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# Automated Cryopreparation of Apheresis Material

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## Abstract

This application note[1] demonstrates the use of the Cue® Cell Processing System for the automated, closed system cryopreparation of fresh leukapheresis starting material for further manufacture.

## Background

The manufacture of autologous Cell and Gene Therapy (CGT) products is not only a process development challenge, but a logistical one. Cryopreservation of the starting apheresis material can help provide a higher-quality and more standardized cellular product that is more stable for transport. This reduces risk in logistics-related delays and can help ensure the availability of manufacturing resources after receipt.

The success of autologous CGT, however, has placed higher burden on cell collection sites to accommodate apheresis collections to support an ever-increasing number of commercial products and clinical trials. Many collection sites are not well equipped to accommodate custom cryopreservation protocols, meaning sponsors often need to compromise and qualify legacy institutional protocols, sacrificing standardization of starting material. Automated systems, such as Fresenius Kabi's Cue Cell Processing System is flexible and can accommodate many custom cryopreservation processes on a single platform, making it easy for collection sites to process material according to several protocols or for sponsors to standardize cryopreservation protocols across multiple sites.

In this study, fresh leukapheresis material was processed according to a model cryopreparation protocol to demonstrate the quality and consistency of the prepared apheresis material.

## Methods

Apheresis material was collected from healthy non-mobilized donors according to institutional protocols. Cells were shipped or held at 4 °C and processed within 24 hours of collection.

Just prior to the procedure, starting material was sampled and analyzed. White Blood Cell (WBC) and Red Blood Cell (RBC) counts (platelets omitted) and starting material volume were entered onto the Cue Cell Processing System, tubing set installed, and wash/formulation solutions connected.

The automated cryopreparation procedure was executed according to the instrument protocol settings listed in the "Protocol Settings" section, below.

Briefly, the collection container was sterile welded to the Cue tubing set and cells were washed with a Plasmalyte-A solution supplemented with 5% Human Serum Albumin to a 2x concentration (100 x$10^6$ WBC/mL). Cells were cooled to 4°C and cryoprotective agent (CPA) containing 10% DMSO was added 1:1 at a controlled 30 mL/min flow rate until a 1x cell concentration (50 x$10^6$ WBC/mL) and a final DMSO concentration of 5% was achieved. Formulated cell suspension was sampled from the bulk bag.

Formulated cells were aliquoted into 250 mL EVA cryobags at either 50 mL or 70 mL fill volumes (targeting 2.5 x$10^9$ or 3.5 x$10^9$ TNC/bag). Bags were air expressed using semi-automated instrument prompts, sealed, placed in cassettes, and promptly placed in a controlled rate freezer (CRF). CRF was programmed to cool at 1 °C/min with a seeding dip at -8 °C and 25 °C/min until -120 °C. Chamber temperature was held at -120 °C for >15 minutes and immediately removed and thawed in a 37 °C water bath. Bags were removed from the water bath when the last ice crystals melted and immediately sampled and analyzed.

All samples were analyzed on an automated hematology analyzer for cell counts. Suspensions were prepared with a no-wash no-lyse protocol for flow cytometry by staining for CD3/CD8/CD45/CD4 and viability and analyzed using a cytometer. Washout was evaluated by measurement of supernatant glucose concentration.

