

Buffy Coat Enrichment of Marrow using the Sepax2 SmartRedux Protocol

Cameron G, Filer K, Low N, Hogge D

Clinical Cell Therapy Laboratory, Vancouver, British Columbia, Canada



Provincial Health Services Authority

BACKGROUND:

Currently marrow requiring buffy coat enrichment for either Plasma or RBC reduction is processed using the COBE 2991. Although the COBE 2991 is in running order it was purchased in 1989 is cumbersome to use and requires two technologists for processing. This Sepax 2 has a small foot print and is currently being used for another protocol in our laboratory. It is a closed system and requires minimal interaction with the technologist while running. This is why this validation was initiated.

METHOD:

The Sepax 2 cell processing system uses a rotating syringe technology that provides both separation through rotation of the syringe chamber (centrifugation) and component transfer through displacement of the syringe piston. The kits are single use and provide a sterile environment once connected to the input cells in a biosafety cabinet. This protocol is used to reduce up to 3300mls of marrow. The output bag volume is a predetermined percentage of the total volume remaining in the processing chamber after the plasma fraction has been extracted. Each cycle will process a maximum of 220mls in about 20 minutes and up to 15 cycles per kit. Each cycle processed will have a reprocessed Buffy Coat option of up to 30mls, and the potential of added back volumes of reprocessed plasma.

Initial Settings:

Initial volume ÷ 12 to determine final volume
30ml of reprocessed buffy coat
10mls of plasma added to product during processing

The initial assessment was done on pooled products that had been collected from PV patients requiring phlebotomy as part of their treatment. Cell counts were performed and the mock product was run on the Sepax 2. This was repeated three times. Once the initial testing was completed, additional runs were done using similar mock products that were greater than 1000mls that are more representative of an actual adult marrow volume. Some adjustments were required to accommodate the larger volume; such as the number used to determine the final volume from 12 to 15 and the volume of plasma (20mls) to make up the final volume. A minimum of two successful runs was required in order to process marrow. Adjustment of settings were determined in collaboration with the manufacturer.

RESULTS:

Results for the initial three samples which were less than 1000ml were very promising, with good recoveries and RBC and Plasma depletion was well within our acceptable limits (>70% TNC recovery, <30mls of RBC and <120mls of Plasma) See Table 1

Table I: Pooled PV Blood <1000mls

Run #	Vol(g)	Start TNC	Final Vol	Final RBC Vol	TNC Rec
1	808	4.69E+09	70	26	94%
2	857	9.51E+09	71	14	94%
3	638	6.32E+09	54	14	86%

The next 8 mock products (Table II) were greater than one litre. The first run yielded RBC volume of 42mls. We decided to adjust the plasma added volume in order to improve the RBC depletion. The following two results used 25ml and 10mls of plasma added which yielded RBC volume of 37 and 32mls. In consultation with the manufacture, the next two products used a combination of variables until we settled on the following settings:

Final Settings:

Initial volume ÷ 15 to determine final volume
30mls of reprocessed buffy coat
20ml of plasma added to product during processing

The last three products were processed using these settings

Table II: Pooled PV Blood >1000mls

Run #	Vol(g)	TNC Start	Plasma Vol/by added	BC reprocessed	RBC Vol	TNC Recovery
4	1075	5.70E+09	12 18mls	30ml	42	87%
5	1025	7.28E+09	12 25mls	30ml	37	98%
6	1060	7.10E+09	15 10mls	30ml	32	85%
7	1321	5.55E+09	15 25mls	30ml	51	79%
8	1060	9.01E+09	15 15mls	30ml	5	73%
9	1102	1.07E+10	15 20mls	30ml	27	108%
10	1189	7.73E+09	15 20mls	30ml	21	80%
11	1066	1.05E+10	15 20mls	30ml	15	97%

Encouraged by the TNC recovery and RBC volume we proceeded to process 5 marrows using the final settings.

Marrow Processing using the Smart Redux Protocol

Vol (g)	TNC Start	RBC Vol	TNC Recovery	Final Volume	Time Processed
1411	3.08E+10	27	73%	95	3:12hr
972	2.87E+10	19	76%	70	2:03hr
1200	2.36E+10	12	74%	81	2:34hr
1187	2.17E+10	13	80%	80	2:44hr
1088	2.27E+10	28	76%	86	2:30hr

Marrows processed had varying initial volumes. Although the RBC volumes were within range, the TNC recoveries were on the low side of our acceptable range of 70% as compared to the mock products. Cell counts were done on the RBC discard bag where most of the remaining cells could be found. Cell recoveries when using the COBE 2991 yield an average of 86% with final volumes between 80-120mls and a run time of no more than 60 minutes.

CONCLUSIONS:

The Sepax 2 can be used to process marrow successfully using the SmartRedux protocol although the time it takes to process is somewhat longer than the COBE 2991. The price of the kits are also somewhat higher than the COBE 2991 kits. The ease of use and the minimal technologist intervention is an incentive to switch to the Sepax. We continue to work with manufacturer to improve TNC recoveries.

References:
GE Healthcare - Biosafe users manual

