considering as significant an association with a probability of error less than 5% (p < 0.05).

Results: Significant association between different plasma types and the AR frequency reported considering severity grade 2-3 and imputability level 3-4 was neither detected during the 2005-2011 analyzed period (p = 0.692) nor during the 2008-2011 period (p = 0.344) nor during 2009-2011 (p = 0.808). Analyses carried out on annual incidents did not show significant differences between different plasma processing methods. Even though the rate was not compared because global analyses were not statistically significant, MB-plasma recipients in 2011 had the lowest serious AR incidence when taking into account the total series between 2005-2011, independently of the type of plasma processing system used (1/206,578 units).

Conclusions: Contrary to previously performed analyses with smaller populations, the longer the experience and the greater the number of recipients transfused with pathogen-reduced fresh frozen plasma using the THERAFLEX MB-Plasma system, the greater the evidence demonstrating that there is no difference in the incidence of AR compared to those obtained with other PR systems used in France.
The incidence of allergic reactions related to fresh frozen plasma in France

Dr. Alvarez, Ignacio, Macopharma, SL, Alcobendas, Madrid, Spain
Reichenberg, S., Macopharma International GmbH, Langen, Germany
ISBT Congress 2013, Amsterdam, P-634

Background: The allergic reactions related to the transfusion of fresh frozen plasma (FFP) treated with pathogen inactivation systems and plasma from quarantine have extensively generated an intensive haemovigilance control in France leading to multiple analysis and to different opinions.

Aims: Evaluate the allergic reaction incidence of the different processes used in France to secure FFP before transfusion during the 2005-2011 period considering the different populations “at risk” and taking into account the different processes used during this seven-year period.

Methods: All allergic/anaphylactic reactions (AR) data related to the transfusion of secured plasma in recipients, have been extracted from the 2005-2011 Official Haemovigilance Reports published by the French regulatory body named ANSM (previously AFSSAPS). In accordance with previous studies we took into account only allergic reactions of severity grade 2-3 (moderate-severe) and imputability level 3 (certain) incorporating the latest harmonization of the severity grading proposed and adopted by the AFSSAPS Working Party (GT Allergie-CNHv 2010). The potential difference in the incidence of AR between the different therapeutic plasma types was analysed by two-sided Fisher's exact test, since it met all the application criteria. A probability of error less than 5% (p < 0.05) was considered to indicate a statistically significant difference. The Bonferroni correction for paired multiple comparisons was used when a significant association was found.

Results: Annually, a significant association (p = 0.025) was found between the three different secured plasma types (Methylene Blue-treated plasma, Solvent-Detergent plasma and Quarantine plasma) and the incidence of AR reported in 2008 only. However, the paired comparisons between groups were not significant (p > 0.05). The incidences during comparable periods of time were analysed using at least two different methods to secure the plasma, and a representative number of transfused units of more than 25,000, simultaneously used in France: 2005-2007, 2008-2011, 2009-2011 and 2010-2011. There were not significant differences observed in the incidence of moderate/severe AR among the different types of secured FFP.

Conclusions: When recipients at risk of developing allergic reactions are analysed in defined time periods with relevant numbers of transfusions, the incidences of moderate/severe allergic reactions related to the transfusion of the different secured fresh frozen plasma used in France between 2005 and 2011 were not significantly different. Moreover, the incidence rates were very low.
The Blueflex filter of the THERAFLEX MB-Plasma system (Macopharma) efficiently eliminates different bacteria species from therapeutic plasma

Ute Gravemann, Stefan Reichenberg, Chryslain Sumian, Axel Seltsam

*ISBT Congress 2013, Amsterdam, P-201*

**Background:** Pathogen reduction systems for fresh frozen plasma (FFP) such as the methylene blue (MB)/light-based THERAFLEX MB-Plasma system (Macopharma) were developed to increase viral safety of plasma transfusions. However, FFP may also get contaminated with bacteria in rare cases. The Blueflex filter is an integral part of the THERAFLEX MB-Plasma system and reduces the concentration of MB and its derivatives in the treated plasma. It was unknown whether it can also filter bacteria and thereby protect patients against infection by bacterially contaminated FFP.