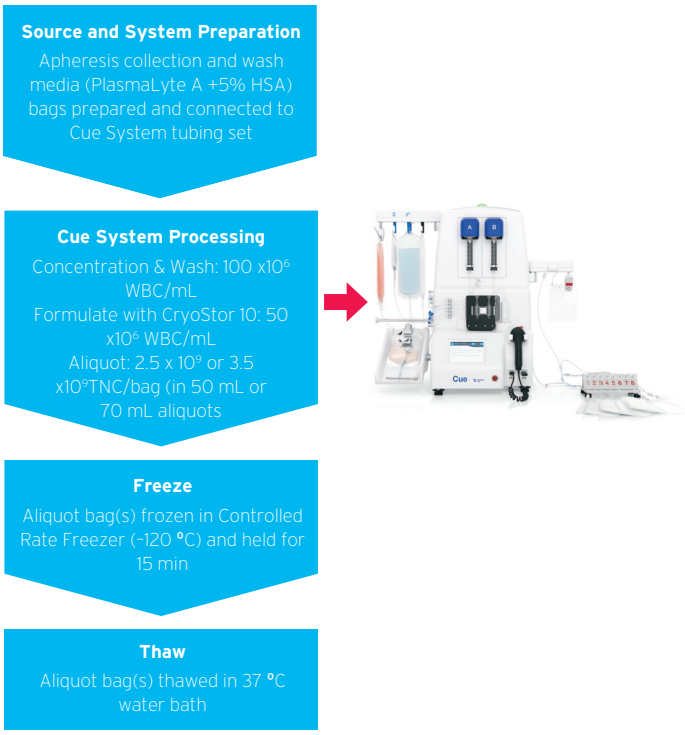


Figure 1. Depiction of the cryopreparation protocol followed in this study. All steps are completed with the assistance of the Cue Cell Processing System.

# Results and Discussion

## Starting Material

Starting material composition varied from procedure to procedure with the Cue processing a range of 3.3 x $10^9$ to 10.6 x$10^9$ total WBCs. All source products were processed on the system under the same protocol (refer to "Protocol Settings", below).

| Parameter | Mean (STD) | Range |
|---|---|---|
| **Volume**, mL | 137.7 (±6.3) | [126.8 - 142.5] |
| **WBC** | | |
| Concentration, x$10^6$/mL | 42.0 (±22.9) | [23.4 – 76.6] |
| Yield, x$10^9$ | 5.8 (±3.2) | [3.3 – 10.6] |
| CD3+, % | 60.2 (±4.0) | [53.3 – 63.1] |
| CD4+, % | 37.0 (±12.3) | [24.0 – 53.6] |
| CD8+, % | 19.5 (±8.1) | [8.1 – 28.6] |
| Viability, % | 99.4 (±0.6) | [98.3 – 99.9] |
| **RBC** | | |
| Concentration, x$10^6$/mL Yield, x$10^9$ | 408 (±148)<br>56.4 (±23.9) | [280 – 660]<br>[35.5 – 97.1] |
| **Platelet Conc.** | | |
| Concentration, x$10^6$/mL | 1010 (±1130) | [168 – 2510] |
| Yield, x$10^{11}$ | 1.40 (±1.57) | [0.21 – 3.49] |

Figure 2. Incoming leukapheresis starting material composition.

## Recovery

WBC recovery, measured as total WBC pre-freeze compared to source material, showed minimal to no cell loss and was consistent across all five runs. On average, approximately half of the source RBC's (55.1%) and less than one fifth of the source PLTs (17.8%) remained in the formulated product. The results demonstrate the Cue System's ability to process a wide range of source material while preserving WBCs.

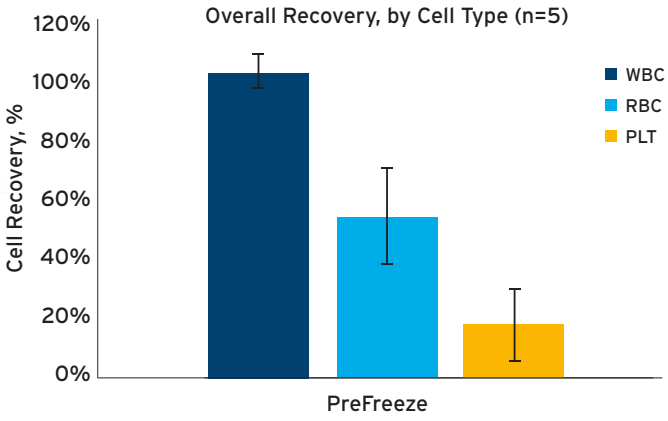


Figure 3. Pre-freeze mean (±SD) cell component recoveries for white blood cells (WBC), red blood cells (RBC), and platelets (PLT) as a percentage of the total in the incoming leukapheresis material showed 104.3% (±6.1), 55.1% (±16.2), and 17.8% (±12.7), respectively.

## Viability

Viability of CD45+ WBCs were measured at multiple points throughout the Cue procedure and post-thaw. No statistical difference was observed for any timepoint from source through post-thaw ($p > 0.05$). Additionally, all viability measurements were above 95% for all

procedures across all samples. The high WBC viability coupled with high WBC recovery provides quality cells post-thaw for further upstream or downstream processing.

