参考資料3

マコファルマ社
提出資料（再配布）

参考資料3（マコファルマ社）

SAFETY OF BLOOD PRODUCTS

THERAFLEX MB Plasma

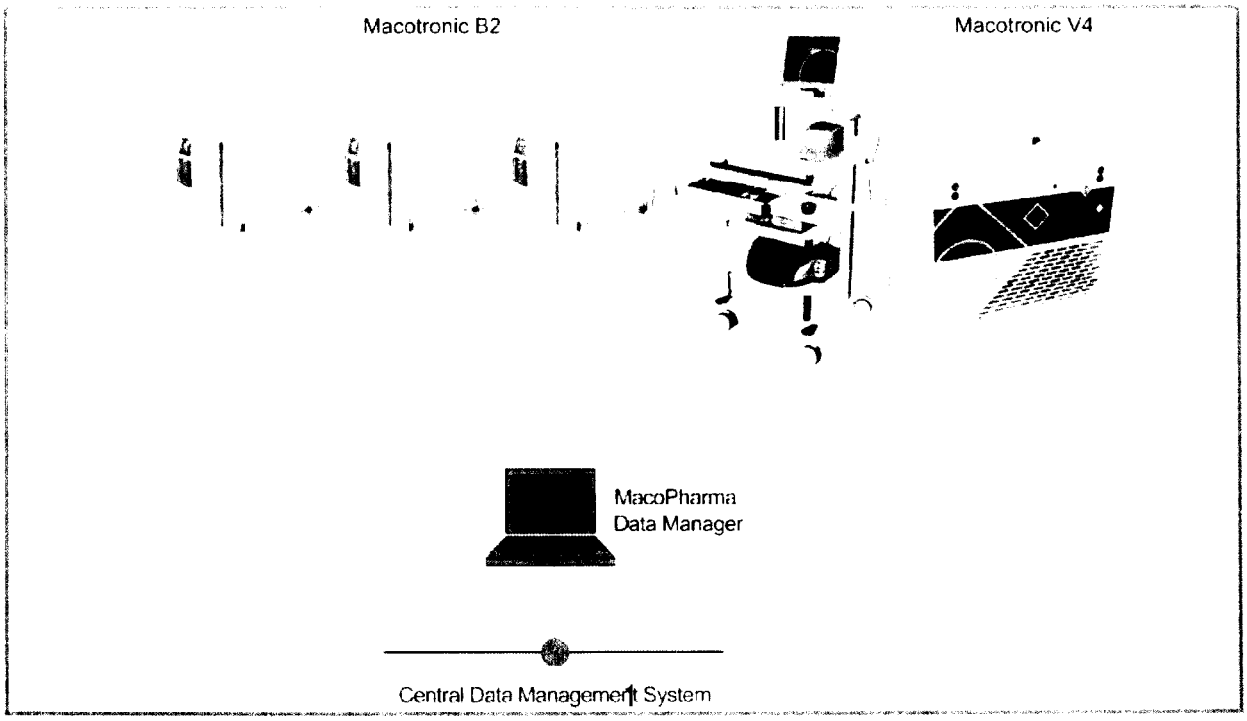
PATHOGEN INACTIVATION SYSTEM FOR PLASMA

Contributing to 10 years of safer Pathogen Inactivated Plasma

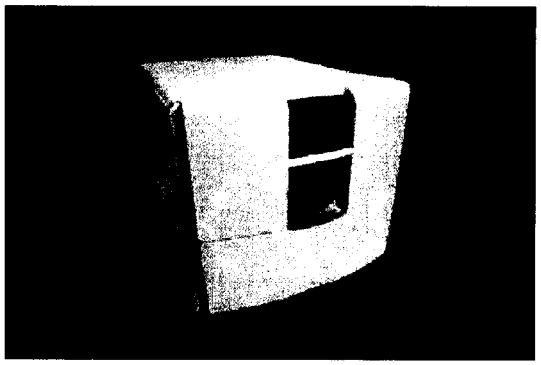
- Routine use worlwide: over 4 million MB Plasma units transfused
- Proven clinical efficacy
- In-house processing
- User friendly technology

New Developments:
Macotronic B2, next generation of illumination equipment

- New light source: LED (Light-Emitting Diode)
- Optimal wavelenght (630nm)
- Reduced illumination time (15mn)
- Touchscreen operating system
- Integrated RFID technology
- Full GMP-Procedure
- CE Marked by December 2007



MacoPharma



MacoTronic B2

Plasma illumination device for the THERAFLEX-MB Plasma pathogen reduction process

Ref. 9MB2000

Specifications :

Dimensions :	47 x 68 x 44 cm (width x depth x height).
Weight :	~ 35 kg
Power supply :	110-240V, 50-60Hz
Lighting system :	96 light-emitting diodes (LED), 4 modules of 24 LED each, 2 modules per bag (double-side exposure)
Wave length :	627 +/-10 nm
Connectivity :	4 USB ports (rear panel : 3, front panel : 1), 1 Ethernet port (network connection)
Screen :	5,5 inch VGA colour touch screen
Cooling system :	Ventilation of illumination chamber by laminar air flow

