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Automated Aliquoting Accuracy of the Cue® Cell Processing System Using the Cue Manifold Set – 8-Lead

Zachary DeSalvo, Johnwesly Kanagaraj, Shena Geisinger
Fresenius Kabi, Transfusion Medicine and Cell Therapies, 3 Corporate Drive, Lake Zurich, IL

Abstract

This application note[1] highlights the minimal variability between aliquots dispensed by the Cue Cell Processing System.

Background

In autologous cell therapy development, source material is initially acquired from patients' immune cells, which undergo upstream processing including selection, isolation, genetic modification, and expansion. After this exhaustive process, the cells are then prepared for final formulation via downstream processing, which includes wash and dilution to the required dose level before reinfusing back into the patient.

These upstream and downstream processes are typically performed at an off-site manufacturing location, which places significant stress on the manufacturing process to maintain high quality cell products stable for transport. Thus, one of the critical and time-dependent steps in the manufacturing process is the preparation of cell suspensions for cryopreservation.

Without cryopreservation, the logistics of producing standardized high quality cell therapy products becomes incredibly challenging. To accommodate this necessity, Fresenius Kabi's Cue Cell Processing System is designed to standardize the cryopreservation process of source material used in both upstream and downstream manufacturing steps.

Methods

Jurkat cells were cultured using Wilson Wolf G-REX 100M Bioreactors with supplemented RPMI media (10% FBS, 1% Pen/Strep) at 37°C and 5% CO_2 for 7-8 days. Supplemented RPMI Waste Media was drained from the G-REX 100M Bioreactors, pooled, and stored in a 1-liter transfer pack to be used for dilution solution on the Cue Cell Processing System. Cells were harvested and the source material was sampled for pre-processing cell concentration and total source volume. Cell concentrations and starting volumes were entered on the Cue Cell Processing System, the Cue Cell Processing Set was installed, and supplement RPMI Waste Media was connected to the Solution 1 line.

The procedures were executed according to the protocol settings listed in the "Protocol Settings" section of Table 4. Three procedures, referred to as runs, were completed. Each aliquot was sampled immediately after completion. The Jurkat cells were harvested into a 600mL-capacity transfer pack and sterile connected to the Cue Bulk Bag line. The source material in the new Bulk Bag was diluted using Supplemented RPMI Waste Media to a total volume of 600mL.

Cells were then sampled and analyzed for cell viability and total count. Source material was cooled to 4°C during the procedure and continuously mixed (via rocker) on the Bulk Bag cooling tray. The resuspended source material was aliquoted into 150mL-capacity Fresenius Kabi transfer packs and 5mL-capcity test tubes, respectively. A total of 16 aliquots were collected per procedure. The 70mL aliquots were collected into the 150mL-capacity transfer packs, and 2mL aliquots were collected into the 5mL-capacity test tube. The 2mL aliquots were collected in the 5mL test tube for accuracy sampling from aliquot container. The protocol designed for this study originally indicated that the 2mL aliquots be collected into 150mL-capacity transfer packs, however this proved difficult in sampling accuracy, therefore a change to use 5mL-capacity test tubes instead of the 150-mL capacity transfer packs was made for the 2mL aliquots. Air was expressed from all aliquot containers using automated air management (150mL transfer packs only), then disconnected from the aliquot line. Two samples were taken from each aliquot for cell count and viability analysis.

All samples were analyzed for viability via AO/PI stain by Nexcelom Auto 2000 Cellometer, and cell counts were analyzed by Sysmex KX-21N. All population comparisons were performed using a 2-variance test in Minitab 21. Aliquot containers were weighed before and after each aliquot for the final fill volume measurement. Aliquot fill times were extracted from the Cue procedure log files. Aliquot fill time was defined by the start of the system filling the aliquot container until the system completed the automated air management.

A post-aliquot pause of three minutes was included in Run 2 to determine whether additional dwell time impacted cell concentration or viability of the aliquots. This pause occurred after each aliquot was filled and a 3-minute timer was started after automated air management was complete. After three minutes passed, the aliquot was sealed, and a sample was taken for cell count and viability. The next aliquot was started following sealing of the previous aliquot. Runs 1 and 3 did not include a post-aliquot pause, therefore each aliquot was completed one after another.

Results and Discussion

Starting Material

Starting material cell concentration and viability ranged from $2.63x10^6$ cells/mL ± $0.37x10^6$ cells/mL (14%) to $10.89x10^6$ cells/mL ± $1.82x10^6$/mL (17%). All starting material was processed on the Cue Cell Processing System (SN: WC03001) under the same protocol. Refer to Table 4 for the protocol settings.

