plasma (65%), with 26.5 percent, 1.8 percent, and 0.8 percent associated with platelets, RBCs, and polymorphonuclear cells, respectively.[13] Our results indicate that the majority of WBCs can be detected after thawing, and these are removed to undetectable levels after the WBC-reduction step of the MB process. However, the method we employed to detect WBCs predominantly measures WBC nuclei (unpublished data), and therefore provides little information on cellular integrity. The majority of WBCs in freeze-thawed plasma are detectable with PI without prior permeabilization,[26,27] suggesting that freeze-thawing alters WBC membrane integrity. The increase in levels of α_1-proteinase inhibitor: PMN elastase complexes after thawing of plasma spiked with WBCs shows that PMN degranulation is occurring, but the postthaw levels remain below 100 µg per L, which is not suggestive of large-scale PMN disintegration. Furthermore, in the absence of platelets, levels of LDH did not increase substantially after freezing, suggesting that WBCs do not disintegrate. We were unable to assess WBC fragments due to the insufficient sensitivity of available methods.

When platelets were spiked into plasma, there was an increase in platelet-derived microparticles after freeze-thawing of plasma, which probably explains the small decrease in platelet count detected by flow cytometry because these events would not be included in the platelet count. This fall was not detected by hematology analyzer, possibly because cell fragments can be detected as platelets by impedance-based methods. These fragments were reduced to or below the level in WBC-reduced fresh plasma after the WBC-reduction step of the MB process. However, we also analyzed cell microparticles based on the binding of purifed annexin V, which has a high affinity for anionic phospholipids. Freeze-thawing resulted in an increase in annexin-V-positive microparticles, which appear to be mainly derived from platelets and were only partially removed by WBC reduction. The increased detection of microparticles by this method compared with using an antibody against the platelet receptor CD61 is probably attributable to the greater number of molecules per platelet of anionic phospholipid (1×10^6) compared with CD61 ($4\text{-}8 \times 10^4$).[28,29] The presence of RBC and WBC microparticles (which will also bind annexin V) may also help to explain this difference, but this seems unlikely because in the absence of platelets the differences between methods were less pronounced. The number of annexin-V-positive microparticles found in non-WBC-reduced plasma that has been frozen-thawed and then filtered is not appreciably higher than would be found in plasma that we currently produce.

The effect of loss of coagulation factor activity due to MB treatment on the in vivo efficacy of the component is difficult to assess because there are no published randomized, controlled clinical trial data comparing MB to either standard FFP or S/D-treated FFP. However, 2.5 million units of MB FFP have been transfused internationally without obvious clinical sequelae.[30,31] In Spain, the switch from standard to MB-treated FFP has been associated with an increase in demand for FFP and cryoprecipitate,[32] which the authors attribute to loss of coagulation activity. However the increase in use (56%) appears to be disproportionate to the decrease in coagulation factors, suggesting that other factors, such as perception of a safer component, may have been influencing usage. It is also reported that the use of MB FFP is associated with a higher number of plasma exchanges compared with untreated FFP for the treatment of thrombotic thrombocytopenic purpura,[33] although we found no difference in the levels of VWF cleaving activity, the presumed therapeutic moiety in plasma treatment of thrombotic thrombocytopenic purpura, in MB FFP.[21] It is critical that transfusion services introducing pathogen inactivation of components monitor ongoing trends in usage as well as having a system for hazard reporting. At the time of writing, MB-treated and -removed FFP is routinely produced in England and Wales for transfusion to children and neonates born after 1995, with similar arrangements in other parts of the UK. However, in the near future, plasma to be pathogen inactivated for this patient group throughout the UK will be imported from volunteer donors in North America. Processes currently available for the pathogen inactivation of plasma all result in a decrease in coagulation factor activity. Improvements in the safety of blood need to be balanced against some likely reduction in the component potency. Single-unit systems for pathogen inactivation of plasma that have less effect on coagulation factor activity are clearly desirable.