- **Operating mode :**

Delivered energy :	Preset as per THERAFLEX-MB Plasma procedure
Bag loading mode :	Manual opening of the drawer
Capacity per cycle :	2 bags per cycle
Temperature of use :	Air-conditioned room (20-22°C)

- **Operating controls :**

Light sensors :	4 control photo-diodes (1 per light module)
Temperature sensors :	2 pyrometers for direct measurement on each bag surface and for ambiant temperature in the illumination chamber
Alarms :	Flashing logo for operating status, sound alarm for errors

- **Process control & traceability :**

Barcode reading :	Bag ID and batch, product code, operator, post-labelling barcode control
Report printing :	Cycle illumination report providing energy, intensity and temperature records
Cycle record :	Up to 8000 illuminations files stored in the internal memory
Backup :	Transfer of illumination files by USB key or MacoTrace (Data Manager)
Network connection :	TCP/IP protocol, assignable IP adress through MacoTrace
Import/export :	Connection to the IT Management System (LIS) through MacoTrace

- **Accessories :** USB barcode laser reader, thermal transfer label printer, report printer, MacoTrace licence, ethernet cable, RFID module

- **Regulations :**

CE Marking :	Conformity with MDD 93/42/EEC expected for end Q1 2008
Electrical safety :	Conformity with EN 61010-1 expected for end Q1 2008
Electromagnetic certification :	Conformity with EN 61326-1 expected for end Q1 2008

9MB2000_A_C 22/02/2008

Certified ISO 9001 Certified ISO 13485
MacoPharma
F - 59420 MOUVAUX

First investigations on a newly developed LED illumination device for the treatment of MB-plasma

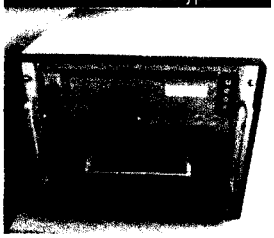
U. Gravemann[1], M. Behague[2], S. Reichenberg[3], H. Mohr[1], W. H. Walker[3], T. Verpoort[2], T.H. Mueller[1]

[1]Blood Center of the German Red Cross Chapters of NSTOB, Institute Springe, Germany
[2]Maco Pharma S.A., Tourcoing, France [3]Maco Pharma International, Langen, Germany

Purpose

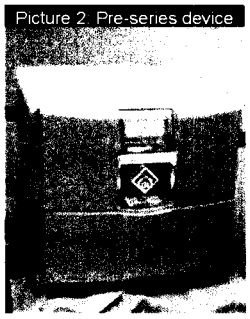
Treatment of fresh frozen plasma (FFP) with MB (methylene blue) and light is a procedure used for the inactivation of blood-borne viruses for more than 15 years. MB plasma is currently produced in several European countries using the MacoPharma Theraflex MB plasma system. High-intensity, long-lived light-emitting diodes (LEDs) are now available on the market, which might replace the sodium vapour lamps currently used in the Macotronic device (MacoPharma). The purpose of this study was the first evaluation of a newly developed LED illumination device (picture 1) with respect to virus inactivation capacity and plasma quality.

Picture1: Prototype



Methods

Picture 2: Pre-series device



Treatment was done in the Theraflex MB plasma bag system (MacoPharma), containing leucocyte-depletion filter Plasmaflex, MB pill (85 µg MB), illumination bag, MB depletion filter Blueflex and plasma storage bag. For investigating plasma quality 3 plasma pools were each prepared from 3 different single donor units. Plasma was divided into three illumination bags and illuminated on the LED device. Samples were taken at different time points of illumination and factor VIII and fibrinogen (Clauss) were determined.

For investigating virus inactivation capacity Pseudorabies virus (PRV) was spiked into FFP (n = 4) resulting in a titer of approximately 10^6 tissue culture infectious doses/ml ($TCID_{50}$/ml). The viral titer was determined from samples taken after 5, 10, 15 and 20 minutes of illumination on the LED device. Infectivity was determined by endpoint titration using a Vero cell CPE (cytopathic effect) assay. In a preliminary run the currently used Macotronic was compared with the newly developed LED device.

Results

Virus inactivation was investigated using the PRV, an enveloped, double-stranded DNA virus, which is used as model virus for HBV. MB/light treatment using the LED-based illumination device resulted in an inactivation of > 4 log steps of PRV after 10 – 15 min of illumination (diagram 1). The device is at least as effective as the Macotronic device routinely used at present.

Table 1: Inactivation kinetics of PRV (n = 4)

sampling time [min]	0	5	10	15	20
$\log_{10}$ $TCID_{50}$/ml (mean · SD)	6.16 · 0.23	4.64 · 0.68	≤ 2.41 · 1.65	≤ 1.75 · 0.38	≤ 1.54 · 0.00
$\log_{10}$ reduction factor		1.52	≥ 3.75	≥ 4.41	≥ 4.62

Plasma quality was only slightly affected by illumination. Factor VIII was decreased by 17% and fibrinogen (Clauss) by 14% during an illumination of 20 min (Diagram 2). Results are at least comparable to or might even be better than those for the Macotronic device.