**Aim:** In this study, the capacity of the Blueflex filter to remove bacteria from contaminated FFP was investigated.

**Methods:** Plasma units (315 mL) were spiked with bacteria suspensions having titers of approx. $1 \times 10^6$ CFU/mL (*S. aureus, B. diminuta*) or approx. $1 \times 10^5$ CFU/mL (*B. subtilis* spore preparation). The plasma was filtered using the Blueflex filter under manufacturer recommendations. Samples were taken after spiking and after Blueflex filtration. The bacteria titers were determined by plating on agar plates and the log$_{10}$ reduction factor for the filtration was calculated.

**Results:** The three different bacteria species were efficiently removed from plasma by Blueflex filtration. *S. aureus* ($n = 2$) was completely eliminated, resulting in a reduction factor of $\geq 5.9$ log steps. *B. subtilis* spores ($n = 2$) were also completely removed, resulting in a log reduction of $\geq 4.7$ log steps. *B. diminuta* ($n = 4$) which has a diameter smaller than other bacteria strains tested was not completely eliminated but removed by more than 4 log steps from FFP.

**Conclusion:** The Blueflex MB removal filter has the capacity to efficiently remove bacteria from contaminated FFP. In a previous study it was shown that the Plasmaflex filter, the leukocyte depletion filter of the THERAFLEX MB-Plasma system, is also capable of reducing bacterial contamination. As the concentration of bacteria in contaminated therapeutic plasma is usually low, the overall reduction capacity of THERAFLEX MB-Plasma system will be sufficient to prevent transfusion-transmitted bacterial infections.
Blood Safety
Poster Abstracts

THERAFLEX UV-Platelets

ISBT Amsterdam 2013
Influenza A virus H3N2 is efficiently inactivated by the THERAFLEX UV-Platelets system


ISBT Congress 2013, Amsterdam, P-200

Background: The THERAFLEX UV-Platelets system (Macopharma) uses UVC light (wavelength: 254 nm) for reduction of pathogen infectivity in platelet concentrates (PCs) without the need of any additional photoactive compound. UVC treatment selectively affects the nucleic acids of pathogens but also of leukocytes while proteins are largely preserved. It was shown previously that a variety of different viruses can be inactivated by this procedure.

Aims: Aim of the study was to investigate the inactivation capacity of the THERAFLEX UV-Platelets procedure for human Influenza A virus, serotype H3N2.

Methods: First, donor sera were screened for the presence of neutralizing influenza antibodies (NIA). A double apheresis, plasma-reduced PC in additive solution SSP+/35% plasma) was obtained from a donor, who tested negative for NIA. The PC was split into two equal units that were spiked with virus suspension (10% v/v). The two PCs (375 mL each) were UVC-irradiated on the Macotronic UV machine (Macopharma) and samples were taken at different time points post irradiation. The infection titre of the H3N2 virus was determined by endpoint titration in microtitre plate assays on MDCK-2 cells. In most cases samples were titrated immediately, except for the “hold” sample which was stored until the end of the experiment and then titrated. For some of the samples also large volume plating was performed to lower the detection limit of the read out assay.

Results: H3N2 was highly sensitive to the THERAFLEX UV-Platelets pathogen reduction procedure. Already with a quarter (0.05 J/cm²) of the full UVC dose (0.2 J/cm²) a mean inactivation factor of 3.37 log₁₀ was achieved for UVC-treated PCs. At a UVC dose of 0.15 J/cm² and higher the virus was inactivated below the limit of detection, resulting in a H3N2 virus reduction factor of greater than 5 log steps (see table).

