WHOLE BLOOD FILTER RZ-2000 EFFICIENTLY LEUKODEPLETES POOLED PLASMA PRIOR TO PATHOGEN INACTIVATION WITH INTERCEPT™

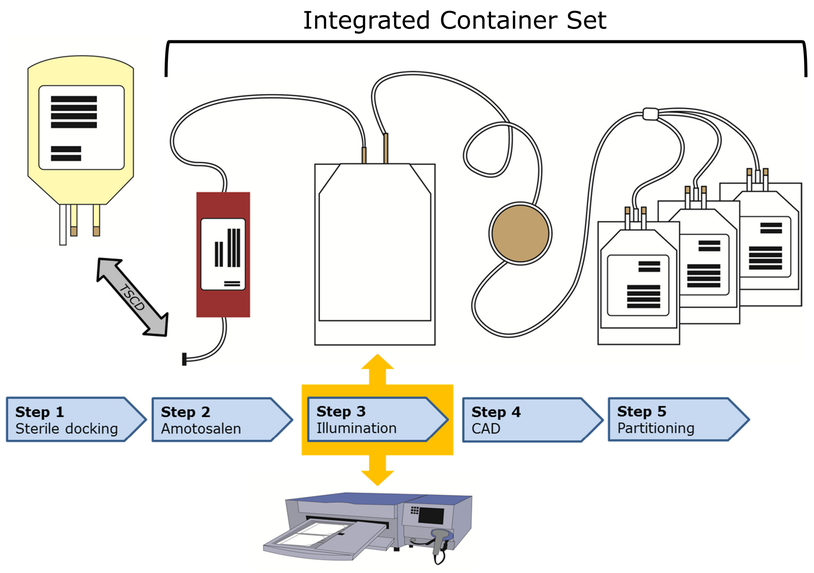
D. Goslings, A. Valek, and B. M. Frey

Blutspende Zurich, Rütistrasse 19, 8952 Schlieren, Switzerland
www.blutspendezuerich.ch

Background

The blood transfusion service Zurich introduced the pathogen inactivation (PI) method INTERCEPT™ for platelets in 2011 and considers now to introduce INTERCEPT™ for plasma as well (Fig. 1). Pathogen inactivated fresh-frozen-plasma (PI-FFP) has several advantages compared to quarantine plasma. Apart from further reducing the risk of transfusion related infections, plasma management is facilitated and availability of FFP increases since PI-FFP can be delivered for transfusion immediately after freezing. To use INTERCEPT™ plasma processing sets with recovered plasma several units need to be pooled prior PI. Ideally a pool of 5 units (approx. 1300mL) is evenly distributed on 2 PI-sets. To reduce cost and optimize workflows only the fraction of recovered plasma intended for transfusion is leukodepleted with a filter integrated into the pooling-set. However, no such set is commercially available yet (Fig. 2). Since whole blood filters are designed to cope with much higher amounts of residual cells than plasma filters for component filtration, we decided to build a pooling-set prototype based on a whole blood filter.

Figure 1: The INTERCEPT™ Process for Plasma



Step 1: Sterile connection between plasma and INTERCEPT™-set (process entry criteria for plasma are volume 385 – 650mL and RBCs < 4x10⁶mL), Step 2: Plasma flows through a small bag containing the reactive compound amotosalen, Step 3:Plasma containing amotosalen gets illuminated with UVA light (DNA/RNA crosslinks irreversibly), Step 4: Removal of excessive amotosalen by filtration, Step 5: Partitioning PI-plasma - ready for freezing (volume approx. 200mL)