Diagram 1: Inactivation of PRV by LED device (n=4) and Macotronic (n=1)

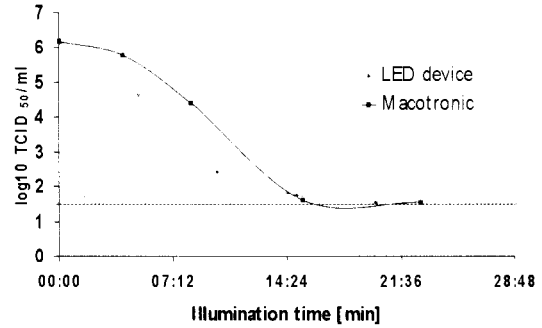
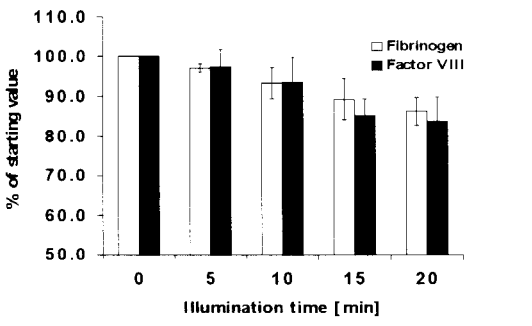


Diagram 2: Influence on coagulation factors (n = 3)



Conclusions

A new, compact illumination device based on long-lived LEDs was developed. The preliminary data suggest that this LED device is comparable to the Macotronic device with respect to virus inactivation capacity and preservation of plasma quality. Illumination time might even be shortened by using this high intensity illumination device.

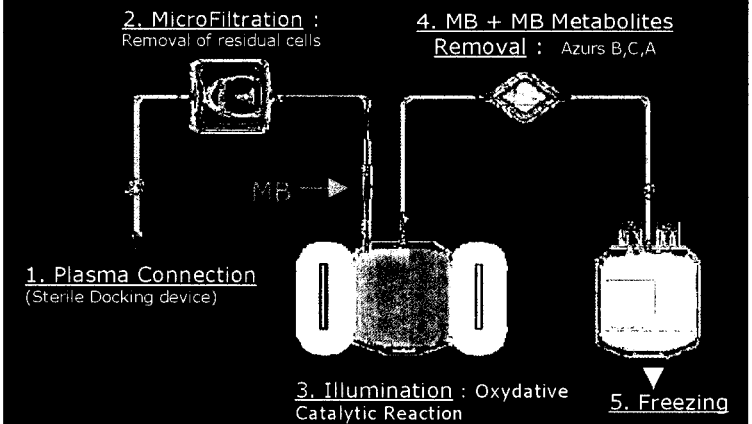
Three years' haemovigilance of methylene blue-treated fresh frozen plasma : no increase in transfusion reaction incidence

Deneys V.[1,2], Lambermont M.[2], Latinne D.[1], Guerrieri C.[1], Baele P.[1]
[1] Tranfusion Committee, Cliniques Universitaires Saint Luc, 1200 Brussels, BELGIUM
[2] Service Francophone du Sang, Belgian Red Cross, BELGIUM

Background

Due to stringent donor selection, laboratory testing and pathogen inactivation procedures, fresh frozen plasma (FFP) offers a high degree of viral safety. In Belgium, only non remunerated volunteers are recruited as plasma, platelets and blood donors. Pathogen inactivation is a proactive strategy designed to inactivate a pathogen before it enters the blood supply. Methylene blue-photoinactivation was choosen by the Belgian Red Cross two years ago (Theraflex MB plasma - MacoPharma)

MB-viroinactivation (Theraflex-MB plasma MacoPharma)



Methods

Until mid 2004, only solvent-detergent FFP (SD-FFP) was used in our teaching hospital. BM-FFP began to be transfused to our patients thereafter. Since nearly 10 years, all transfusions of labile blood products are checked for the appearance or not of an adverse event.
In this study, the incidence and the seriousness of adverse event after BM-FFP transfusion were compared with those observed with SD-FFP.

Results

(a) : benign allergic reaction (n=4)
moderate allergic reaction (n=1)
NHFR (n=1)
(b) : benign allergic reaction (n=5)
moderate allergic reaction (n=3)
No TRALI episode (any previous pregnancy / transfusion = contra-indication for plasma donation)

	SD-FFP	BM-FFP
Period	2003 - 2004	mid 2004 - 2005
Number of FFP units transfused	5101	5660
Number of adverse reactions after FFP Tf°	6 (a)	8 (b)
Incidence of reaction	0.12 %	0.14 %

Discussion and conclusion

No significant increase in adverse event incidence was observed after BM-FFP transfusion (odds ratio 1.2) and the seriousness of these events was comparable. The clinical efficacy of both FFP was similar : both procedures have limited effects on coagulation factors (especially on fibrinogen and factor VIII for MB, on protein S and alpha 2-antiplasmin for SD).
Finally, plasma pooling may have the undesirable effect of increasing the risk of transmitting viruses that are either resistant to the process or have escaped the virucidal process.
The hallmarkof MB technology is that it allows the viral inactivation of single donor units of FFP, offering reassurance that no increased infectious risks are added due to pooling.
This a crucial point in term of public health safety.