Conclusion: Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate Influenza A virus (H3N2) in potentially contaminated PCs.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>UVC dose [J/cm²]</th>
<th>mean titre [log₁₀ TCID₅₀/mL]</th>
<th>mean log₁₀ reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>0</td>
<td>5.57 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Hold sample</td>
<td>0</td>
<td>5.25 ± 0.00</td>
<td>0.32 ± 0.42*</td>
</tr>
<tr>
<td>1</td>
<td>0.05</td>
<td>1.88± 0.00</td>
<td>3.37 ± 0.39**</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>≤ 1.5</td>
<td>≥ 3.75 ± 0.19**</td>
</tr>
<tr>
<td>3</td>
<td>0.15</td>
<td>≤ -0.06</td>
<td>≥ 5.31 ± 0.19**</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>≤ -0.06</td>
<td>≥ 5.31 ± 0.19**</td>
</tr>
</tbody>
</table>

* in comparison to the load sample, ** in comparison to the “hold sample”
In vitro quality of platelets treated in the THERAFLEX UV-Platelets system is well preserved during storage

U. Gravemann, P. Pohler, T.H. Müller, A. Seltsam

ISBT Congress 2013, Amsterdam, P-208

**Background:** The THERAFLEX UV-Platelets system (Macopharma) uses UVC light (wavelength: 254 nm) for pathogen reduction of platelet concentrates (PCs) without the need of any additional photoactive compound. UVC treatment selectively affects the nucleic acid of pathogens as well as of leukocytes while proteins are largely preserved.

**Aim:** In the current study, the accumulation of cytokines during storage was investigated. The ThromboLUX (LightIntegra Technology) score as an in vitro measure for PLT quality and function was determined, which previously was shown to correlate with the corrected count increment (CCI) of PCs as a measure of posttransfusion PLT response.

**Methods:** PCs with a residual plasma content of 35% were prepared from buffy coats using the additive solution SSP+ (Macopharma). PCs were pooled and split; one unit (350 mL) was left untreated while the other one (350 mL) was irradiated on the Macotronic UV machine (MacoPharma) with a UVC dose of 0.2 J/cm². PCs (n=6) were stored for 7 days after preparation and samples were taken on days 2, 5 and 7. PLT- (CD 40L, RANTES, sCD62P, VEGF) and white blood cell (WBC)-associated cytokines (IL-1β, IL-6, IL-8) were measured by enzyme-linked immunosorbent assay. The ThromboLUX score was determined by dynamic light scattering using the ThromboLUX technology (LightIntegra Technology, Inc.). The score combines data on PLT size, number of microparticles, and PLT temperature response, which is a PLT viability indicator.

**Results:** All PCs (control and UVC-treated) showed low levels of WBC-associated cytokines. UVC treatment led to an only mild increase in the levels of different PLT-derived cytokines in PCs during storage. The level of RANTES in the UVC treated group was increased by 25% compared to the control group (243.6 ± 37.3 ng/mL vs. 305.5 ± 31.9 ng/mL, p<0.001) at the end of storage. Also CD40L and sCD62P were slightly higher in the UVC treated group on day 7 (CD40L 7.4 ± 1.5 ng/mL vs. 8.9 ± 1.7 ng/mL, p=0.002; sCD62P 124 ± 15 ng/mL vs. 137 ± 22 ng/mL, p=0.018). No significant differences in the ThromboLUX score were detected between groups. In particular, there was no statistically significant difference in microparticle concentration of the control versus the UVC-treated PCs.

**Conclusion:** There is only a small increase of PLT-derived cytokines in UVC-treated PCs during storage for 7 days. In addition, the ThromboLUX score of UVC-treated PLTs is well maintained. These results confirm the results of previous studies that the in vitro quality of UVC-treated PLTs is well preserved during storage.
The efficacy of UVC pathogen inactivation on the reduction of Babesia divergens in buffy coat derived platelets

Emma Castro¹, Luis Miguel González², Jose Miguel Rubio², Raquel Ramiro² Nuria Gironés³ and Estrella Montero²

¹Centro de Transfusión de Cruz Roja Española en Madrid, Spain
²Servicio de Parasitología. Centro Nacional de Microbiología. Instituto de Salud Carlos III, Madrid, Spain
³Centro de Biología Molecular, CSIC-UAM, Universidad Autónoma de Madrid, Madrid, Spain.