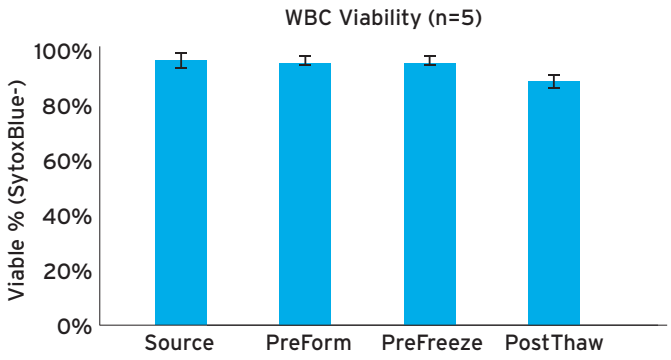


Figure 4. Viability of CD45+ white blood cells (WBC) as measured by SytoxBlue- for incoming leukapheresis source material, pre-formulation (pre-CPA addition), pre-freeze (post-CPA addition), and immediate post-thaw.

## Fill Accuracy

As described in the Methods section above, aliquots were filled at a target fill volume of 50 mL or 70 mL at a target concentration of 50 x10$^6$/mL. The Cue System dispensed aliquots with an average error of 1.2 mL (n=4) when targeting a 50 mL fill volume and 1.5 mL (n=4) when targeting 70 mL fill volume. The Cue System achieved a freeze concentration of within 3.3% of the target 50 x10$^6$/mL for all aliquots (n=8). Samples were taken post-thaw and analyzed and confirmed the target cell concentration of 50 x10$^6$/mL was maintained through the freeze-thaw process. The data supports the Cue System's reproducibility and ability to standardize the cryopreservation process given a large range of source inputs.

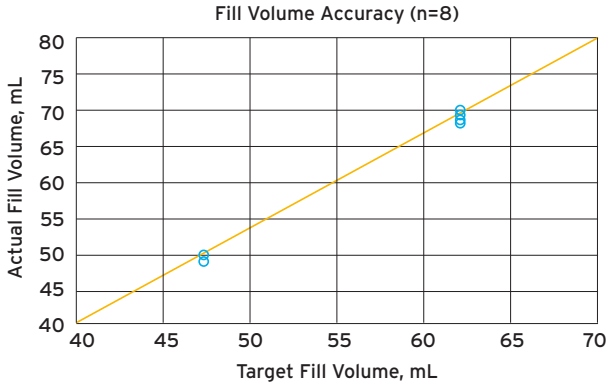


Figure 5. Volumetric fill accuracy of formulated doses. For 50 mL (n=4) and 70 mL (n=4) fill targets, the system prepared volumes within 1.2 mL (2.3%) and 1.5 mL (2.2%) of target, respectively. The yellow line depicts parity (actual fill = target fill).

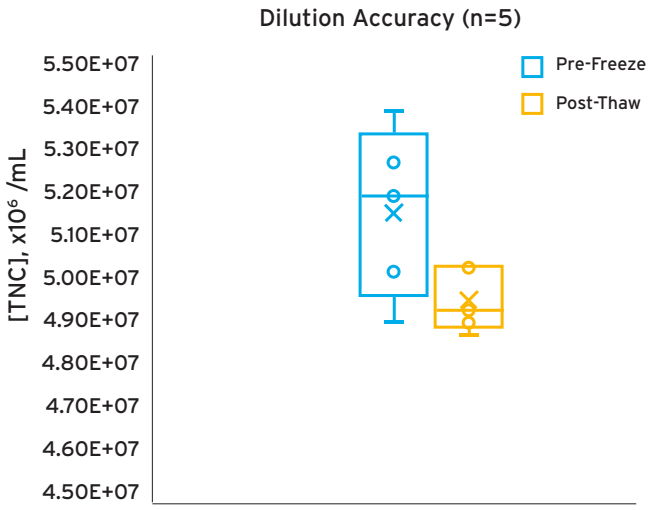


Figure 6. Using source material cell concentration inputs, the system targeted a freeze concentration of 50x10$^6$ WBC/mL. Cells were formulated within 0.4x10$^6$ WBC/mL (3.3%) of target.