ACKNOWLEDGMENTS

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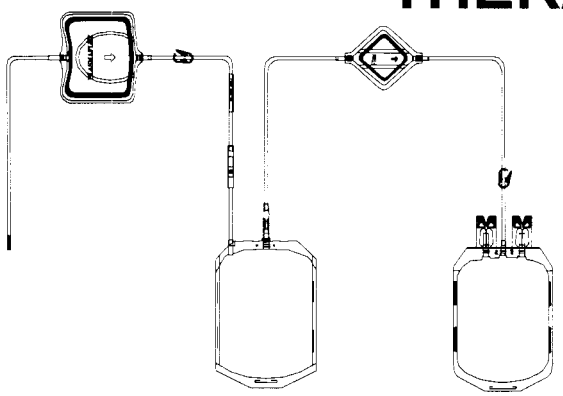
Pathogen Reduction System
THERAFLEX - MB PLASMA



Pathogen Reduction of Leucodepleted Plasma Methylene Blue Removal by Filtration

CE 0459 **Ref. SDV0001XQ**

Specifications

. **Filters** : Plasmaflex PLAS4, Blueflex filter

. **Bags** : 2 PVC

. **Included Items** : Methylene Blue pill (85µg)

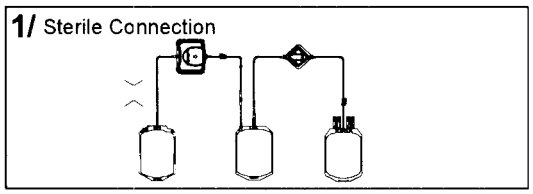
. **Label** : English, French, German, Dutch

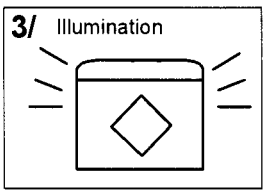
. **Sterilisation** : Steam

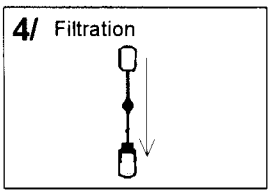
. **Shelf life** : 2 years

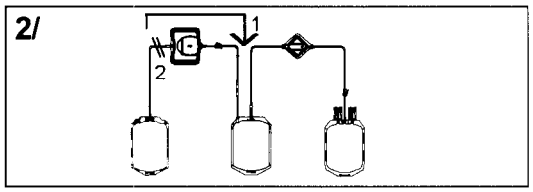
. **Packaging** : 2 packs/peelable sachet - 24 packs/box

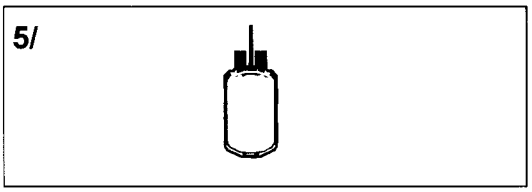
Use











SDV0001XQ_A_A 08/03/2006

■ Whole Blood ■ RCC □ PLASMA □ MB PLASMA □ PRP ⊡ Platelets □ SAG-M PAGGSM □ NaCl ■ Buffy Coat ▩ Serum □ Supernatant □ Cryoprecipitate

Certified ISO 9001 Certified ISO 13485

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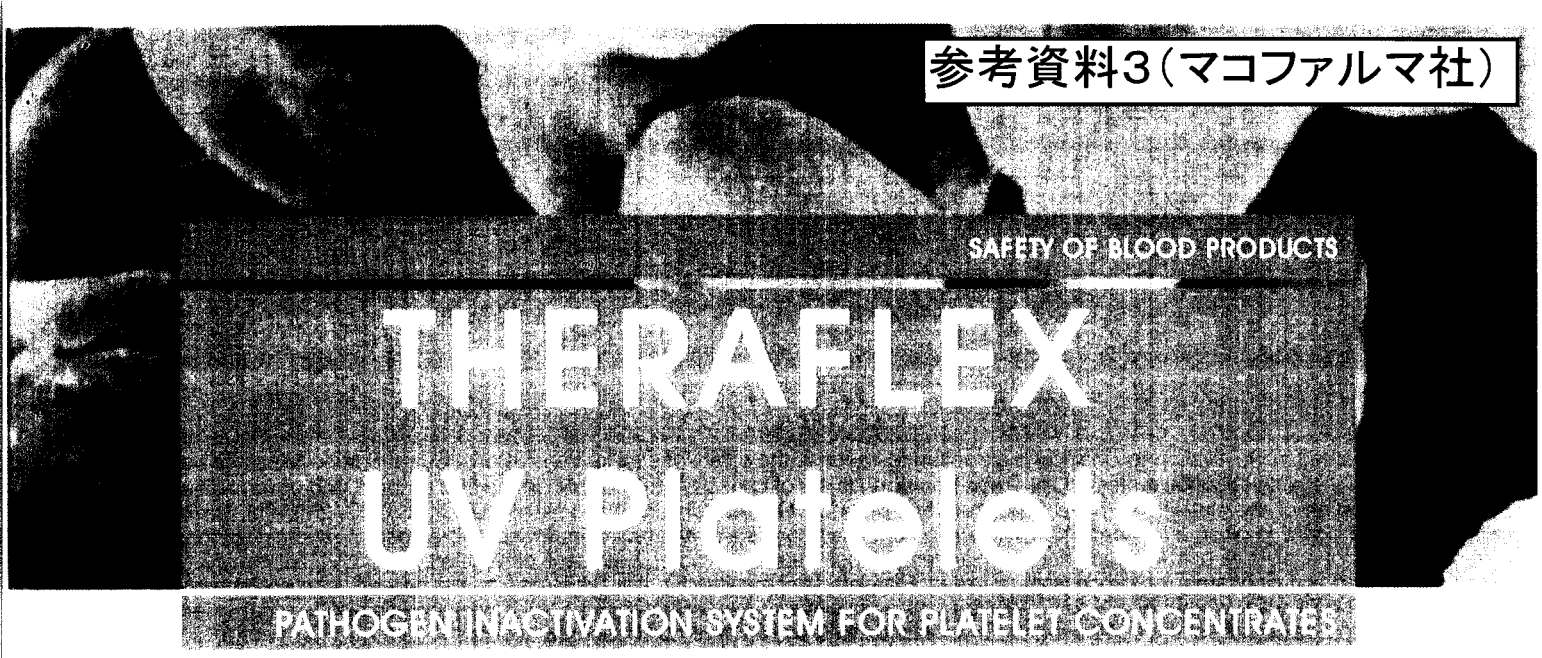
参考資料3（マコファルマ社）