ISBT Congress 2013, Amsterdam, P-199

Background and Aim: Babesia ssp. are intraerythrocytic parasites that cause human babesiosis. These emergent parasitic infections are naturally transmitted through a tick bite, but alternative routes of transmission, such as transplacental and transfusion, have also been reported. The number of transfusion transmitted babesioses (TTB) is increasing, however, currently there are no FDA-licensed blood donor screening tests. Other alternatives for the prevention of TTB are pathogen inactivation systems (PI) for labile blood components. Recently, an innovative short-wave ultraviolet light (UVC, 254nm) pathogen reduction technology (THERAFLEX UV-Platelets, Macopharma) has been developed which does not require any additional photoactive reagent.

The aim of this study was to evaluate the effectiveness of this PI system in reducing Babesia divergens-infected buffy coat derived platelet concentrates (PC).

Study design and methods: Fifteen buffy-coat-derived PC suspended in SSP+ (Macopharma), with a volume of approximately 350 ml, were artificially inoculated with 7.5x10⁸ RBCs, of which 1.3% were parasitized with B. divergens (iRBC), i.e. a concentration of 1x10⁷ iRBC per bag. Negative control samples were taken from each platelet bag prior to inoculation. Positive control samples were also taken immediately after inoculation. Infected platelets were subsequently submitted to THERAFLEX UVC-treatment. All negative and positive control samples, as well as UVC treated samples, were plated for in vitro cultures and monitored for 8 weeks. Growth kinetics were controlled by incorporating 3H thymidine into parasitic DNA.

Results: The detection limit of B. divergens in culture was previously established at a parasitemia level of 0.1x10⁻⁶%. No growth was detected in any of the 15 negative control samples. Conversely, all 15 positive control sample cultures grew after 3 days. In 12 out of 15 infected/UVC-treated PC, no parasites were detected in vitro after an 8 week follow-up. Parasitemia had been reduced to below the level of detection, yielding in a reduction of >6 logs. In 3 out of 15 UVC treated platelets, a certain level of growth was detected after 10 days in culture. Therefore, B. divergens was only reduced to the level of detection (0.1x10⁻⁶%) with a calculated reduction of 5 logs.

Conclusion: UVC treatment is capable of reducing infective forms of B. divergens down to or below the detection limit of the viability assay. Therefore, UVC based PI systems can be considered a suitable tool to prevent the transmission of human babesiosis via platelet transfusions.
The efficacy of UVC pathogen inactivation on the reduction of Plasmodium falciparum in buffy coat derived platelets.

Background and Aim: Plasmodium ssp. are intraerythrocytic parasites that cause malaria in humans. Due to immigration and travel, this has become a re-emergent parasitic infection in non-endemic areas. It is naturally transmitted by infected female anopheline mosquito bites, however alternative transmission routes, such as transfusion (TT), have been reported. TT malaria cases are mainly related to RBCs, but platelet components have also been implicated. Currently there are no FDA-licensed blood donor screening tests, but EIA tests are used in several European countries to detect IgG antibodies and shorten deferral periods. Another alternative preventive measure is a pathogen inactivation system (PI) for labile blood components. Recently, an innovative short-wave ultraviolet light (UVC, 254nm) pathogen reduction technology (THERAFLEX UV-Platelets, Macopharma) not requiring any additional photoactive reagent has been developed.

This study aimed to evaluate the effectiveness of this PI system in reducing Plasmodium falciparum-infected buffy coat derived platelet concentrates (PC).

Study design and methods: Sixteen buffy-coat-derived PC (approximately 350 ml) suspended in SSP+ (Macopharma) were artificially inoculated with RBCs parasitized with P. falciparum (iRBCs) at a final concentration of 2.0-3.45 x 10^5 iRBCs/ml. Negative control samples were taken from each platelet bag prior to inoculation. Positive control samples were also taken immediately after inoculation. Infected platelets were subsequently submitted to THERAFLEX UVC-treatment. Negative and positive control samples, as well as UVC treated samples, were tested with three different viability assay arrays: 1) Molecular detection of P. falciparum DNA, 2) Molecular detection of P. falciparum RNA expression and 3) P. falciparum in vitro cultures following the method of Radfar et al. (2009). The post-PI treatment logarithmic reduction in the parasite burden was calculated using the formula: Log Red = Log (parasites pre-PI – parasites post-PI).