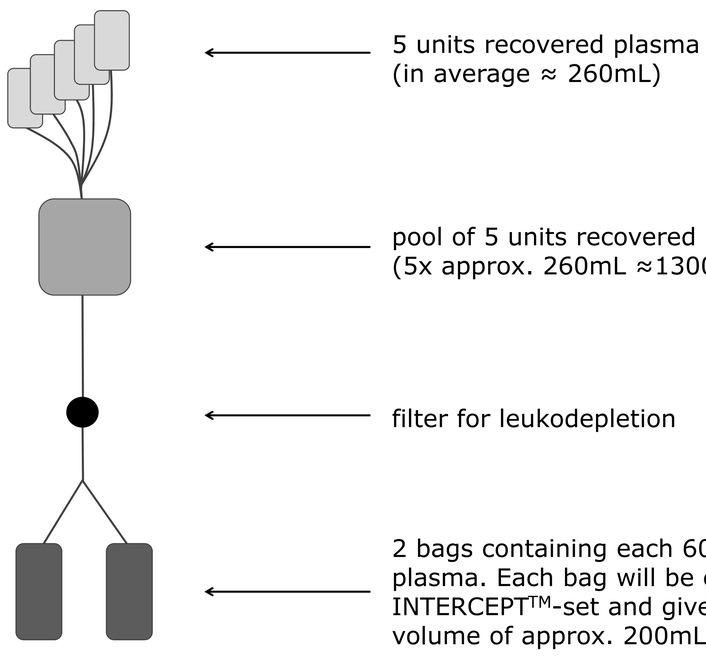
Aims

The objective of this study was to identify a filter suitable to quickly process 1300mL non-filtered recovered plasma to meet process entry criteria for INTERCEPT™ (guard bands: vol. 385-650mL, <4x10⁶ RBC/mL) and Swiss specifications for FFP with respect to residual cells and Factor VIII (<1x10⁶ WBC/unit, <5x10⁹ RBC/unit, <50x10⁹ platelets/L, Factor VIII ≥0.7IU/mL).

Methods

A pooling-set was built by connecting a 2000mL-bag (R4R2041, Fenwal) with the Asahi RZ-2000 filter taken from Fenwal set NGR6449. The RZ-2000 outlet was connected via y piece to a dual-bag-set (2x500mL, VDE40003XA, MacoPharma). 6 Plasma-pools (each containing 5 non-filtered recovered plasma units) were processed with this prototype (Fig. 3). Each of the 2 bags of the dual-set is supposed to be connected to an INTERCEPT™-set. However, for economic reasons of evaluation, PI was performed with 1 bag only resulting in 3 PI-FFP per INTERCEPT™ -set used. Time from donation to freezing was 5-8h. 1 PI-FFP unit per pool was thawed for analysis at first month of storage. Residual cells and Factor VIII were assessed by FACS and photometry, respectively.

Figure 2: Principle of Plasma Pooling prior to INTERCEPT™



Five plasma units are filtrated after pooling to remove leucocytes and then evenly distributed on two bags. Each bag must contain 600 – 650mL plasma to use full capacity of the set and contain less than 4x10⁶ RBC/mL to fulfill process entry requirement for INTERCEPT™. In addition WBC content must be < 1x10⁶ per final FFP and plts concentration must be less than 50x10⁹/L to meet Swiss specifications.

Results

Before filtration average pool-volume (n=6) was 1335mL (1310-1358) and mean WBC concentration in pools was 0.0233x10³/µL (range 0.0087-0.0697x10³/µL) which is equivalent to 4.7x10⁶WBC/200mL-FFP. RBC ranged 0.19-0.65 x10⁶/mL, platelets 11.91-23.67x10⁹/L and Factor VIII was 1.14IU/mL (0.97-1.40, n=5). After filtration (max. 9min) volume loss was 56-69mL and WBC ranged 0.0000-0.0001x10³/µL (equivalent to 0.0-0.02x10⁶WBC/200mL-FFP). RBC were reduced to 0.07-0.36x10⁶/mL and platelets to 0.13-0.62x10⁹/L while Factor VIII was still in average 1.14IU/mL (1.00-1.29, p=0.686). PI was performed with 611-634mL filtered plasma and resulted in 3 PI-FFP per run. Factor VIII of thawed PI-FFP was in average 0.78IU/mL (0.70-0.94) (Tab. 1-4). While loss of Factor VIII through freezing was not significant (p=0.102), PI significantly decreased Factor VIII by 25% (p=0.042) (Fig. 4).

Parameters Measured During Production of PI-FFP with Prototype

Table 1: Parameters before Filtration with RZ-2000

	Average	Median (	Range	)	Specification*	pass/fail
Volume [mL]	1335	1338	(1310 -	1358)	n.a.	n.a.
WBC						
[1x10⁶/200 mL]	4.660	3.150	(1.740 -	13.940)	< 1	100% fail
[1x10³/µL]	0.0233	0.0158	(0.0087 -	0.0697)	n.a.	n.a.
RBC [1x10⁶/mL]	0.40	0.36	(0.19 -	0.65)	< 4	100% pass
Plts [1x10⁹/L]	18.53	19.54	(11.91 -	23.67)	< 50	100% pass
Factor VIII [IU/mL]	1.14	1.06	(0.97 -	1.40)	≥ 0.7	100% pass

Table 2: Parameters after Filtration with RZ-2000

	Average	Median (	Range	)	Specification*	pass/fail
Volume [mL]	625	627	(611 -	634)	385 - 650	100% pass
WBC						
[1x10⁶/200 mL]	0.007	0.000	(0.000 -	0.020)	< 1	100% pass
[1x10³/µL]	0.0000	0.0000	(0.0000 -	0.0001)	n.a.	n.a.
RBC [1x10⁶/mL]	0.20	0.15	(0.07 -	0.36)	< 4	100% pass
Plts [1x10⁹/L]	0.31	0.23	(0.13 -	0.62)	< 50	100% pass
Factor VIII [IU/mL]	1.14	1.09	(1.00 -	1.29)	≥ 0.7	100% pass