## Washout

Washout efficiency was measured as a function of supernatant glucose concentration. The HSA/Plasmalyte wash solution contained no glucose. Source materials averaged 261.8 mg/dL (± 9.2) whereas washed samples showed 15.6 mg/dL (± 7.7), demonstrating a >16-fold decrease in glucose concentration. Calculating for total glucose mass balance, washed samples contained ~2.1% of the starting glucose amount, showing an average washout efficiency of >97.8%. These data were generated with a standard 20 mL wash buffer volume used per harvest, however, higher wash volumes can be targeted to achieve higher washout efficiencies.

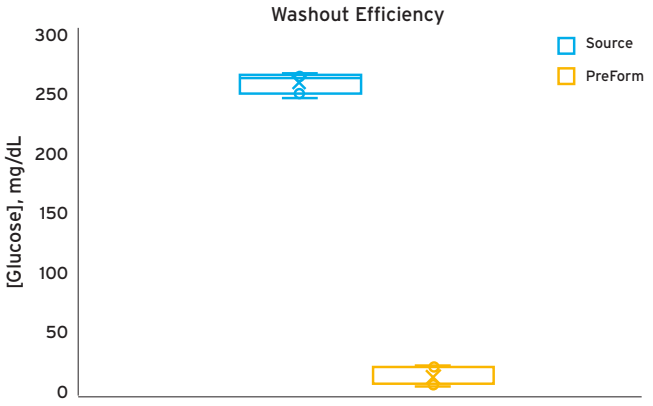


Figure 7. Using supernatant of prepared suspensions, samples showed a >16-fold reduction in glucose concentration (261.8 mg/dL source material reduced to 15.6 mg/dL) using a standard 20mL wash volume. This equates to a >97.8% washout efficiency by glucose mass.

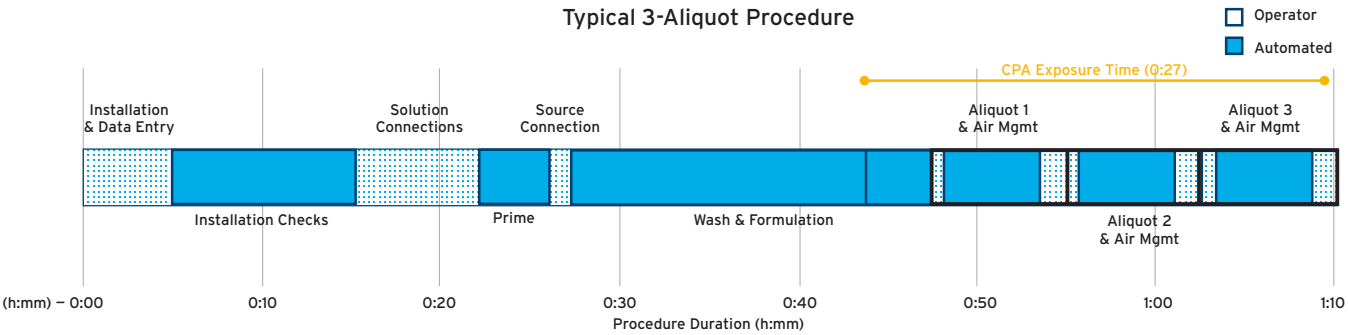


Figure 8. Median procedure durations for each segment of the cryopreparation protocol. Filled areas indicate portions of the protocol that are fully automated. Open areas indicate portions of the protocol where operator interaction is required. Total procedure time of a 3-aliquot median protocol are approximately 1 hour, 11 minutes. Operator interaction accounted for a total of 20 minutes of the procedure and cells were exposed to cryopreservation agent (CPA) for 27 minutes prior to being transported to the CRF.

## Time

Procedure time was measured at the beginning of disposable installation through the point the last filled aliquot was disconnected from the system. The typical 3-aliquot procedure was 1 hour, 11 minutes with an average of 27 minutes of cryopreservation agent (CPA) exposure time. Operator interactions on the system (including tasks such as disposable installation, solutions connection, semi-automated air management) accounted for 20 minutes of the total 1 hour, 11 minute duration. Limited user interaction may provide the opportunity to process additional Cue Systems in tandem and increase overall throughput. However, further testing will need to be carried out to verify this hypothesis.