Fill Accuracy

The protocol was configured to fill eight 70mL target volume aliquots and then eight 2mL target volume aliquots. Each run varied in source concentration (Table 1). The Cue System dispensed aliquots with an average error of 0.05mL (n=24) when targeting 70mL fill volume and 0.43mL (n=24) when targeting 2mL fill volume across all three procedures as shown in Figure 1. The data supports the Cue System's ability to consistently achieve target volume with minimal error.

Cell Concentration Variation

The cell concentration was measured using Sysmex KX-21N Automated Hematology Analyzer after completing each aliquot. Average dose-to-dose concentration variation per procedure is shown in Table 2: Aliquot Cell Concentration Coefficient of Variation. 70mL aliquot variation ranged between 1.98%–5.91% for all runs. 2mL aliquot variation ranged between 3.65%–6.45% for all runs. Concentration variation is higher for small volume aliquots (2mL) when the source material is a higher cell concentration; minor volume differences in small volume aliquots will impact the final dose concentration of the aliquot.

Table 1
Procedure Source Material Information

Procedure	Source Volume* (mL)	Post-dilution System Reported Volume (mL)	Post-dilution Concentration† (10^6 cells/mL)	Post-dilution Percent (%) Viable‡)	Post-aliquot Dwell Time (min)
Run 1	86	600	$2.35x10^6$	90.15	0
Run 2	241	600	$9.93 x10^6$	89.53	3
Run 3	239	600	$9.95 x10^6$	86.10	0

*The Source Volume refers to the starting Bulk Bag volume.

†Cell Concentration was measured via Sysmex KX-21N.

‡Cell Viability was measured via Nexcelom Auto 2000 Cellometer.

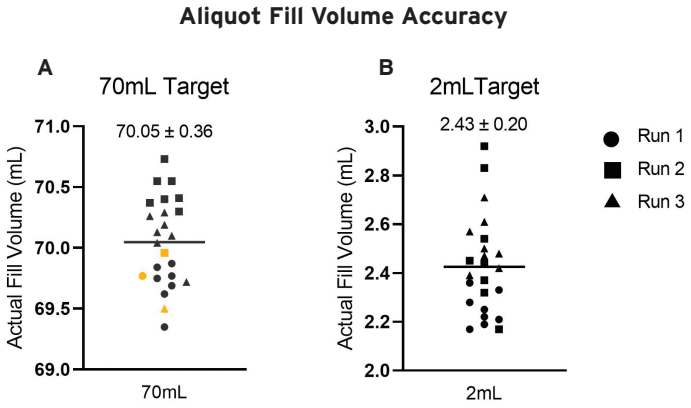

Fig 1. Aliquot fill volume accuracy. 70mL and 2mL aliquot volumes were targeted (n=24 per target volume across 3 runs). The first aliquot filled per run is noted by an orange symbol. The protocol was configured to fill the 70mL aliquots (n=8) followed by the 2mL aliquots (n=8), therefore the 70mL target volume aliquot is the first aliquot filled. The aliquot fluid pathway in the Primary Cue Set Cassette is not primed prior to the first aliquot configured in the protocol. The horizontal line indicates the average fill volume. The Cue Cell Processing System aliquot volumes were within 0.05mL of the targeted 70mL volume, and 0.43mL of the targeted 2mL volume. Note, a Cue Aliquot Manifold was not used.

Post-aliquot dwell time did not impact cell concentration, determined by comparing a 3-minute post-aliquot dwell time procedure (Run 2) to a no post-aliquot dwell time procedure (Run 3) (Figure 2C and 2D). A 2-population variance test showed no statistical difference in concentration variation between Run 2 and Run 3 ($p>0.05$).

Cell Viability Variation

Cell viability was measured after Bulk Bag dilution and again for each aliquot upon completion. Average dose-to-dose viability variation is shown in Table 3: Aliquot Cell Viability Coefficient of Variation. 70mL aliquot variation ranged between 0.91%–1.46% for all runs. 2mL aliquot variation ranged between 1.14%–1.39% for all runs.

Post-aliquot dwell time did not impact cell viability, determined by comparing a 3-minute post-aliquot dwell time procedure (Run 2) to a no post-aliquot dwell time procedure (Run 3) (Figure 3C and 3D). A 2-population variance test showed no statistical difference in concentration variation between Run 2 and Run 3 ($p>0.05$).