Results: P. falciparum DNA was significantly reduced after UV-treatment (Log reduction factor 4.2 to 5.1 (average 4.9)). P. falciparum RNA expression was also reduced after PI (log reduction factor 3.0 to 5.0 log (average 4.2)). All 4 P. falciparum positive control cultures grew, however the post-PI samples did not. Based on the results of P. falciparum growth kinetics in culture, the reduction achieved was established at 8 log.

Conclusion: Based on NAT viability assays, UVC treatment is capable of reducing infective forms of intraerythrocytic P. falciparum by 3.0 to 5.0 logs. The parasite burden reduction was remarkably higher, with an 8 log reduction, when in vitro culture assays were used (i.e. PI treatment impeded parasite growth, even in the two samples that showed the lowest reduction level on RNA expression assays). The implication of residual RNA after UVC treatment is unclear, but may represent dead parasites unable to replicate.
The effect of volume on platelet quality during storage after UV-C treatment

Peter Schubert, Brankica Culibrk, Tamiko Stewart, Frank Tolksdorf and Dana V. Devine

Background: Pathogen reduction technologies (PRT) were developed to improve the safety of blood products; however, this treatment appears to accelerate the development of the platelet storage lesion (PSL). The THERAFLEX UV-Platelets method (Macopharma) uses short-wave UV light (UV-C, 254nm) for pathogen reduction of plasma-reduced platelet products, without the use of any photoactive compound.

Aims: In an attempt to evaluate extended specifications for UV-C treatment, the influence of different component volumes on platelet quality following this PRT treatment was examined.

Materials and Methods: Pooled platelet concentrates in SSP+ (Macopharma) were prepared by the buffy coat method to generate component volumes of 250, 350 or 450 mL (n = 3 for each). In a pool-and-split design, one unit was treated with UV-C on day 1; the other was an untreated control. All components contained similar platelet concentrations and were stored in the same bags (Ref. XUV4005XU) at 20–24 °C with agitation. Units were sampled on day 1, 2, 5, 7 and 9. Metabolic parameters and pH were measured on a Gem Premier 3000 Blood Gas Analyzer. CD62P expression was measured by flow cytometry. Extent of shape change (ESC) and hypotonic shock response (HSR) were measured on Chronolog 2000.

Results: Platelet count and mean platelet volume were not affected by UV-C treatment. A significant difference between treated and untreated units was seen for pH (p< 0.05), with lower pH following UV-C treatment (day 9: 7.3±0.0 vs. 6.9±0.0; 7.3±0.0 vs. 7.2±0.0; 7.2±0.1 vs. 7.1±0.0 for 250, 350 and 450 mL volumes, untreated vs. treated, respectively). Glucose dropped as is commonly seen during storage, with a significantly greater difference with treatment (p < 0.05). The greatest glucose consumption was in the 250 mL units; by Day 9, the level of glucose in the treated arm was as low as 0.4 ±0.5 mM vs. 3.3 ± 1.0 mM in the untreated units. This trend was reflected in the lactate levels, which were highest in UV-C treated 250 mL units (Day 9: 18.6 ± 4.5 mM vs. 14.5 ± 3.4 mM in the untreated units). CD62P expression increased throughout storage in all volumes, and this increase was significantly greater following UV-C treatment (p < 0.05). Platelet response to the agonist ADP (ESC) was significantly lower upon UV-C treatment (p < 0.05) in all volumes, with minimal responsiveness remaining by Day 9 (1.2±0.4 %, 3.8±2.0 % and 4.1±1.1 % in 250, 350 and 450 mL, respectively). Similar to ESC, the decrease in HSR was greater in the treated units (p < 0.05), and was most pronounced and occurred immediately following treatment in 250 and 350 mL units; this was not seen in the 450 mL units.

