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

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## 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.*

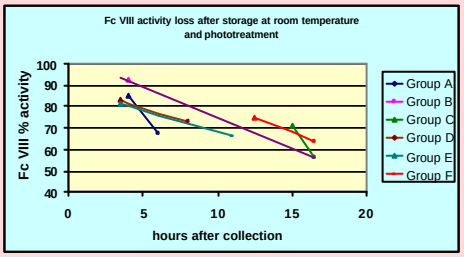


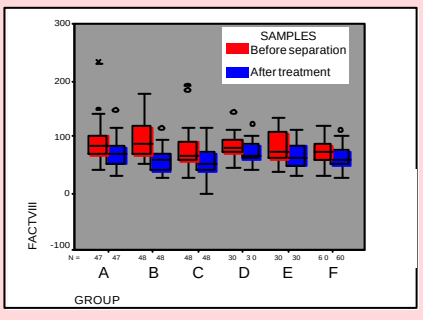
## RESULTS

*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.*

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