Table 3: Parameters after Pathogen Inactivation

	Average	Median (	Range	)	Specification*	pass/fail
Factor VIII [IU/mL]	0.85	0.82	(0.70 -	1.01)	≥ 0.7	100% pass

Table 4: Parameters after Freezing and Thawing

	Average	Median (	Range	)	Specification*	pass/fail
Factor VIII [IU/mL]	0.78	0.76	(0.70 -	0.94)	≥ 0.7	100% pass

*Tables 1-4: * Specifications for volume and RBCs according to INTERCEPT™ process entry criteria and specifications for WBCs and Factor VIII according to Swiss regulations (Limits for WBCs are per unit; 1 unit ≈200mL); n=6 except for Factor VIII (n=5); WBC=White Blood Cells; RBC=Red Blood Cells; Plts=platelets; WBC were reduced from out of specification values to levels around detection limit. Levels of RBCs and Plts were reduced as well. Factor VIII content was reduced but acceptable.*

Figure 3: Prototype in Use

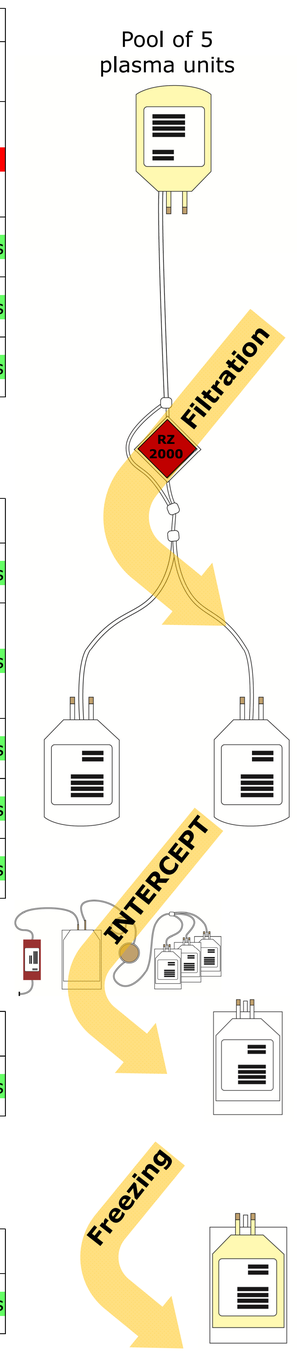
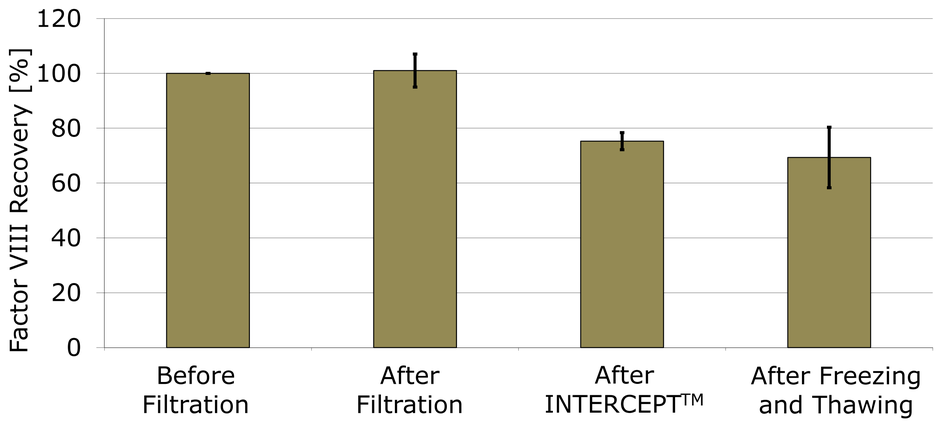


Figure 4: Factor VIII Recovery



Recovery of Factor VIII in plasma at different processing steps (n=5); Average Factor VIII content before filtration = 100%; Decrease through filtration and freezing is not significant (p=0.686 and p=0.102) while decrease through PI is significant (p=0.042)

Summary/Conclusions

All plasma-pools met guard bands after filtration and all PI-FFP met Swiss specifications. The RZ-2000 seems to be well suited to filter 1300mL plasma for INTERCEPT™ without having any negative impact on Factor VIII. Due to these promising results further development of plasma pooling-set using RZ-2000 is desirable.

32nd International Congress of the ISBT in joint cooperation with the 10th Congress of AMMTAC, July 7–12, 2012 (Cancun, Mexico)