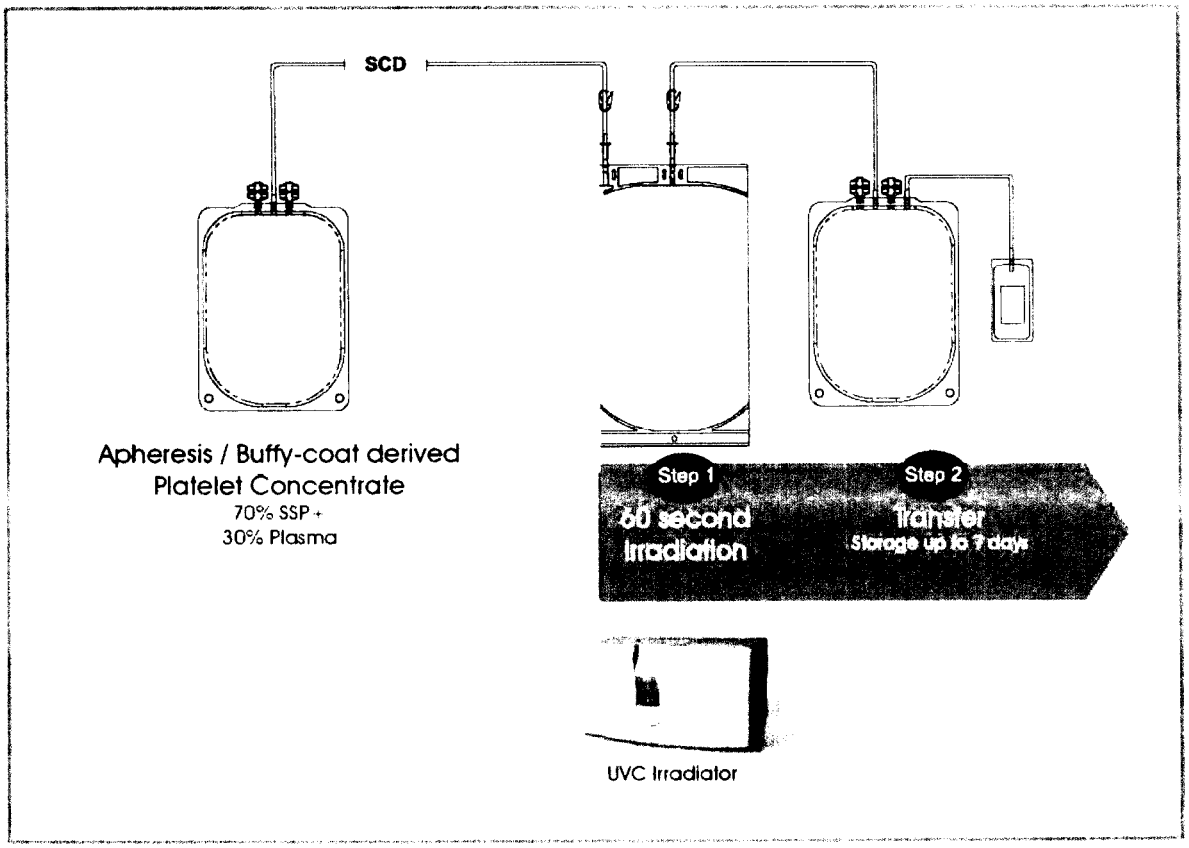
MacoPharma's latest development in the Safety of Blood Product Range:

- No photosensitizer
- Two step process
- 60 second UVC irradiation

Performance Targets:

- Apheresis and Buffy-coat derived platelet concentrates
- Efficacy on Bacteria, Spores, Non-ervelopped and Envelopped Viruses, Leucocytes, Parasites
- Storage up to 7 days with SSP+

THERAFLEX UV Platelets: 2 step process



A NOVEL TECHNIQUE FOR PATHOGEN INACTIVATION AND ITS EFFECT ON THE QUALITY OF PLATELET CONCENTRATES: THERAFLEX UV PLATELETS

Walker W H[1], Tolksdorf F[1], Mohr H[2], Gravemann U[2], Müller T H[2],
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[2] German Red Cross Chapters of NSTOB, Springe, Germany

ISBT Regional Congress Asia, Hanoi, November 2007

INTRODUCTION

The use of a pathogen inactivation technology is an option to enhance the safety of platelet transfusions. Current procedures need chemicals to be added to the platelet concentrates (PCs). These compounds are of concern if they remain in the final product. Moreover, treatment may cause deterioration of the platelets. A novel procedure has been developed using only short-wave UV light (UVC, 254 nm) that effectively inactivates pathogens in plasma-reduced PCs. The equipment used consists of an irradiation device with a specific mechanism for agitation. Its capacity is one platelet unit (random donor or apheresis) per treatment cycle. Treatment parameters, e.g. UVC intensity, UVC dose, temperature and agitation, are microprocessor-controlled. Platelets are processed in the THERAFLEX twin-bag kit, which comprises a highly UV-transparent polyolefin acetate bag[1] for irradiation and a platelet storage container (Fig.1, 2). It was investigated to what extent platelet integrity and storage stability of the treated products were influenced by this new inactivation procedure.

MATERIALS & METHODS

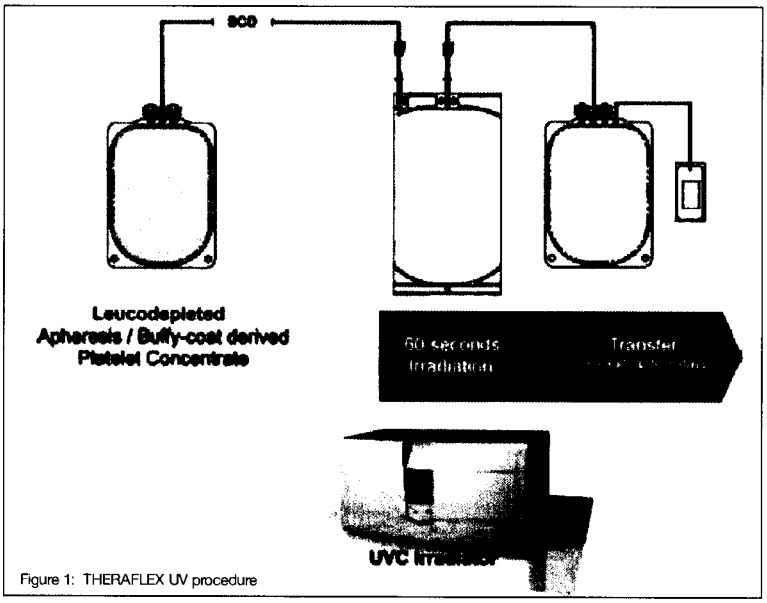

Figure 1: THERAFLEX UV procedure

Plasma-reduced PCs were treated with the THERAFLEX UV procedure (Fig.1) and stored until day 8 after blood donation (day 6 after treatment). The in vitro quality of UVC treated PCs was evaluated in comparison to non-treated control platelets. Control PCs were stored for the same time period. PCs in storage medium SSP+ (MacoPharma) were prepared from pools of 5 buffy coats. The average volume treated was 350 mL. Plasma concentration was approx. 30%. PCs were transferred into irradiation bags (Fig.2) and treated with UVC light at a dose of 0.4 J/cm² (approx. 60 sec) (Fig.3). They were strongly agitated during irradiation.


