

Automated manufacturing of CD34-enriched cell products from cryopreserved mobilized apheresis on the CliniMACS Prodigy®

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Background

CD34-selected stem cell boost (SCB) is a valid treatment option for poor graft function (PGF) and long-term cytopenia, which are severe complications after allogeneic hematopoietic stem cell transplantation and CAR T cell therapy.[1,2]

PGF is associated with increased morbidity and mortality from severe infections and hemorrhagic complications.[1,3] A boost of selected CD34+ stem cells from the initial donor, without further conditioning and graft-versus-host disease (GVHD) prophylaxis, has been described as an option to overcome persistent PGF.[3–6] SCBs applied independent of donor type, transplantation protocol, or failed therapy with growth factors, have shown 72–100% rapid and sustained lineage recovery with low rates of GVHD, even in high-risk and mixed chimerism patients.[1]

SCB with enriched CD34+ cells has also been used to treat long-term cytopenia after CAR T cell therapy. Different case studies show that the time to recovery from neutropenia and transfusion independence ranged from 14 to 33 days after a CD34+ SCB.[2,7–9]

The enrichment of CD34+ cells is usually performed on fresh mobilized apheresis products. Unfortunately, fresh products are not always available as a cell source for SCB. Using cryopreserved starting materials, however, requires the removal of DMSO in a separate manual or automated cell wash before the cell separation process that enriches the CD34+ cells. Here, we describe an integrated approach that incorporates DMSO removal directly into the automated enrichment of CD34+ cells on the CliniMACS Prodigy (fig. 1).

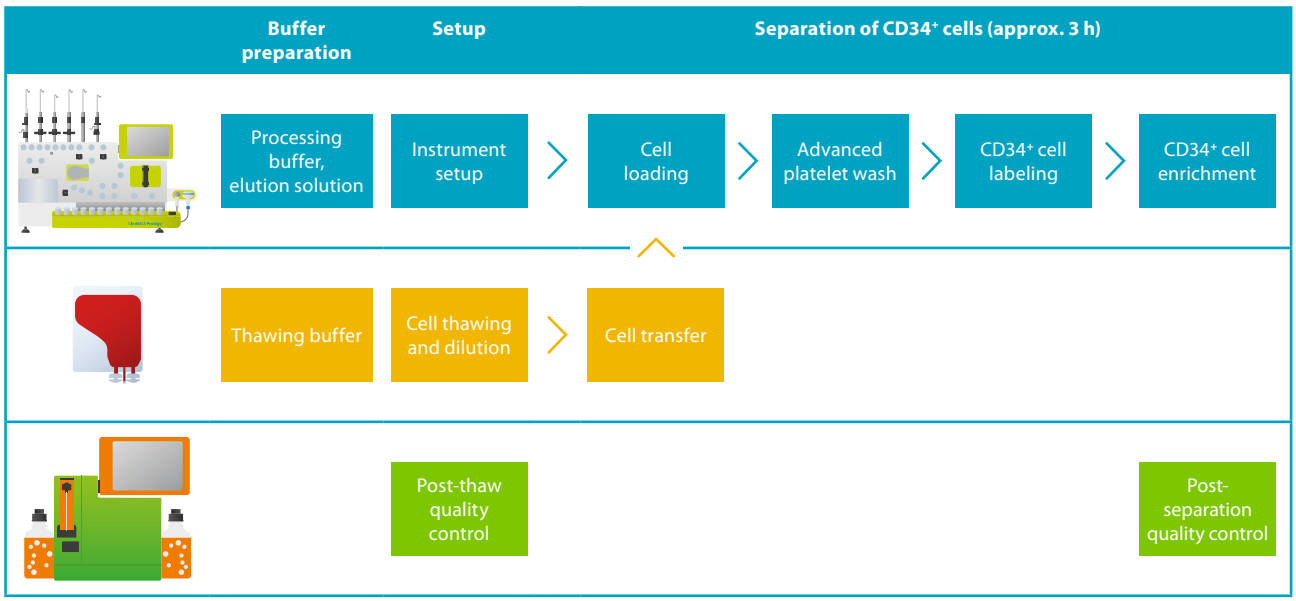


Figure 1: Timing and alignment of CliniMACS Prodigy preparation and use, cell product thawing, and relevant quality control analysis for manufacturing CD34-enriched cell products.

Materials and methods

The method we developed builds upon the well-established process for enriching CD34+ cells on the CliniMACS Prodigy. The cryopreserved mobilized apheresis product is thawed and diluted before loading onto the CliniMACS Prodigy, and the removal of DMSO occurs during an advanced platelet wash automatically performed on the CliniMACS Prodigy (fig. 1). Below, we describe how to prepare the necessary buffers, thaw and dilute the cryopreserved mobilized apheresis product before loading onto the CliniMACS Prodigy. The product table on the last page lists the required instruments and reagents.