Safety Of Methylene Blue Treated Plasma

Pohler P[1], Leuschner J[2], Gravemann U[1], Reichenberg S[3], Walker W H[3], Mohr H[1]

[1]DRK NSTOB, Springe, Germany, [2]LPT KG, Hamburg, Germany, [3]Maco Pharma International GmbH, Langen, Germany

AABB Annual Meeting 2007, Anaheim, USA

OBJECTIVES

The procedure using methylene blue (MB) to inactivate viruses in therapeutic plasma is well established worldwide. It includes membrane filtration (Plasmaflex, 0.65 µm pore size), addition of MB (dry pill, 85 µg, resulting in 1 µmole/L at 266 ml), illumination (approx. 20 min, 590 nm), and filtration of MB and photoproducts (Blueflex). More than 4 million units of plasma were transfused without any unusual adverse event reported.

Aim of this study was to prove the toxicological safety of MB, its photoproducts azure A, B and C, and that of MB-treated plasma.

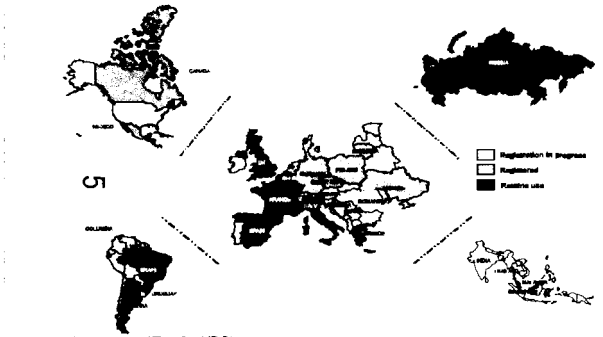


Figure 1: Global status of Theraflex MB-Plasma use

METHODS

Adsorption, distribution and excretion of 14C-labeled MB following 24 h infusion were investigated at a dose level of 20 mg/kg body weight (b.w.) in rats. Observation time was 96 hours.

Studies on teratogenic effects were done by intravenous bolus injection of MB into rats and Beagle dogs. MB was administered daily to the dams at 4, 12, 36 mg/kg b.w. (rat) and 2, 6, 18 mg/kg b.w. (rabbit).

In a tolerance test 5 ml/kg b.w. of autologous light-treated plasma (1 or 10 µM MB) was administered to 5 male Beagles per group by intravenous administration. After 21 d 3 dogs/group were treated again and sacrificed 24 h later. Hematology, clinical biochemistry, and electrocardiogram were examined. A complete histopathology was done.

MB and Azure A/B/C were tested in: bacterial reverse mutation test (Ames test), in vitro mammalian cell gene mutation test, in vitro mammalian chromosome aberration test with human lymphocytes, in vivo micronucleus test with rat bone marrow and peripheral blood cells (20 mg/kg b.w., 24 h infusion), in vivo unscheduled DNA synthesis test in rats (20 mg/kg b.w., bolus infusion).

RESULTS

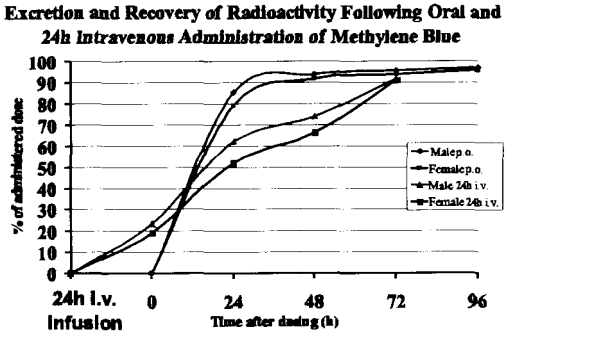


Fig. 2 **Recovery of ^{14}C-labeled Methylene blue (MB).** The recovery was examined in Sprague-Dawley rats following oral administration (p.o.) and 24 h i.v. infusion at a dose level of 20 mg MB/kg body weight. Urine, faeces, organs, expired air, rinse water and infusion site were analysed. The radioactivity recovery rate in organs and at the infusion.

1. Pharmacokinetics of ^{14}C-labeled MB after 24 h infusion were determined in T_{max}, $T_{1/2}$ α and $T_{1/2}$ β It indicated:

- biphasic elimination of MB with an initial half-life of 3 min and a longer terminal half-life of 12.6 h (male) and 16.0 h (female)

- less than 1 % radioactivity in plasma and examined organs

- Excretion of radioactivity was almost complete after 96 h

- no accumulation or storage of MB

2. The no observed effect level (NOEL) for the fetal organism was 4 mg and 6 mg/kg b.w./day in rats and rabbits.

3. Clastogenic effects of MB and Azure B were found in vitro.

4. No genotoxic effects on bone marrow, peripheral blood cells and hepatocytes after application of 20 mg/kg b.w. MB and Azure B.

