

# THERAFLEX MB-PLASMA PROCEDURE: PLASMA QUALITY AFTER 15 MONTH STORAGE

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## Introduction

**Background:** Although in the last decades thanks to the implementation of several methods like donor selection and testing procedures the risk of virus transmission from plasma has decreased, infection of patients still exists. Additionally new viruses like West Nile Virus enter the transfusion chain. Therefore, the treatment of therapeutic plasma with methylene blue (MB) is a technique used in several European countries for virus inactivation. MacoPharma has developed the proprietary photodynamic Theraflex MB-Plasma bag system including a MB pill, an illumination system (Macotronic) with visible light, and a final MB filtration step with the Blueflex filter (Williamson et al. Transfusion 2003;43:1322-1329).

**Aims:** Aim of this study was to show the reduction of MB and photoproducts due to the Blueflex filter and to prove the reproducibility of the filtration efficiency. Additionally the quality of the plasma after 15 month storage was checked.

## Materials & Methods

18 plasmas were treated at three different days. At different steps of the Theraflex MB-Plasma procedure the MB and photoproduct content was measured by HPLC, which was described previously (Verpoort et al. 2003; ISBT Istanbul P246). Measurement was done after dissolution of the MB pill, after illumination, and after filtration (Fig. 2).

At each day plasma was pooled and divided into several storage bags (storage temperature <-30 °C). At different time points a palette of plasma factors was measured.

1. global tests (Quick, INR, aPTT, thrombin time)
2. coagulation factors (Fibrinogen, F II, F V, F VII, F VIII:c, F IX, F X, F XI, F XII, FXIII, vWF Ristocetin Co-Factor)
3. Inhibitors (AT III, Protein C, Protein S)
4. Fibrinolysis (Plasmin inhibitor, alpha-1-Antitrypsin, Plasminogen)
5. Complement (CH50, C3a)
6. Activation (TAT, F XIIa, D-Dimer)

## Results

Test	Limits	Unit	0 month	6 month	9 month	15 month
<b>1. Global tests</b>						
Quick	80 - 130	%	98 ± 2	105 ± 3	94 ± 2	93 ± 3
INR	1.0 - 1.30		1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
aPTT	30 - 60	sec	34 ± 1	35 ± 1	34 ± 1	35 ± 1
Thrombin time	14 - 21	sec	22.7 ± 0.7	23 ± 1.0	23 ± 1.0	22 ± 1.0
<b>2. Coagulation factors</b>						
Fibrinogen	1.8 - 3.5	g / l	2.4 ± 0.1	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.1
F II	70 - 130	%	99 ± 4	104 ± 4	106 ± 7	105 ± 12
F V	65 - 150	%	101 ± 3	99 ± 6	103 ± 9	101 ± 10
F VII	70 - 130	%	103 ± 8	119 ± 9	108 ± 3	102 ± 4
F VIII:c	0.50 - 2.00	I.U. / ml	0.81 ± 0.15	0.83 ± 0.12	0.92 ± 0.16	0.87 ± 0.11
F IX	0.70 - 1.30	I.U. / ml	1.00 ± 0.05	0.92 ± 0.04	0.98 ± 0.04	0.94 ± 0.04
F X	70 - 130	%	106 ± 6	108 ± 5	111 ± 7	98 ± 3
F XI	50 - 130	%	82 ± 2	92 ± 3	83 ± 3	83 ± 4
F XII	70 - 130	%	87 ± 10	101 ± 11	99 ± 10	88 ± 11
F XIII	70 - 130	%	81 ± 11	77 ± 7	75 ± 4	85 ± 9
vWF (Ristoc. Co-F)	50 - 140	%	85 ± 8	81 ± 19	94 ± 13	95 ± 7
<b>3. Inhibitors</b>						
AT III	0.80 - 1.30	I.U. / ml	1.12 ± 0.08	1.02 ± 0.08	1.06 ± 0.07	0.99 ± 0.06
Protein C	70 - 150	%	110 ± 7	109 ± 6	116 ± 6	110 ± 3
Protein S	70 - 140	%	75 ± 7	70 ± 2	76 ± 2	81 ± 2
<b>4. Fibrinolysis</b>						
Plasmin inhibitor	80 - 120	%	103 ± 4	101 ± 3	103 ± 6	105 ± 10
alpha-1-Antitrypsin	0.70 - 1.50	I.U. / ml	1.14 ± 0.03	1.12 ± 0.07	1.24 ± 0.09	1.20 ± 0.08
Plasminogen	75 - 140	%	102 ± 7	105 ± 8	12 ± 11	104 ± 10
<b>5. Complement</b>						
CH50	70 - 100	%	116 ± 14	110 ± 11	108 ± 5	114 ± 8
C3a	123-2228	ng / ml	1029 ± 323	1106 ± 596	898 ± 332	921 ± 217
<b>6. Activation</b>						
TAT	1 - 4.1	µg / l	2.1 ± 0.2	2.4 ± 0.5	2.0 ± 0.0	2.2 ± 0.7
F XIIa	< 3.0	ng / ml	1.0 ± 0.2	0.9 ± 0.1	1.3 ± 0.6	1.0 ± 0.0
D-Dimer	64 - 246	µg / l	241 ± 120	239 ± 131	199 ± 97	225 ± 122