Preparing buffers

Two buffers needed to be prepared: a thawing buffer and a processing buffer. The MACS® GMP Tyto® Running Buffer (PBS/$MgCl_2$) served as the basis for both.

The thawing buffer consisted of 1 L MACS GMP Tyto Running Buffer (PBS/$MgCl_2$) supplemented with 1% (w/v) human serum albumin (HSA) and 0.4% sodium citrate. We used the thawing buffer to dilute the apheresis product after thawing and adding DNase. We used Pulmozyme® because it is readily available and clinically approved.

We also prepared the processing buffer from MACS GMP Tyto Running Buffer (PBS/$MgCl_2$) supplemented with 1% HSA (w/v) and 10 U/mL DNase (Pulmozyme) for cell processing on the CliniMACS Prodigy. Six 1 L bags were required.

Finally, we prepared an elution solution from a sodium chloride solution (0.9%) supplemented with 1% HSA (w/v) to elute the enriched cell fraction.

Setting up the CliniMACS Prodigy

Setup of the CliniMACS Prodigy followed the User Manual instructions for preparing the process to enrich CD34+ cells and installing the CliniMACS Prodigy Tubing Set (TS) 320 with the following adjustments:

1. We connected a one-liter bag of processing buffer (see "Preparing buffers") to the CliniMACS Prodigy TS 320, and the remaining five bags were connected in a daisy chain configuration to the first bag.
2. We also connected a bag with 500 mL elution solution.
3. We then performed the priming procedure on the instrument.
4. We entered the cell product parameters for the thawed and diluted apheresis product (see "Thawing and diluting apheresis product"), ensuring that we stayed within the normal scale process capacity.
5. When instructed to transfer the apheresis product, we continued the setup without loading the cells.
6. We transferred IgG and the CliniMACS CD34 Reagent to their respective reagent bags and registered these.

Thawing and diluting the apheresis product

1. We dry-thawed the frozen cell product.
2. While ice crystals were still visible, we added DNase (2.5 mL/20 mL apheresis product).
3. Once the apheresis product had thawed completely, we took a sample for quality control analysis and then diluted the cells at least 1:3 by adding thawing buffer, ensuring that the final volume did not exceed 600 mL. If required, we transferred the cells to a 600 mL Transfer Bag for dilution, taking care not to generate foam.

Enrichment of CD34+ cells on the CliniMACS Prodigy

We transferred the diluted apheresis product into the Application Bag of a CliniMACS Prodigy TS 320 installed on the CliniMACS Prodigy and initiated the enrichment process. Upon completing cell processing, we took samples from the Target Cell Bag and Non-Target Cell Bag for quality control analysis according ISHAGE Guidelines.[10]

Results

Efficient enrichment of CD34⁺ cells

We conducted three enrichment runs, each with one cryopreserved mobilized apheresis product. All three apheresis products were successfully thawed, prepared, and enriched for CD34⁺ cells. We started from an average of 13.2×10⁹ white blood cells (WBC) containing 1.55% CD34⁺ cells, and after enrichment, we were able to provide 100 million CD34⁺ cells per run. Cell counts and viability values for the sampled cell fractions are listed in table 1.

All steps of the method, from thawing and diluting to processing the cells on CliniMACS Prodigy, went smoothly. No aggregates or deposits were visible in the separation columns, the Application Bag, or the Filtration Bag (fig. 2).

Mean values listed in table 2 demonstrate that all three runs on the CliniMACS Prodigy showed excellent separation performance, providing highly pure (93%) and viable (97%) CD34-enriched cell products with high target cell yields (53%). The CD3 log depletion was outstanding (–5.18).[11]

Sampled cell fraction		Total WBC (×10⁹)	CD34⁺ cell fraction (%)	CD34⁺ cell count (×10⁶)	CD34⁺ cell viability (%)
Thawed apheresis product	Run 1	14.07	1.34	188.7	84
	Run 2	15.64	0.96	150.4	64
	Run 3	9.90	2.36	233.9	70
	Mean	**13.20**	**1.55**	**191.0**	**73**
Target cell fraction (CD34-enriched)	Run 1	0.114	93.6	106.8	97
	Run 2	0.090	90.5	81.4	99
	Run 3	0.123	93.6	115.0	96
	Mean	**0.110**	**92.6**	**101.1**	**97**
Non-target cell fraction	Run 1	10.72	0.45	0.50	–
	Run 2	9.63	0.70	0.67	–
	Run 3	6.17	1.30	0.80	–
	Mean	**8.84**	**0.82**	**0.70**	**–**

Table 1: Numbers and viability of cells in different pre-enrichment and post-enrichment fractions.

