Development of an automated cryopreservation process for leukapheresis to support CGT supply chain

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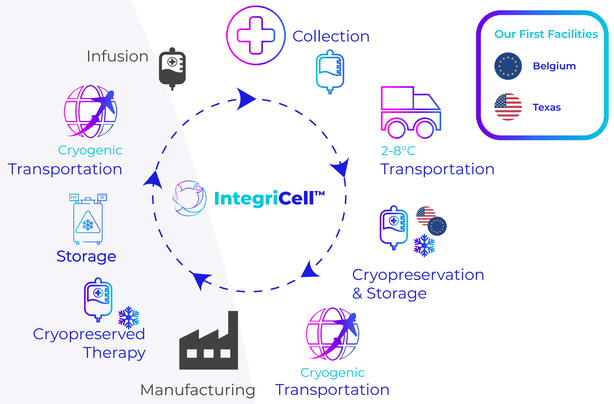
IntegriCell™ platform

Cryoport Systems is the global market leader in temperature-controlled supply chain solutions for critical materials in the life sciences industry and is currently supporting the Cell Therapy Industry with IntegriCell™ – an Integrated Leukapheresis Supply Chain Platform **(Figure 1).** IntegriCell™ aims to deliver consistent, compliant, high-quality leukapheresis starting material for use in the manufacture of cell-based therapies leading to risk and cost reduction through optimized manufacturing capacity planning.

Cell therapies require an optimized cryopreservation process for starting material to achieve consistent results. Cryoport Systems, through the IntegriCell™ platform, is building a network of cryopreservation centers across the US and EU to ensure close proximity to patients, and to optimize and standardize cryopreservation of leukapheresis together within an integrated storage and distribution network.

Figure 1. IntegriCell™ platform

- Leukapheresis collection support
- Leukapheresis pick up at collection site
- Industry leading temperature-controlled transportation to cryopreservation sites
- Standardized cryopreservation <24h with appropriate quality control
- Cryopreservation slots booked with collection timing
- Storage and controlled worldwide transportation to destination



Development of automated closed cryo-process for fresh leukapheresis

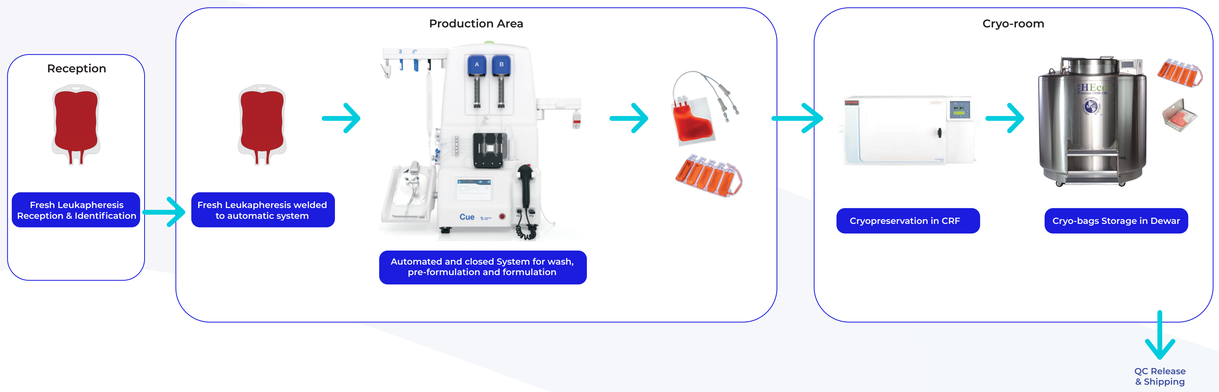
Here, we illustrate the development of an automated closed process using the Cue® Cell Processing System from Fresenius Kabi. This process is subdivided into several critical steps: buffer preparation; Cue system processing, post processing and freezing. Cue system processing involves leukapheresis cells resuspension, wash by spinning membrane filtration, buffer exchange and cryo-formulation. We developed a Quality by Design (QbD) cryo-process upon which Critical Process parameters (CPPs) were identified based on risk assessment. Built on those prerequisites, the IntegriCell™ automated closed cryo-process was developed by optimizing CPPs such as wash buffer solution content, cell concentration and spinner wash flow rate upon processing.

