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 However, the method we employed to detect WBCs predominantly measures WBC nuclei (unpublished data), and therefore provides little information on cellular integrity.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.[24] 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.

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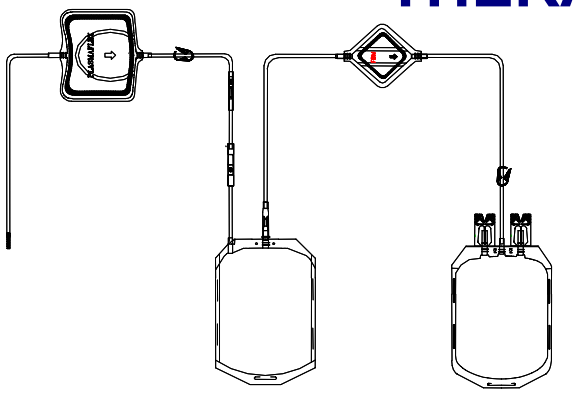
Pathogen Reduction System
THERAFLEX - MB PLASMA



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)

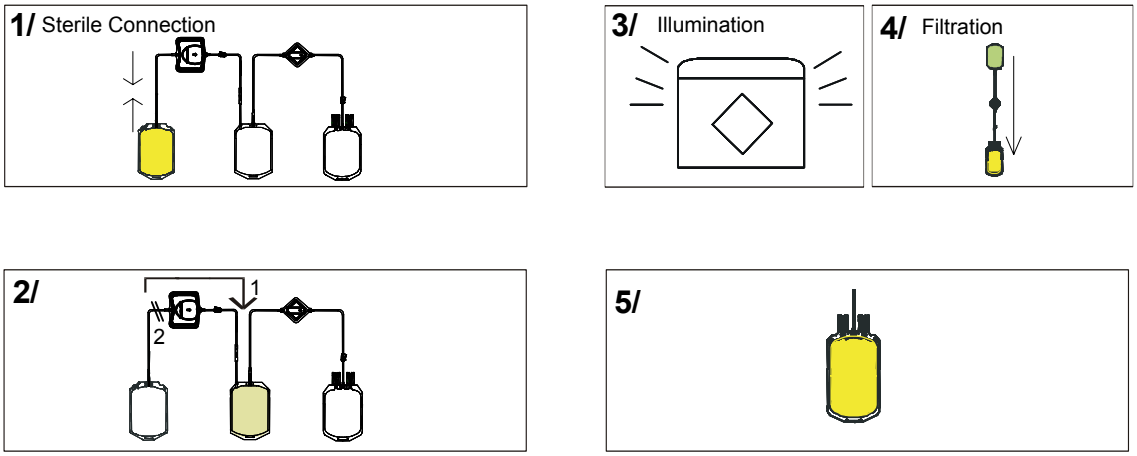
. **Label** : English, French, German, Dutch

. **Sterilisation** : Steam

. **Shelf life** : 2 years

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

Use



Whole Blood | RCC | PLASMA | MB PLASMA | PRP | Platelets | SAG-M PAGGSM | NaCl | Buffy Coat | Serum | Supernatant | Cryoprecipitate

SDV0001XQ_A_A 08/03/2006

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