Table 2
Aliquot Cell Concentration Coefficient of Variation

Aliquot Volume	70 mL	2 mL
Run 1	5.91%	3.65%
Run 2	1.98%	6.45%
Run 3	2.73%	3.67%

Table 3
Aliquot Cell Viability Coefficient of Variation

Aliquot Volume	70 mL	2 mL
Run 1	1.46%	1.39%
Run 2	1.22%	1.27%
Run 3	0.91%	1.14%

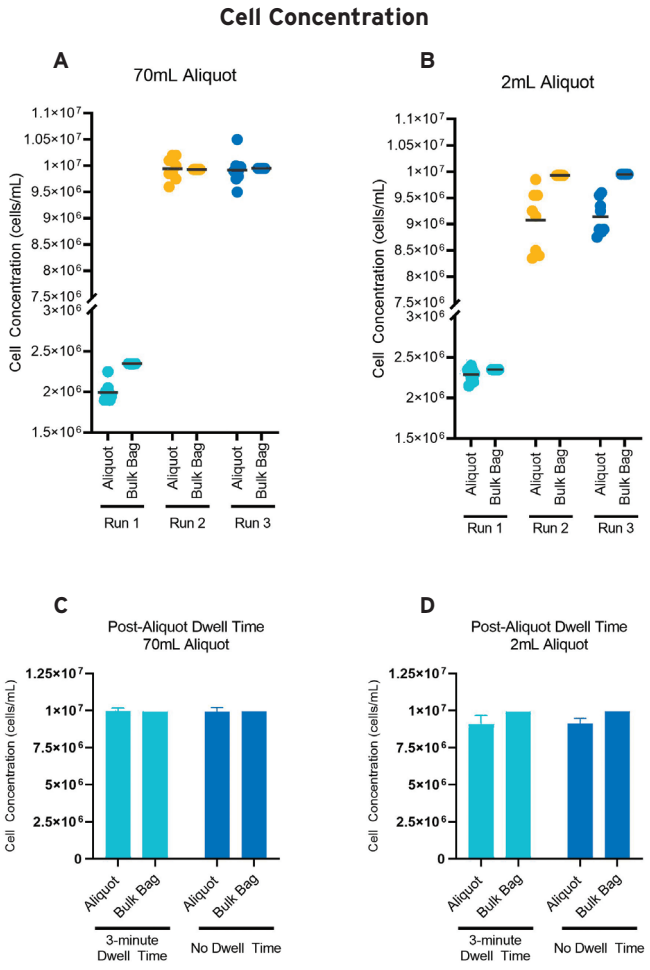

Fig 2. Cell Concentration of each aliquot across three procedures measured using the Sysmex KX-21N Automated Hematology Analyzer. The horizontal line indicates the average cell concentration (A and B). Post-aliquot dwell time does not impact cell concentration when comparing a procedure including a 3-minute post-aliquot pause (Dwell Time) versus a procedure with no pause configured (No Dwell Time) Error bars indicate mean with SD (C and D).

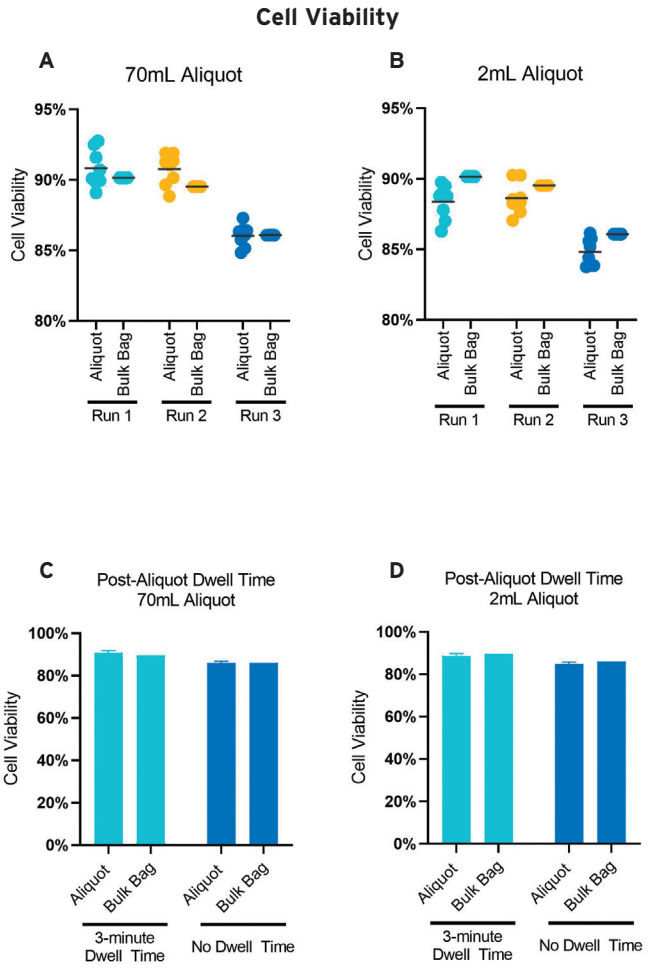

Fig 3. Cell viability of each aliquot measured by AOPI stain. The horizontal line indicates the average cell viability (A and B). Post-aliquot dwell time does not impact cell viability when comparing a procedure including a 3-minute post-aliquot pause (Dwell Time) versus a procedure with no pause configured (No Dwell Time) Error bars indicate mean with SD (C and D).