The developed Cryo-process is designed in an automated closed system. Automation and process closing from the surrounding environment reduces the need for critical interventions in open phases and associated contamination risks while increasing reproducibility.

The Automated Cryo-process entails the following subsequent steps **(Figure 2):**

- Leukapheresis preparation: the fresh material is controlled. Leukapheresis is visually checked, transport temperature is verified, and documentation is controlled for release.
- Washing: before the washing step, sampling are performed for QC testing and the leukapheresis volume is measured. Afterward, cells are washed within the washing buffer by spinning membrane filtration. Once washing step is complete, in-process control sampling is performed for cells count measurement allowing the cell concentration adjustment at the next process step.
- Pre-formulation: cell concentration is automatically adjusted by the Cue equipment in pre-formulation buffer to reach appropriate cell concentration.
- Formulation: DMSO based cryoprotectant is added to reach a target cell concentration. Then, formulated cells are transferred in Cryo-bag and final QC sampling are performed.
- Freezing: the processed cells are frozen with automated Controlled Rate Freezer (CRF) equipment ensuring that freezing cycle parameters are performed in a reproducible and controlled manner.

Figure 2. IntegriCell™ automated cryo-process for fresh leukapheresis



Performance of the IntegriCell™ cryo-process

Several process parameters were assessed upon IntegriCell GMP compatible leukapheresis cryo-process development. Among them, critical process parameters (CPPs) such as wash buffer solution content, cell concentration upon processing and spinner wash flow rate of leukapheresis were assessed. In total, three groups of CPP conditions were optimized.

Figure 3. shows Viable Nucleated Cell (VNC) recovery **(Fig. 3a)** and viability **(Fig. 3b)** for 3 optimized CPP conditions assessed on: 1. fresh leukapheresis before processing 2. post spinning membrane filtration wash and 3. post-formulation (i.e. pre-freezing). Among the three different CPP conditions analyzed, CPP condition C demonstrated highest cell recovery and viability post-formulation (mean±SD of 89.8±5.2 and 98.7±0.5 respectively). Based on those results, CPP corresponding to condition C were selected for further evaluation of the automated cryo-processing of fresh leukaphereses. Importantly, as highlighted on **Figure 3c.**, lymphocyte population was preserved during the CPP optimized cryo-processes.

In order to assess the developed automated cryo-process as comparing to a manual cryo-process, we investigated post-thaw recovery and viability from both processes as shown in **Fig. 4a and 4b.** Cryo-vials or Cryo-bags were transferred into controlled thawing device before washing and resuspension for assessment. Cell recovery and viability of manual and automated cryopreserved products were measured using automated cell counter. Both processes provides high VNC recovery and viability (90,2±8,7% and 91,8±6,6% recovery and 98,3±0,7% and 97,7±0,5% viability for Manual and automated cryo-process respectively). Interestingly, whereas manual cryo-process showed higher recovery variations with outliers potentially associated with operator variability, automated cryo-process ensured the robust performance within the defined CQA.

To further understand the retained profile of each immune cell subtype, leukapheresis cell populations were assessed by flow cytometry before and after cryo-processing derived from 2 different donors. As illustrated on **Fig. 4c**, lymphocytes population was slightly reduced after cryo-preservation and thawing compared to fresh leukapheresis. Importantly, T cell population was maintained while only slight reduction in B lymphocyte population was observed. Finally, the potential impact of hematocrit / White blood cell ratio of fresh leukapheresis on process recovery and cell viability was assessed. HCT/WBC ratios of 12 different donors (with ratio between 0,39 and 1,33) were assessed and no correlation on process CQA was observed **(Fig. 4d).**