Figure 2: Twin-bag kit

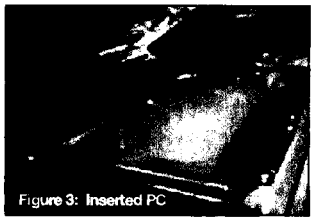
Figure 3: Inserted PC

RESULTS

In vitro characteristics were hardly influenced by the THERAFLEX treatment. HSR reactivity was only slightly reduced whereas collagen induced aggregation was moderately increased. Glucose consumption and lactate formation were found to be marginally higher in the treated PCs. Thus, pH slightly dropped but remained above 7.0 until day 8 after donation. The mean platelet loss due to UVC treatment was 4% (Tab.1).

Day 3	Plts [x10⁹/mL]	HSR [%]	pH	Spontaneous aggregation [%]	Collagen-ind. aggregation 100 µg/mL [%]	Glucose [mg/dL]	Lactate [mmol/L]
Control	10.2 ± 1.6	68 ± 4	7.13 ± 0.05	11 ± 3	94 ± 3	122 ± 8	7.5 ± 1.0
Treated	9.6 ± 1.3	64 ± 5	7.07 ± 0.07	14 ± 2	89 ± 5	118 ± 6	7.7 ± 0.8
Day 6							
Control	9.9 ± 1.0	66 ± 2	7.24 ± 0.13	12 ± 2	74 ± 9	86 ± 10	10.8 ± 1.0
Treated	9.5 ± 1.3	64 ± 8	7.09 ± 0.06	14 ± 3	81 ± 9	68 ± 10	12.8 ± 1.5
Day 8							
Control	9.4 ± 1.6	68 ± 1	7.29 ± 0.12	10 ± 1	62 ± 7	63 ± 9	12.5 ± 0.9
Treated	9.1 ± 1.3	61 ± 8	7.09 ± 0.05	16 ± 4	69 ± 7	41 ± 8	15.2 ± 1.0

Table 1: Platelet parameters of untreated an treated PCs on day 3, 6 and 8 after blood donation (mean +/- SD; n=4)

CONCLUSIONS

Plasma-reduced PCs were only slightly affected when treated with the THERAFLEX UV system for pathogen inactivation. In vitro parameters and storage stability were well preserved until day 8 after blood donation.

[1] Polyolefineacetate bags for pathogen inactivation and for storage of platelet concentrates, H Mohr, TH Müller, F Tolksdorf, WH Walker, Vox Sang 2004; 87 (Suppl. 3): 70

Funded by Forschungsgemeinschaft der Blutspendedienste des Deutschen Roten Kreuzes e.V. and Maco Pharma.

PATHOGEN REDUCTION IN PLATELET CONCENTRATES USING UVC LIGHT IN COMBINATION WITH STRONG AGITATION: EFFECT ON ACTIVATION MARKERS AND STORAGE STABILITY

Tolksdorf F[1], Walker W H[1], Mohr H[2], Gravemann U[2], Mueller T H [2]
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AABB Annual Meeting 2007, Anaheim, USA

INTRODUCTION

Pathogen Inactivation technologies are proactive measures to enhance the safety of platelet transfusions. Their use can be effective also against emerging unknown pathogens. Current procedures need chemicals like photosensitizers. We developed a procedure, which allows an efficient reduction of pathogens (e.g. bacteria and viruses) in plasma-reduced platelet concentrates (PCs) using short-wavelength UV light (UVC) in combination with strong agitation, i.e. there is no photoactive compound needed (THERAFLEX UV Platelets technology). The irradiation device developed is emitting UVC light at a wavelength of 254 nm. Moreover a mechanism for orbital agitation is installed. UVC irradiation is microprocessor-controlled. Relevant treatment parameters are monitored throughout the entire treatment thus allowing a fully documented and reproducible process. PCs are treated in a twin-bag kit, which comprises of a highly UV-permeable irradiation bag and a container for extended platelet storage. In the present study we investigated the influence of the THERAFLEX UV treatment on activation parameters and on the storage stability of PCs.