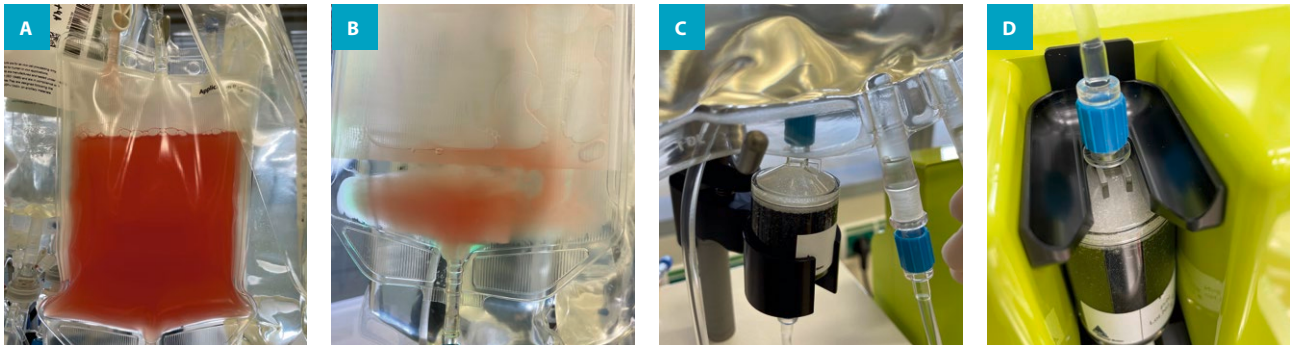


Figure 2: Application Bag (A), Filtration Bag (B), and columns (C and D) were aggregate and deposit-free throughout cell processing.

Parameter	Run 1	Run 2	Run 3	Mean
Purity of CD34⁺ cells (%)	94	91	94	93
Yield of CD34⁺ cells (%) compared to post-thaw cell counts	57	54	49	53
CD3 log depletion compared to post-thaw cell counts	–5.14	–5.02	–5.36	–5.18
Viability of CD34⁺ cells (%)	97	99	96	97

Table 2: Performance statistics for the enrichment of CD34⁺ cells.

Conclusions

This application note underscores the flexibility, ease of use, and robustness of enriching CD34⁺ cells on the CliniMACS Prodigy, even when processing frozen cells. We presented a method to thaw, dilute, and process cryopreserved mobilized apheresis products without the aggregate and deposit formation that commonly plagues this material type.

The enriched CD34⁺ cells were highly pure and viable, with a mean of 93% and 97%, respectively. The process also provided excellent T cell (CD3) depletion (–5.18 log). As expected, the average yield of 53% CD34⁺ cells from thawed products was slightly lower than the 66% yield averaged from fresh apheresis (internal verification data).

With the integrated cell enrichment workflow, DMSO is removed from the thawed apheresis product during the advanced platelet wash on the CliniMACS Prodigy. This on-board step eliminates the need for a separate wash, saving 1–1.5 hours of preparation time. Thus, the enrichment process, including the DMSO removal, takes only three hours and requires minimal manual intervention, freeing up time and resources during cell manufacturing.

The reduced number of washing steps is also gentler on cells and reduces the risk of cell loss. Although functional assessments have yet to be made, the quicker separation of target cells suggests that they may be in better condition, which could offer therapeutic benefits.

Overall, the high purity and viable cell count of the CD34-enriched cell products demonstrate that this method effectively gains high numbers of quality stem cells from cryopreserved products. This capability enables SCB to be used as a treatment option for PGF after hematopoietic stem cell transplantation or long-term cytopenia following CAR T cell therapy, without needing to re-invite and re-mobilize the donor.

References

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Product tables

Miltenyi Biotec products
CliniMACS Prodigy Instrument
CliniMACS Prodigy Tubing Set 320
CliniMACS CD34 Reagent
MACS GMP Tyto Running Buffer (PBS/$MgCl_2$), 1 L bags
Flow cytometer, like a MACSQuant® Analyzer
Transfer Set Coupler/Coupler (or equivalent)
Three-way Tube Adapter (optional)

Other materials and equipment
Human serum albumin (HSA), pharmaceutical grade
Sodium chloride solution (0.9%), for infusion
Transfer Bag (600 mL)
5% IgG solution
Sodium citrate solution, pharmaceutical grade
DNase (Pulmozyme)
Luer Lock syringes (10, 30, and 50 mL) and hypodermic 20-gauge needles
Sterile docking device
Cell counter
Blood product thawing device

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