5. No signs of intolerance or sensitization after infusion of 1 µM or 10 µM MB light-treated plasma before removal of MB and photoproducts were observed.

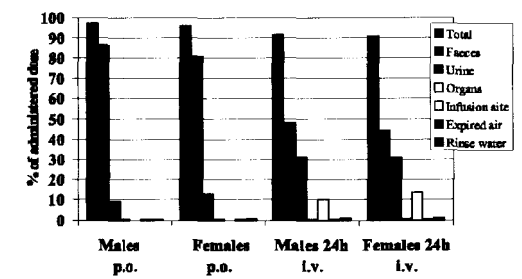


Fig. 3 Mean recovery of radioactivity after oral (gavage) application and 24 h i.v. infusion of 20 mg MB/kg body weight in rats.

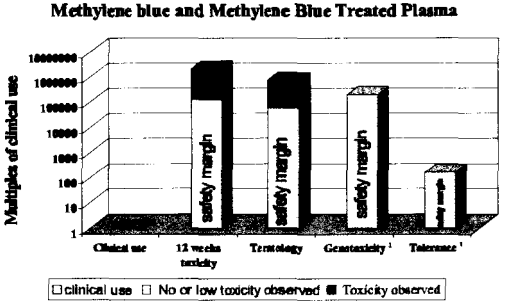


Fig. 4 Safety margins for toxicity from in vivo studies with emthylene blue. Calculations are based on the treshold NOEL: no observed effect level (slight methaemoglobinaemia) (12 weeks toxicity) and a normal clinical exposure of 0.1 µg MB/kg body weight (=6 units MB plasma). [1] no toxicity occured; therefore the safety margins are based on the highest dose tested.

CONCLUSIONS

Thresholds for no or low toxic properties which occurred after administration of MB in preclinical studies varied depending on the amount of MB applicable in the specific test system. They are > 160 to 200,000 fold higher than the estimated clinical exposure of MB after infusion of 6 units MB-light treated plasma.

REFERENCES

1 Williamson et al. 2003 Transfusion 43:1322-1329

2 Pohler et al. 2004 Transfus Med Hemother 31 (supp 3):1-84. PS305

CHARACTERISTICS OF MACO PHARMA THERAFLEX MB-PLASMA

S. Reichenberg[1], U. Gravemann[2], P. Pohler[2], W. H. Walker[1]
[1]Maco Pharma, Langen, Germany
[2]Blood Center of the German Red Cross Chapters of NSTOB, Institute Springe

ISBT Congress, Madrid, June 2007

Background: During the last 15 years the method using methylene blue (MB) to inactivate viruses in plasma was constantly improved. Invented by the Blood Center of the German Red Cross, chapters of NSTOB, Institute Springe, the initial procedure included: Freezing and thawing to release intracellular viruses from leucocytes, addition of a proportional amount of a MB stem solution to a final concentration of 1 µM, and subsequent one-side illumination for one hour with fluorescent tubes.

Aim: The aim was to improve the original method to facilitate the implementation in the blood bank.