Conclusions: The most pronounced development of the PSL was seen in the 250 mL volume components, and treatment with UV-C appeared to contribute to this accelerated development. The differences seen among the 3 volumes may be related to the different surface area to volume ratios which could affect gas exchange and diffusion, as well as the effective irradiation dose.
Clot forming capacity and storage stability of platelets after UVC pathogen inactivation

Nahreen Tynngård, Marie Trinks, Gösta Berlin

ISBT Congress 2013, Amsterdam, P-194.

**Background:** The storage of platelet concentrates (PCs) for 5-7 days at room temperature enables contaminating bacteria to replicate and to cause serious transfusion reactions in the recipients. Pathogen inactivation (PI) procedures can prevent the replication of pathogens. PI with UVC-light (THERAFLEX UV-Platelets, Macopharma) is a fast and easy method for inactivation of pathogens making it suitable for routine use provided that the platelets have a preserved haemostatic function.

**Aims:** This study compares the in vitro function of platelets (including haemostatic properties) during 7 days of storage after PI with UVC-light. Non-irradiated platelets and gamma-irradiated platelets were used as controls.

**Methods:** PCs in 30% plasma and 70% SSP+ (Macopharma) were prepared using OrbiSac (Terumo BCT). Three PCs with the same ABO group were pooled and divided into three identical parts with a volume of 354±8 mL containing 354±25 x10⁹ platelets. One part was UVC-treated, the second gamma-irradiated and the third served as a non-irradiated control. In total 10 PCs of each type were prepared. In vitro assays were performed on Day 1 (directly after UVC- or gamma-irradiation), Day 5 and Day 7 of storage. Swirling, number of platelets, blood gases and metabolic parameters were analysed. Surface expression of platelet activation marker P-selectin was measured by flow cytometry. The haemostatic properties of the platelets were analysed by free oscillation rheometry (FOR) assessing clotting time and clot elasticity of the coagulum.

**Results:** Swirling was well preserved and pH was above 6.4 during the storage period for all groups. Glucose concentration decreased and lactate concentration increased from Day 1 to Day 7 (p<0.05). UVC-treated platelets had higher glucose consumption (p<0.05) and expressed higher level of P-selectin (p<0.05) than control platelets at the end of storage. The clot forming capacity (clotting time) was similar for UVC-treated platelets and control platelets. Storage resulted in a small increase in clot elasticity for all groups but this change was less pronounced for the UVC-treated platelets.

**Summary/Conclusions:** UVC-treatment of platelets resulted in an increased consumption of glucose and increased expression of activation marker (P-selectin) at the end of storage compared to gamma-irradiated and non-irradiated platelets. However, the ability to promote clot formation and clot retraction was well maintained.
Removal of exogenous prion infectivity in leucoreduced red blood cells unit by P-CAPT™ prion removal filter

N. Lescoutra-Etchegaray¹, C. Sumian³, A. Culeux¹, V. Durand², J.P Deslys², E. Comoy²

¹Macopharma, Fontenay-aux-Roses, France
²CEA, Prion Research Group, DSV/IMETI/SEPIA, Fontenay-aux-Roses, FRANCE
³Macopharma, Tourcoing, FRANCE

ISBT Congress 2013, Amsterdam, P-382

Background: Five cases of variant Creutzfeldt-Jakob Disease (vCJD) infections were probably linked to infusion of contaminated blood components, turning to real the inter-human transmissibility of this prion disease from asymptomatic carriers. Corresponding preventive policies are currently limited to exclusion from blood donation, but also take advantage of leucoreduction initially implemented against leucotropic viruses. In the absence of available antemortem diagnostic tests, the updated prevalence of silent vCJD infections (1/2,000 in the UK) urges the necessity to enforce blood safety with more efficient active measures able to remove remaining infectivity.

Aims: Several affinity resins were proved to experimentally reduce high levels of brain-spiked infectivity from human leucoreduced red blood cell concentrates (L-RBC). One was integrated in a device adapted to field constraints (volumes, duration) of human transfusion. We aimed here to assess the ability of the final device, in its real conditions of use, i.e. the real conditions of filtration with human leucoreduced red cell concentrates (L-RBC), to remove infectivity from human L-RBC unit spiked with scrapie-infected hamster brain.