Fill Time

Aliquot fill times were extracted from the Cue procedure log files. Aliquot fill time was defined by the start of the system filling the aliquot container until the system completed the automated air management. A 70mL aliquot fill time averaged 3 minutes, 15 seconds, ± 9 seconds (n=24). A 2mL aliquot fill time averaged 1 minute, 31 seconds, ± 5 seconds (n=24). Operator interactions on the Cue system (including tasks such as installing the aliquot container, removal of the aliquot container, semi-automated air management) are not accounted for in the aliquot fill time metrics.

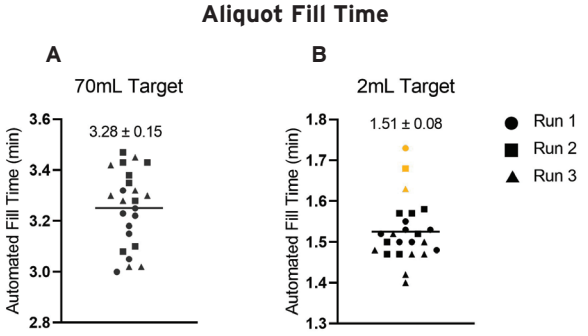

Fig 4. Automated aliquot fill time for the targeted volumes of 70mL and 2mL. The last aliquot filled per run is noted by a blue symbol. The protocol was configured to fill the 70mL aliquots (n=8) followed by the 2mL aliquots (n=8), therefore a 2mL target volume aliquot is the last to be completed. The last aliquot includes an automated air chase to ensure all fluid is in the bulk bag; approximately 10 seconds. The horizontal line indicates the average fill time.

Protocol Settings

Cue Aliquot Only Protocol Configuration

Protocol Setting	Parameter	Value
Procedure Parameters	Spinner Idle Revolution Rate	1500 RPM
	Skip Concentration and Wash	Yes
	Aliquot Only Protocol	No
Post-Final Dilution	Dilution Flow Rate	100 mL/min
	Post-Final Dilution Entry Method	Volume
	Post-Final Dilution Bulk Volume	600 mL
	Post-Final Dilution Pause Configured	Yes
Thermal Mixing Tray Parameters	Temperature Control Configured	Yes
	Target Temperature	4°C
	Temperature Low Limit	2°C
	Temperature High Limit	8°C
	Mixing Configured	Yes
	Mixing Rate	2
	Mixing Angle	10°
Final Product and Aliquots	End in Bulk Bag Configured	No
	Bulk Bag Type	New
	Bulk Bag Capacity	1000 mL
	Aliquot Air Chase Volume	20 mL
	Aliquot Air Pull Back Volume	0 mL
	Aliquot Flow Rate	100 mL/min
	Required Bulk Excess Volume	0 mL
	Aliquot Type: 70mL	
	Aliquot Bag Type	150mL Transfer Pack
	Aliquot Number	8
	Aliquot Volume	70 mL
	Aliquot Type: 2mL	
	Aliquot Bag Type	Test Tube
	Aliquot Number	8
	Aliquot Volume	2 mL

Materials Used

Not all products listed or shown are available, or approved for sale in all countries.

Equipment

Cue Cell Processing System, SW 1.1 (v1.0.16.247; experimental build commercially equivalent) (WC03001, Fresenius Kabi)

Automated Hematology Analyzer (KX-21N, Sysmex)

Cell Counter (Cellometer Auto 2000 Cell Viability Counter, Nexcelom)

Soft Goods

Cue Primary Processing Set – 4µm (FTC-5014; RUO)

G-Rex 100M-CS (closed system) - (RU81100-CS, Wilson Wolf)

Transfer Pack container with Coupler – 150 mL (4R2001, Fresenius Kabi)

Transfer Pack container with Coupler – 600 mL (4R2023, Fresenius Kabi)

Test Tube with Cap (12 x 75mm, Globe Scientific

Reagents

Jurkat Cell Line, Clone E6-1 (ATCC #TIB-152) Culture Media RPMI 1640 (12633-012, Gibco) PenStrep (15140-122, Gibco) Fetal Bovine Serum (FBS) (FBS001, Neuromics) ViaStain AOPI Staining Solution

(CS2-0106-5mL, Nexcelom)

1. Cue Software 1.1 Aliquot Accuracy Review: 224-DER-082150 [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 these products.

For additional information, please visit **www.choosecue.com**

Data on file Fresenius Kabi USA
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