Results

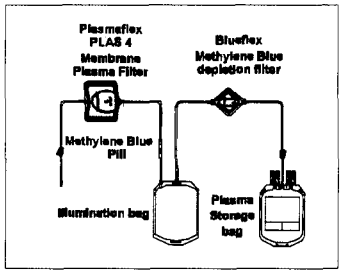


Figure 1: bag system

Figure 2: Illumination device Macotronic

Plasma quality

Test	Unit	Range	Init. value	after prep.	3 month	9 month	18 month	27 month
Thrombin time	[s]	14 - 21	15,2 ± 0,3	17,4 ± 0,9	15,9 ± 1,1	16,2 ± 1,1	18,5 ± 0,5	20,2 ± 0,9
Fibrinogen (Clauss)	[mg/dl]	200 - 450	262,3 ± 8,5	190,5 ± 12,6	194,0 ± 14,0	190,8 ± 11,3	254,5 ± 13,4	227,0 ± 11,7
Factor II	[%]	70 - 130	104,8 ± 2,1	101,9 ± 2,6	96,6 ± 1,4	103,0 ± 5,3	109,3 ± 5,2	104,5 ± 2,9
Factor V	[%]	60 - 130	87,1 ± 6,0	105,6 ± 8,8	108,1 ± 3,8	105,0 ± 8,8	99,4 ± 6,4	107,6 ± 3,8
Factor VIII	[%]	60 - 150	88,6 ± 17,9	72,5 ± 15,7	82,3 ± 17,6	72,9 ± 13,8	73,5 ± 14,8	81,5 ± 13,1
Factor IX	[%]	60 - 130	100,4 ± 5,8	92,8 ± 3,1	90,9 ± 6,8	97,8 ± 4,8	77,5 ± 5,3	99,6 ± 8,1
Factor XI	[%]	60 - 130	98,6 ± 5,4	78,4 ± 7,1	80,1 ± 4,5	79,3 ± 5,9	71,5 ± 4,3	87,5 ± 3,4
vWF:RCo	[%]	60 - 150	96,5 ± 5,3	100,5 ± 15,5	110,3 ± 20,7	112,8 ± 22,2	101,8 ± 15,8	110,8 ± 19,8
free Protein S	[%]	55 - 130	104,3 ± 6,4	103,5 ± 7,0	81,8 ± 7,2	98,8 ± 10,5	99,0 ± 5,9	98,8 ± 7,0
Protein C	[%]	70 - 140	97,8 ± 7,7	89,5 ± 5,9	85,0 ± 7,8	81,0 ± 18,5	114,0 ± 10,3	97,0 ± 6,5
AT III	[%]	80 - 120	91,3 ± 3,3	90,5 ± 3,3	95,5 ± 2,6	91,8 ± 3,8	110,8 ± 4,1	102,0 ± 6,8
a_1-Antitrypsin	[mg/dl]	90 - 200	98,5 ± 1,3	97,5 ± 2,5	98,3 ± 1,5	99,3 ± 2,1	106,5 ± 2,4	100,8 ± 3,4
a_2-Antiplasmin	[%]	80 - 120	95,0 ± 2,8	94,0 ± 2,6	92,8 ± 4,4	84,8 ± 2,1	96,0 ± 4,2	100,5 ± 4,4
Factor XIIa	[mU/ml]	< 50	31,3 ± 4,1	33,3 ± 3,9	33,4 ± 6,2	35,1 ± 6,4	36,3 ± 6,8	36,3 ± 4,7
CH100	[U/ml]	392 - 1019	689,6 ± 157,4	579,1 ± 31,7	949,3 ± 85,7	771,3 ± 161,5	798,8 ± 179,7	978,0 ± 74,1

Figure 3: Plasma quality after treatment during storage for 27 month

Virus reduction capacity

Sensitivity of enveloped viruses		
HIV-1	Retro	5,45 [2]
WNV	Flavi	5,78 *2
BVDV	Flavi	5,44 *2
Hog cholera	Flavi	5,92 *1
PRV	Herpes	5,48 *2
Herpes Simplex	Herpes	5,50 *1
Bovine herpes	Herpes	8,11 *1
Semliki Forest	Toga	7,00 *1
Sindbis	Toga	9,73 [1]
Influenza	Orthomyxo	5,1 [1]
HBV (Duck model)	Hepadna	> 6 [3]
Vesicular Stomatitis	Rhabdo	4,89 *1
Sensitivity of non-enveloped viruses		
Adeno	Adeno	4 [1]
Calici	Calici	3,9 *1
SV 40	Papova	4 [1]
Parvo B 19	Parvo	5 [1]

[1] Mohr et al. Immunological Investigations 1995, 24(1&2):73-85
[2] tested by Analysis Biomedizinische Test GmbH
[3] tested by Prof. Christian TREPO et al., INSERM Unit 271, Lyon, France
* tested under production conditions
- Reduction below the limit of detection

Figure 4: Virus reduction capacity

Safety margins for toxicity from *in vivo* studies with MB and MB-treated plasma

■ clinical use □ No or low toxicity observed ■ toxicity observed

Safety margins for toxicity from in vivo studies with methylene blue. Calculations are based on the thresholds NOEL: no observed effect level (teratology) and LOEL: lowest observed effect level (slight methaemoglobinemia) (12 weeks toxicity) and a normal clinical exposure of 0.1 µg MB/kg body weight (= 6 units of MB plasma). ¹No toxicity occurred, therefore the safety margins are based on the highest dose tested.

Pohler et al. 2004 Transfus Med Hemother 31 (suppl 3):1-84, PS305

Figure 5: Safety margin for toxicity of Methylene Blue

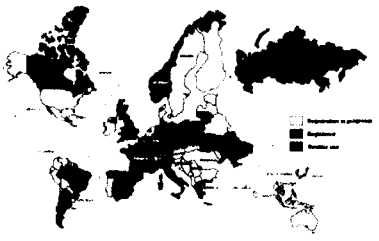
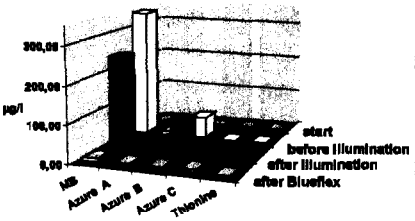


Figure 6: Global status of Theraflex MB-Plasma use

Methylene blue reduction



Reichenberg et al. 2006 Vox Sang 91 (suppl 3): P386

Figure 7: Methylene Blue reduction by Blueflex filtration

Results: With the current Theraflex MB-Plasma procedure provided by MacoPharma the procedure is markedly improved. The elimination of leucocytes is realized by membrane filtration, MB is added as an integrated dry pill, and residual MB and photoproducts are removed by a special Blueflex filter. The specially designed illumination device (Macotronic) ensures treatment under GMP conditions. Illumination dose and intensity are constantly monitored and temperature is controlled. The use of sodium low pressure lamps as improved light sources allowed the reduction of the illumination time to about 20 min.