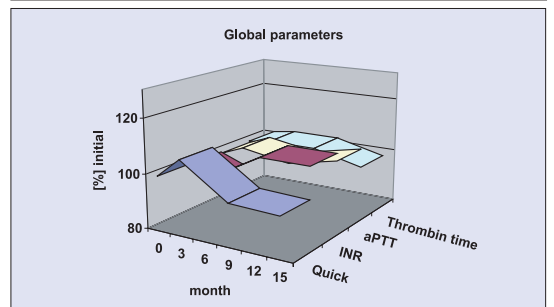
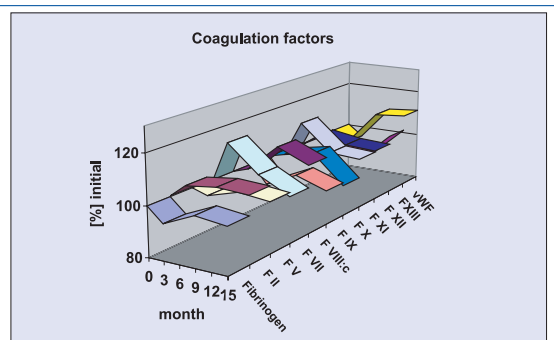


Fig. 1: Storage stability of Theraflex MB-Plasma

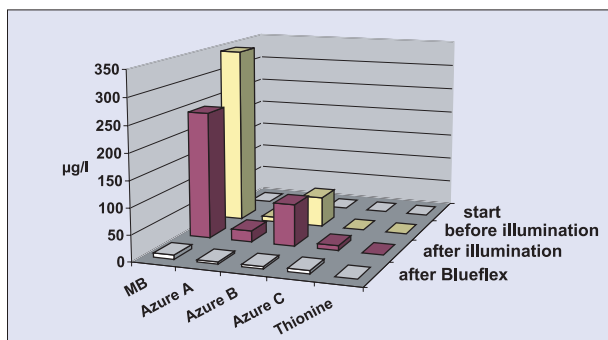


Fig. 2: Methylene blue and photoproduct reduction due to Blueflex filtration

Illumination of MB-containing plasma with visible light using the Macotronic illumination device resulted in the generation of photoproducts as described previously. Mean reduction of the total phenothiazine content was 94.5%. Every single filtration yielded in a filtration efficiency of minimum 91%. The mean reduction capacity for MB was above 99.9%.

There was no significant change in the plasma factor content after treatment during the whole 15 month storage period. Slight variations are within the error of measurement. The only difference between the plasma parameters resulted from the treatment itself. Here, an increase in the INR and aPTT (14.3 %; 18.2 %), decrease of fibrinogen (-19.3 %), factor V (-25.3 %), factor VIII (-21.8 %), factor IX (-25.6 %), factor X (-23.4 %), and factor XI (-16.8 %) was observed. Despite this variations the values were within the ranges found in non-treated plasma.

## Conclusions

The filtration of plasma with the Blueflex filter is a reliable method to reduce the amount of MB and photoproducts substantially. The plasma quality is not changed during the observed storage period and remains within the physiological variation of non-treated plasma.