## Materials Used

### Equipment

Cue Cell Processing System, SW v1.0.0.0 (6R5000, Fresenius Kabi)

Automated Hematology Analyzer (KX-21N, Sysmex)

Flow Cytometer (Novocyte Advanteon 3000VBR, Agilent)

### Soft Goods

Cue Primary Processing Set – 4µm
250 mL Cryobags (CF-250, Charter Medical)

### Reagents

Wash Solution – 5% HSA in Plasmalyte

- 25% Human Serum Albumin (SeraCare)
- Plasmalyte-A (Baxter)

Cryopreservation Agent (CryoStor10, Biolife Solutions)

Flow Cytometry Stain (BD Multitest CD3/CD8/CD45/CD4, BD)

Viability Stain (Helix NP Blue, BioLegend)

## Protocol Settings

The following Cue v1.0.0.0 protocol settings were used to generate the dataset:

| Protocol Setting | Parameter | Value |
|---|---|---|
| **Source Composition** | WBC Cell Volume<br>RBC Cell Volume | 400 fL<br>100 fL |
| **Source Prime** | Source Prime Volume<br>Source Prime Flow Rate | 10 mL<br>50 mL |
| **Procedure Parameters** | Spinner Idle Revolution Rate<br>Skip Concentration and Wash<br>Aliquot Only Protocol | 600 RPM<br>No<br>No |
| **Source Loading Information** | Spinner Loading Revolution Rate<br>Source Inlet Flow Rate<br>Max maPCV%<br>Source Rinse Configured<br>Source Rinse Volume | 2500 RPM<br>50 mL/min<br>50 %<br>Yes<br>10 mL |
| **Spinner Wash Information** | Spinner Wash Configured<br>Number of Spinner Washes<br>Spinner Wash 1 Volume<br>Spinner Wash Solution<br>Spinner Wash Flow Rate | Yes<br>1<br>20 mL<br>Solution 1 (5% HSA + Plasmalyte)<br>50 mL/min |
| **Harvest Information** | Harvest Volume<br>Harvest Flow Rate | 10 mL<br>50 mL/min |
| **Post-Initial and Post-Final Dilution Information** | Post-Initial Dilution Entry Method<br>Post-Initial Dilution Bulk Conc.<br>Dilution Flow Rate<br>Post-Final Dilution Entry Method<br>Post-Final Dilution Bulk Conc. | Concentration<br>125 x$10^6$/mL<br>50 mL/min<br>Concentration<br>100 x$10^6$/mL |
| **Formulation Information** | Formulation Configured<br>Solution 3 Connection Point<br>Ratio (Suspension : Solution 3)<br>Formulation Flow Rate | Yes<br>Before Formulation<br>1:1<br>30 mL/min |
| **Thermal Tray Mixing Parameters** | Temperature Control Configured<br>Target Temperature<br>Temperature Low Limit<br>Temperature High Limit<br>Mixing Configured<br>Mixing Rate<br>Mixing Angle | Yes<br>4 °C<br>2 °C<br>8 °C<br>Yes<br>1<br>20 ° |
| **Final Product and Aliquots** | End in Bulk Bag Configured<br>Bulk Bag Type<br>Aliquot Air Chase Volume<br>Aliquot Air Pull Back Volume<br>Aliquot Flow Rate<br>Required Bulk Excess Volume<br>Aliquot Type<br>Aliquot Bag Type<br>Aliquot Number<br>Aliquot Volume | No<br>Original<br>20 mL<br>18 mL<br>50 mL/min<br>5 mL<br>Final Product<br>CF-250 CryoBag<br>1<br>70 mL |

1. Cue Leukapheresis Cryopreparation Characterization Review: 224-DER-077240 [A] - Data on file

The Cue Cell Processing System is for laboratory use only and may not be used for direct transfusion. Appropriate regulatory clearance is required by the user for clinical use. Refer to the Cue Cell Processing System User's Guide for a complete list of warnings and precautions associated with the use of this device. For additional information, please visit www.choosecue.com



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0101-CUE-01-06/22