The characteristic features of the system are:

1. Virus inactivation of enveloped viruses shows a reduction rate of at least 5 log10 steps. (Figure 4)
2. Plasma quality: Only fibrinogen and factor VIII are reduced by about 20-25%. (Figure 3)
3. Clinical use: More than 4 million MB-treated plasmas were transfused with excellent tolerance and efficacy in several countries all over the world. (Figure 6)
4. MB and photoproducts are eliminated by more than 90% using the Blueflex filter. (Figure 7)
5. Toxicology: Investigation on toxicology of MB and photoproducts showed a high safety margin for the concentration used. (Figure 5)

Conclusions

Conclusions: The MacoPharma Theraflex MB-Plasma represents an efficient, safe, and easy to use system which generates virus-safe plasma of high quality.

THE EFFECT OF METHYLENE BLUE PATHOGEN REDUCTION SYSTEM ON Fc VIIIc IN PLASMA DERIVED FROM WHOLE BLOOD DURING STORAGE

Baeten M[1], Rapaille A[2], Donnay D[2], Van der Beek M[1], Sondag D[1], Vandekerckhove P[1]
[1] Dienst voor het Bloed, Rode Kruis-Vlaanderen, Belgium.
[2] Service du Sang, Croix Rouge de Belgique, Belgium.

BACKGROUND

The virucidal properties of methylene blue have been documented since 1930. In 1991, the Springe Institute developed a photodynamic method to inactivate pathogens, particularly viruses, in human plasma using methylene blue in combination with visible light. MacoPharma has improved this method and developed the THERAFLEX MB-Plasma system consisting of the Macotronic illumination machine, together with an appropriate disposable set for pathogen reduction and removal of residual methylene blue to a level less than 4µg/unit.

This method is known to be effective on viruses as well as other documented pathogens, although reducing slightly the activity of clotting factors such as factor VIII (Fc VIIIc) and Fibrinogen. According to Belgian legislation, plasma should be frozen within 18 hours after blood collection. In the case of pathogen reduced plasma, a level of at least 0.5 IU / ml for Fc VIIIc should be attained.

The aim of this study was to investigate the effect on Fc VIIIc recovery of various time delays, between donation and the photodynamic treatment of plasma derived from whole blood.

MATERIALS AND METHODS

In centre 1, 143 units of whole blood were selected from volunteer male A+ donors. These units were divided into 3 groups. Plasma from group A was separated at 4 hours and treated at 5.5 hours, group B plasma at 4 and 16.5 hours, and group C plasma at 15 and 16.5 hours, respectively.

In centre 2, 120 units of whole blood were selected from volunteer male or female donors of any blood group. These units were also divided into 3 groups. Plasma from group D was separated at 3.5 hours and treated at 8 hours, group E plasma at 3.5 and 11 hours, and group F plasma at 12.5 and 16.5, respectively.

Pathogen reduction was performed using the THERAFLEX MB-Plasma system (disposable with leucodepletion filter and methylene blue removal filter, Macotronic illumination machine). Samples for Fc VIIIc activity assay were taken immediately after separation and after treatment. Fc VIIIc measurement was done using a one-stage aPTT clotting assay with Fc VIIIc deficient plasma. Results of Fc VIIIc recovery are expressed in percentage of activity.

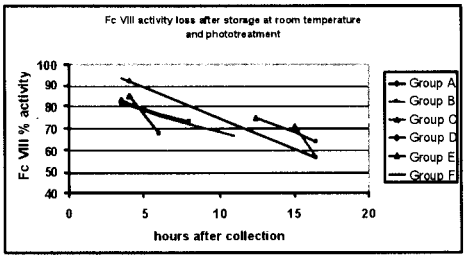
Prior to separation and photo treatment, the whole blood and plasma were stored on eutectic plates to keep the products at a temperature of 20 °C. Results were analysed using repeated measures Anova for general comparison, student t-test for group comparison and paired student t-test when appropriate.

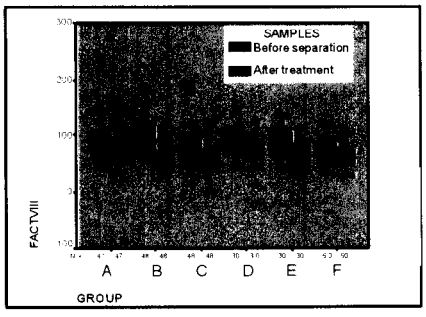
RESULTS

Fc VIIIc pre- and post-photo treatment mean results are presented in table 1.