Methods: A standardized method for preparation of clarified brain homogenate from 263K infected hamsters, allowing elimination of large aggregates of PrPres without modification of apparent infectivity, was selected for spiking human L-RBC unit. Filtration by gravity, according to the manufacturer’s recommendations, of a 0.0001% spiked L-RBC unit was carried out on day 1 at room temperature. Pre- and post- blood filtration sample aliquots were removed for infectivity studies (intracerebral inoculation of hamsters).

Results: Incubation periods of recipient animals suggest around 3 log₁₀ removal of brain-derived prion infectivity by filtration through the P-Capt™.

Conclusion: On brain-derived spiked infectivity, the P-Capt™ filter provided similar performances as columns used for initial proof-of-concept studies (Gregori et al., Lancet 2006), suggesting an appropriate scale-up to efficiently remove infectivity from an individual human blood bag. According to the ability of resin to completely remove apparent endogenous infectivity from hamster leucoreduced blood, the implementation of such a filter, now commercially available, might seriously improve blood safety towards prion.
Update on the ability of the prion capture filter, P-CAPT™, to delay onset of an original myelopathic disease observed in primates exposed to prion infected blood

N. Lescoutra-Etchegaray1*, N. Jaffré1, 2, A. Culeux1, C. Sumian3, V. Durand2, J. Mikol2, S. Luccantoni-Freire2, J.P Deslys2, E. Comoy2

1Macopharma, Fontenay-aux-Roses, FRANCE
2CEA, Prion Research Group, DSV/IMETI/SEPIA, Fontenay-aux-Roses, FRANCE
3Macopharma, Tourcoing, FRANCE

Background: In the United Kingdom, the recent report of four human cases of variant of Creutzfeldt-Jakob disease (vCJD) through transfusion has justified the implementation of measures to secure blood and blood products towards prions. Leucoreduction, implemented against blood-borne viruses, is not sufficient to remove the entire prion blood infectivity, halved between white cells and plasma. In absence of antemortem diagnostic tests, several devices, including P-Capt™ filter, were designed to remove prions. This filter incorporates an affinity resin specific for PrP, which has already demonstrated its efficiency in removing both exogenous and endogenous prion infectivity in the experimental model of hamster infected with the experimental 263K strain. The ability of the P-Capt™ filter was also previously assessed with human L-RBC artificially contaminated with 263K clarified brain extracts and demonstrated comparable efficiency.

Aims: We aimed to complete the evaluation of the P-Capt™ filter with blood-borne infectivity in the cynomolgus macaque considered as an utmost relevant model for the investigation of human prion diseases.

Methods: Two independent experiments were performed one year apart. First, five donor primates were intravenously (iv) inoculated with high amounts of clarified brain homogenate from a BSE-infected primate to maximize their blood infectivity. At the onset of first clinical signs, their blood was drawn and pooled to reach a volume equivalent to a human blood donation. After whole blood leucoreduction, Red Blood Cell Concentrate (L-RBC) was prepared following routine blood human procedures. L-RBC was suspended in plasma according to conditions used for pediatric transfusion in the UK. Twenty-seven milliliters were transfused to two or three recipient primates prior or after P-Capt™ filtration respectively. In the second experiment designed for the evaluation of a combined filter for leucoreduction and prion removal, the same scheme was applied with RBC (suspended in Sag-M) issued from 6 animals intravenously infected with clarified brain homogenate from a vCJD-infected primate.

