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

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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

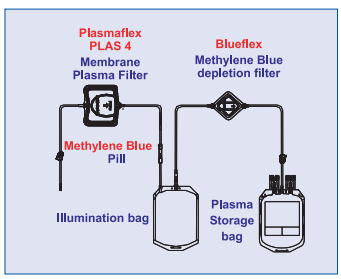


Figure 1: bag system

Figure 2: Illumination device Macotronic

Plasma quality

Test	Unit	Range	Init. value	after prep.	3 month	9 month	18 month	27 month
Global test								
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
Coagulation factors								
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
Inhibitors								
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
Fibrinolysis								
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
Activation								
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
Complement								
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

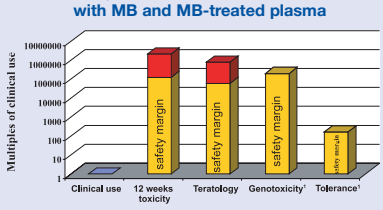
Virus reduction capacity

Sensitivity of enveloped viruses		
Virus	Family	Reduction rate (log_{10})
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		
Virus	Familiy	Reduction rate (log_{10})
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
* 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



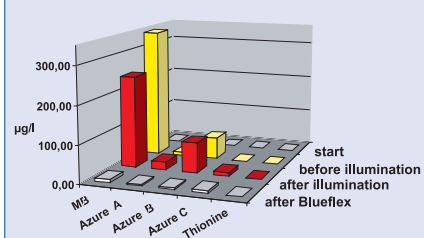
Safety margins for toxicity from in vivo studies with methylene blue. Calculations are based on the thresholds NOEL: no observed effect level (teratology) and LOEL: lowest observed effect level (slight methaemoglobinemia) (12 weeks toxicity) and a normal clinical exposure of 0.1 µg MB/kg body weight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