Figure 3. IntegriCell™ automated cryo-process CPP evaluation

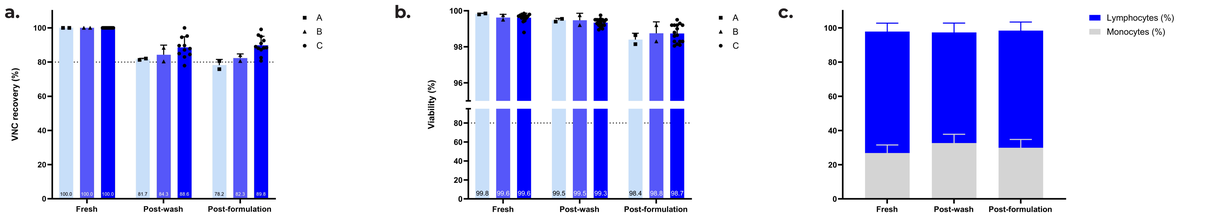


Figure 3. IntegriCell™ automated cryo-processed for fresh leukapheresis maintains high cell recovery rate and viability throughout different CPP testing conditions. CPP condition C demonstrates superior cell recovery rate over condition A and B. Importantly, cryo-processed leukapheresis under CPP condition C preserves lymphocyte population at comparable level to the fresh counterpart. a Viable nucleated cell (VNC) recovery assessment and **b.** Viability assessment by Dapi/Acridine Orange staining in the fresh leukopak, post-wash stage and post-formulation stage under different CPP conditions (A, B, C). **c.** Leukocyte subpopulation flow cytometry analyses in fresh Leukopak, post-wash stage and post-formulation stage under CPP condition C.

Figure 4. IntegriCell™ automated cryo-process post-thaw evaluation

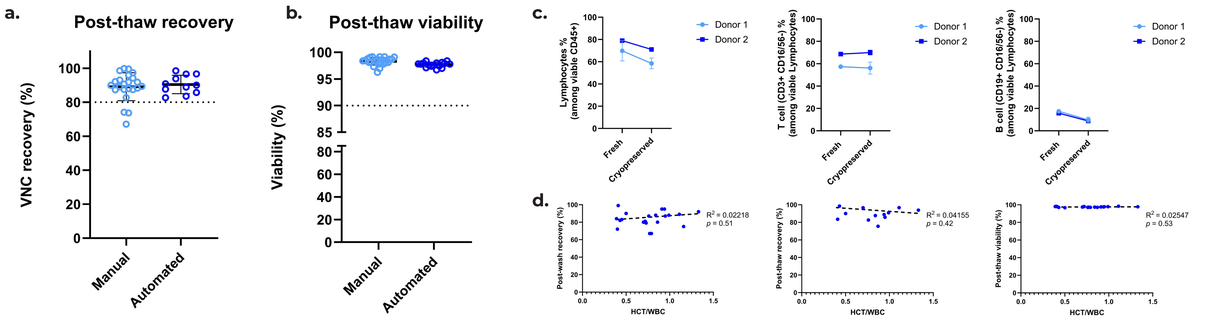


Figure 4. IntegriCell™ automated cryo-processing for leukapheresis ensures the robust performance within the defined post-thawing attributes as compared to manual cryo-processing. Cryopreserved leukapheresis maintains high recovery rate of T cell population post-thawing as compared to the fresh leukapheresis. Additionally, hematocrit/white blood cell ratio in the starting leukopak shows no potential impact on the cell recovery, viability during and after the automated cryo-processing. a. Post-thaw cell recovery and **b.** Post-thaw viability assessment for the automated cryo-processed versus manually cryo-processed leukapheresis. **c.** Flow cytometry analysis for the lymphocyte subpopulation post-thawing versus the fresh counterparts. **d.** Pearson correlation analysis between the HCT/WBC ratio in the fresh leukapheresis and post-wash cell recovery rate, post-thaw cell recovery rate as well as the post-thaw viability respectively.

Conclusion and discussion

Here, we demonstrate the leukapheresis cryopreservation process created by Cryoport Systems for the IntegriCell™ platform. This system facilitates automated closed processing of leukapheresis material, recognized as the initial material for subsequent GMP applications in cell therapy manufacturing. Historically, cryopreservation has been viewed as a “one-size-fits-all” solution that was often performed at the same center as the collection process. However, here we have demonstrated that the IntegriCell™ optimized and standardized process delivers consistency in cell viability and cell recovery while maintaining immune cell populations between multiple donor-derived leukaphereses.

Cryopreservation, when performed appropriately, provides a high-quality starting material for cell therapy manufacturing. Cryoport Systems is currently deploying the GMP compliant automated closed cryopreservation service through the IntegriCell™ platform across the US and EU within Cryoport logistics and GMP-storage network. The goal is to enable increased and improved standardization for high-quality leukapheresis supply to support our clinical and commercial partners globally.

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