	Time before separation (h)	Time before treatment (h)	Pre treatment [1] Fc VIIIc (%)	Post treatment [2] Fc VIIIc (%)
Group A	4	5.5	85	67
Group B	4	16.5	92	56
Group C	15	16.5	71	56
Group D	3.5	8	83	73
Group E	3.5	11	81	66
Group F	12.5	16.5	75	64

Table 1 : [1] Sample taken after separation [2] Sample taken after treatment





- *The difference between the two groups was statistically significant ($p<0.001$).*
- *A significant decline in Fc VIIIc activity was measured in all groups following the photo treatment process ($p<0.001$).*
- *No significant difference between group B and groups C and F after photo treatment was observed ($p=0.87$, $p=0.10$); this suggests no significant difference in the loss of Fc VIIIc activity between the time of separation of whole blood into plasma and photo treatment 16.5 h.*
- *No significant difference between Fc VIIIc activity loss in group A and C ($p=0.66$) and in group D and F ($p=0.53$) suggesting that the time interval between blood collection and separation does not influence the loss of Fc VIIIc activity post photo treatment.*

CONCLUSION

Regarding the Fc VIIIc activity of plasma, Methylene blue pathogen reduction has to be completed within a limited time interval after whole blood donation. The processing / separation of whole blood can be performed at any time between donation and the photo treatment of plasma. Following the attainment of these results, methylene blue pathogen reduction of plasma has been implemented in both centres.

XE7RA01A 07/04

Quality Control Evaluation of Methylene Blue Light Treated Plasma.

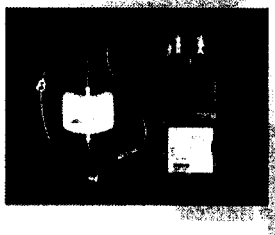
L Larrea[1], A Cerveró[2], M Calabuig[1], A Blanquer[1], P Solves[1], V Mirabet[1], R J Roig[1].
[1]Centro De Transfusion De La Comunidad Valenciana, Valencia, Spain;
2H General Universitario, Valencia, Spain.

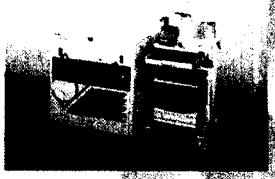
INTRODUCTION

Methylene blue light treated plasma has been used in Spain from 1998. Since then slight variations of the technique have been implemented such as plasma leukoreduction instead of freezing and thawing.
Studies on plasma quality have been published analysing initial methods. We have tested several plasma units to evaluate plasma quality with the current technology.

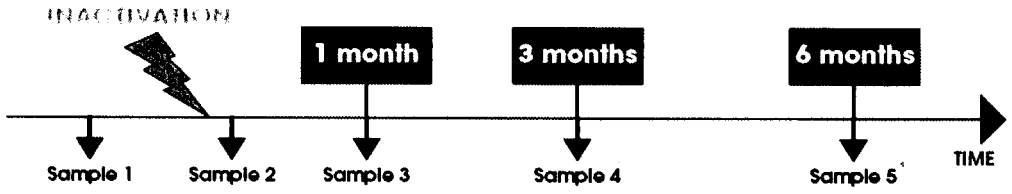
MATERIAL AND METHODS

Briefly speaking, 450±50 mL whole blood units were collected in automatic scales using top & bottom blood bags.
After collecting, units were cooled with 1.4- butanodiol plates and later stored at 22±2°C. Then, whole blood was centrifuged at high speed to obtain a concentrate of red blood cells and plasma, while maintaining the buffy coat in the initial blood bag. For plasma inactivation the Springe modified method was used (Theraflex-MB-Plasma system: Macopharma®). Plasma was joined to the MB system by means of sterile docking and, simultaneously, gravity filtered. In batches of four plasma was illuminated for 20 min. Units for storage were frozen after inactivating before 24 hour postdonation. For the study we inactivated 30 plasma units (10 A, 10 O, 5 B and 5 AB). We took several samples: before inactivation (sample1), just after inactivation (sample2), after 1 month of storage at -30°C (sample3), after 3 months of storage at -30°C (sample4) and after 6 months of storage at -30°C (sample5). After each moment samples were stored at -80°C till the tests were performed.
In every sample we performed the following tests: PT, APTT, FV, FVIII and fibrinogen.





The process of the 5 plasma samples



RESULTS

As published before most affected parameters by the inactivation procedure were fibrinogen and FVIII (18 and 16% respectively decrease from sample 1 to 2). FV was scarcely affected (a 3% decrease from sample 1 to 2). PT an APTT were prolonged only in 2.74 and 5.26% respectively from sample 1 to 2. Results may be seen with more detail in the attached table.

Results of the tests (PT, APTT, Fib, FV and FVIII) for the 5 plasma sample

Sample parameter	(%)↑PT	(%)↑APTT	(%)↓Fib	(%)↓FV	(%)↓FVIII
∧					
∧					
∧					
∧					

PT: Prothrombin rate
APTT: Activated Partial Thromboplastin Time test
Fib: Fibrinogen
FV: Factor V
FVIII: Factor VIII

CONCLUSIONS

The Methylene blue inactivation methodology is very easy to use and the plasma factors after inactivation are preserved. However, during storage there is a certain loss of coagulation factors. If the reason for this is related to the treatment or the storage conditions remains to be evaluated.