Results: In the BSE experiment, both primates injected with L-RBC before filtration developed an original neurological disease 30 and 31 months post inoculation and died two months later. This original neurodegenerative disease is described by E. Comoy et al as an atypical form of prion disease. Conversely, all the three animals transfused with P-Capt™ filtrated L-RBC still remained asymptomatic 54 months post transfusion. In the vCJD experiment, first clinical signs evocative of the myelopathic syndrome were detected in both primates before filtration 20 to 27 months post inoculation. In the vCJD experiment, first clinical signs evocative of the myelopathic syndrome were detected in both primates before filtration 20 to 27 months post inoculation. One of them was subject to euthanasia 42 mpi, and pathological examination confirmed the occurrence of the myelopathic syndrome. The others animals are still asymptomatic.
Conclusion: The P-Capt was shown to be able to retain classical prion strains. Here in two independent experiments, we demonstrate that this filter is also able to retain atypical strains recently identified in primates after exposition to human blood products.
Contaminated blood products induce an atypical prion disease in primates in the absence of detectable abnormal prion protein

Emmanuel E. Comoy1, Nina Jaffré1, Jacqueline Mikol1, Valérie Durand1, Christelle Jas-Duval1,2, Sophie Luccantoni-Freire1, Evelyne Correia1, Vincent Lebon3, Justine Cheval4, Isabelle Quadrio5, Nathalie Lescoutra-Etchegary7, Nathalie Streichenberger, Stéphane Haik Chryslain Sumian7, Armand Perret-Liaudet6, Marc Eloit4,5, Philippe Hantraye3, Paul Brown1 and Jean-Philippe Deslys1

1CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Division of Prions and Related Diseases (SEPIA), Route du Panorama, BP6, 92265 Fontenay-aux-Roses, France; 2EFS-Nord de France, Quai de Jemmapes, 59000 Lille, France 3CEA/I2BM/MIRCen, Route du Panorama, BP6, 92265 Fontenay-aux-Roses, France 4Pathoquest, 28 Rue du Docteur Roux, 75015 Paris, France 5Institut Pasteur, Virology Department, 28 Rue du Docteur Roux, 75015 Paris, France 6Hospices Civils de Lyon, Prion Unit, Neurobiology department, 59 Boulevard Pinel, 69500 Bron, France 7Macopharma, 200, chaussée Fernand Forest, 59200 Tourcoing – France

Background: Concerns about the blood-borne risk of prion infection have been confirmed by the occurrence in the UK of four transfusion-related infections of vCJD (variant Creutzfeldt-Jakob disease), and an apparently silent infection in an hemophiliac patient. Asymptomatic incubation periods in prion diseases can extend over decades in humans, and a typical disease may or may not supervene. Several parameters, including factors driving blood infectivity, remain poorly understood.

Aims: We used a validated non-human primate model of prion disease to evaluate the transfusional risk linked to vCJD. We present here unexpected results of independent individual transmission experiments.

Methods: Cynomolgus macaques were inoculated with brain or blood specimens from vCJD infected humans and vCJD or BSE-infected monkeys. Neuropathological and biochemical findings were obtained using current methods used for human patients.

Results: Six out of 12 primates exposed to human or macaque blood-derived components exhibited an original neurological disease (myelopathy) previously not described either in humans or primates, and which is devoid of the classical clinical and lesional features of prion disease (front leg paresis in the absence of central involvement, lesions concentrated in anterior horns of lower cervical cord, with no spongiosis or inflammation), while the nine brain-inoculated donor animals and one transfused animal exhibited the classical vCJD pattern, and the five other primates exposed to blood-derived components remain asymptomatic. No abnormal prion protein (PrPres) was detected by standard tests in use for human prion diagnosis. No alternative cause has been found in an exhaustive search for metabolic, endocrine, toxic, nutritional, vascular and infectious etiologies, including a search for pathogen genotypes (‘deep sequencing’). In secondary transmission experiments, plasma transfusion transmitted this myelopathic syndrome, whereas intracerebral inoculation of spinal cord induced a prion disease harboring the classical hallmarks, i.e. spongiosis and PrPres.
Conclusion: We describe a new neurological syndrome in monkeys exposed to various prion-infected inocula. Our experimental observations in the absence of evident alternative etiology confirm the prion origin for this myelopathy, which might be compared under some aspects to certain forms of human lower motor neuron diseases including neuromyelitis optica, the flail arm syndrome of ALS and the recently described FOSMN. Similar human infections, were they to occur, would not be identified as a prion disease by current diagnostic investigations.
Lead the way in blood safety

www.bloodsafety.macopharma.com