MATERIALS & METHODS

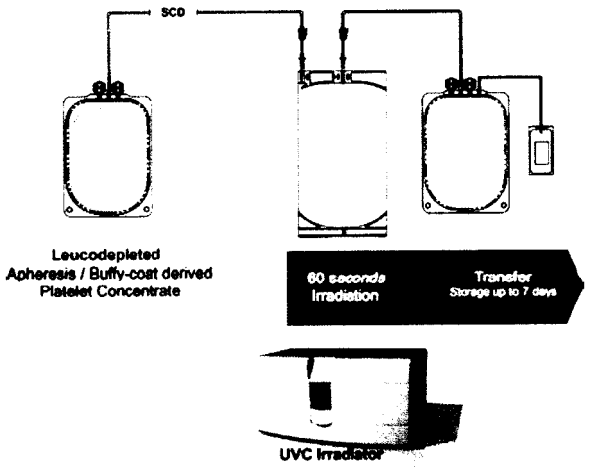

Figure 1: THERAFLEX UV procedure

Plasma-reduced PCs were treated with the THERAFLEX UV procedure (Fig.1) and stored until day 8 after blood donation (day 6 after treatment). The in vitro quality of UVC treated PCs was evaluated in comparison to untreated control platelets. Control PCs were stored for the same time period. PCs in storage medium SSP+/MacoPharma (containing saline, citrate, acetate, phosphate, magnesium and potassium, identical to PAS-IIIM) were prepared from pools of 5 buffy coats. The average volume was 350 mL (platelet concentration approx. 10^9/mL) and the plasma concentration was approx. 35%. PCs were transferred into irradiation bags (Fig.2) for UVC treatment at a dose of 0.4 J/cm² (approx. 60 sec) (Fig.3). They were strongly agitated during irradiation. Relevant treatment parameters, e.g. UV dose, UV intensity, temperature and irradiation time, were microprocessor-controlled.

Figure 2: Twin-bag kit

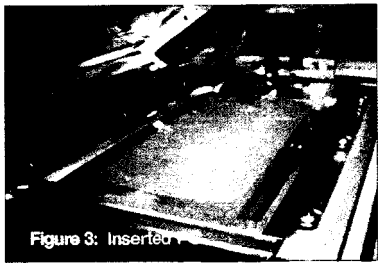

Figure 3: Inserted

RESULTS

Until day 8 of storage in vitro characteristics were only marginally influenced by the THERAFLEX process. Platelet quality was evaluated by measurement of the hypotonic shock response (HSR) and the expression of the activation marker CD62p. HSR reactivity and CD62p levels were only slightly affected by the Theraflex treatment. Annexin V binding percentage, as a marker for apoptosis, remained almost unchanged. Glucose consumption and lactate formation were found to be marginally higher in the treated PCs. pH remained above 7.0 until day 8 after donation (Tab. 1).

Day 6*	Plts [x10⁹/mL]	CD62 [%]	Annexin V [%]	HSR [%]	pH	Glucose [mg/dL]	Lactate [mmol/L]
Control	9.2 ± 1.1	21 ± 6	5 ± 1	71 ± 5	7.29 ± 0.04	62 ± 17	10.2 ± 2.3
Treated	8.5 ± 0.9	32 ± 5	9 ± 4	68 ± 4	7.22 ± 0.05	52 ± 21	10.8 ± 1.6
Day 7*							
Control	8.9 ± 0.8	24 ± 6	7 ± 1	72 ± 3	7.32 ± 0.05	55 ± 19	10.4 ± 1.6
Treated	8.4 ± 0.9	42 ± 13	10 ± 3	68 ± 3	7.22 ± 0.06	42 ± 20	11.8 ± 1.7
Day 8*							
Control	9.4 ± 1.6	30 ± 3	8 ± 4	71 ± 5	7.34 ± 0.06	45 ± 18	11.5 ± 1.6
Treated	9.1 ± 1.3	52 ± 12	10 ± 3	65 ± 5	7.22 ± 0.09	31 ± 17	13.1 ± 1.7

Table 1: In vitro parameters of untreated and treated PCs on day 6, 7 and 8 after blood donation (N = 6, mean ± SD) *after blood donation

CONCLUSIONS

THERAFLEX treatment with 0.4 J/cm² UVC light has only a minor influence on in vitro parameters of PCs and their storage stability until day 8 after blood donation was maintained.

[1] Polyolefineacetate bags for pathogen inactivation and for storage of platelet concentrates, H Mohr, TH Müller, F Tolksdorf, WH Walker, Vox Sang 2004; 87 (Suppl. 3): 70

Funded by Forschungsgemeinschaft der Blutspendedienste des Deutschen Roten Kreuzes e.V. and Maco Pharma.