Leukoreduction System (LRS)

Chambers/Cones
For Research Use

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Product Description

During routine plateletpheresis, platelets are separated from other blood components by centrifugation. Leukoreduction system (LRS) chambers play a direct role in the cell reduction process used to collect the platelets. Upon completion of the donation process, red blood cells, white blood cells, and plasma are returned to the donor. However, a small volume of granulocyte-reduced, concentrated mononuclear cells remains inside the LRS chamber (Figure 1A and Table 1). These chambers are typically discarded following the completion of the platelet donation process, but if they are sterilely separated from the collection kit, the mononuclear cells contained within can be harvested for use in a whole host of research applications[1-4].

Work from our group shows an average of 1.8x10^9 total nucleated cells (TNC, Figure 1B and Table 1) are obtained from a single LRS chamber, with an average of 96.3% of those cells being lymphocytes and monocytes (Mononuclear Cells, Figure 1A and Table 1). CD3$^+$ T cells make up between 30% and 65% of the total LRS chamber contents, depending in the individual (Table 1). The distribution of common mononuclear subpopulations is shown in Figure 1C and listed in Table 1.

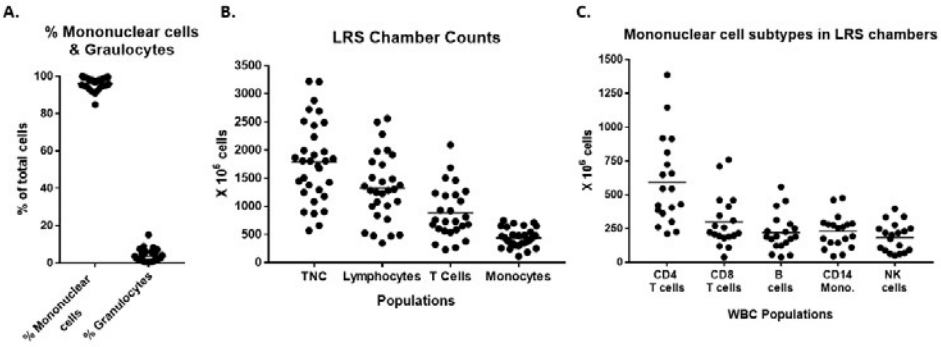

Figure 1: Makeup of LRS chambers from platelet-only donors collected at the Oklahoma Blood Institute. **A.** The percentage of mononuclear cells (lymphocytes and monocytes) and granulocytes (neutrophils, eosinophils, basophils) in LRS Chambers at time of harvest. Percentages obtained from Sysmex Hematology Analyzer. **B.** Absolute cell numbers counted by Sysmex Hematology Analyzer (for TNC [Total Nucleated Cells], Lymphocytes, and Monocytes) or by flow cytometry (T cells). **C.** Absolute number of mononuclear cell subpopulations in LRS chambers, calculated from flow cytometry percentage multiplied by chamber TNC. See Table 1 for mean and range for each population.

	Popualtion	Mean %	Range %	Mean cells x10^6	Range x10^6	N =
Readings from Sysmex Hematology Analyzer	Total Nucleated Cells			1789	565-3224	32
	Granulocytes	3.72	0.3-15.1	54.9	5-288	29
	Mononuclear Cells	96.3	84.9-99.7	1769	532-3152	29
	Lymphocytes	68.1	54.0-81.8	1323	347-2561	29
	Monocytes	24.8	13.6-45.4	442	113-752	29
Readings & calculations from flow cytometer	CD3+ T Cells	47.6	30.7-65.1	889	229-2092	26
	CD4+ T Cells	31.8	22.1-43.1	595	212-1328	20
	CD8+ T Cells	15.8	4.6-23.9	299	40-761	20
	B Cells	11.7	3.7-18.3	221	41-558	20
	CD14+ Monocytes	13.1	6.5-30.1	230	47-477	20
	NK Cells	10.4	2.8-20.8	187	52-398	20

Table 1: Percentages and absolute cell numbers of LRS chamber populations. Total Nucleated Cells, Granulocytes (neutrophils, eosinophils, basophils), Mononuclear Cells (Lymphocytes, Monocytes) were measured by Sysmex hematology analyzer. T Cells (CD3+, CD4+, CD8+), B Cells, CD14+ Monocytes, and NK Cells were measured by flow cytometry and absolute cell numbers were calculated by multiplying the percentage by the total nucleated cell count. The mean and range for percentage and absolute number are reported for all populations.

Additional References:

1. Dietz AB, et al. 2006. *Transplantation and Cellular Engineering*. 46:2083.

2. Strasser E, et al. 2007. *Transfusion.* 47:1943.
3. Weidinger TM, et al. 2011. *Transfusion*. 51:2049.
4. Pfeiffer IA, et al. 2013. *Immunobiology*. 218:1392.

Storage

Cells harvested from LRS chambers in media or plasma may be stored at 4°C for 24-96 hours and maintain up to 90% total viability (Figure 2A). Chambers may be stored, unharvested for up to 48h at 4°C with >95% total viability expected upon harvest or for up to 96h at 4°C with >90% total viability expected upon harvest (Figure 2B).

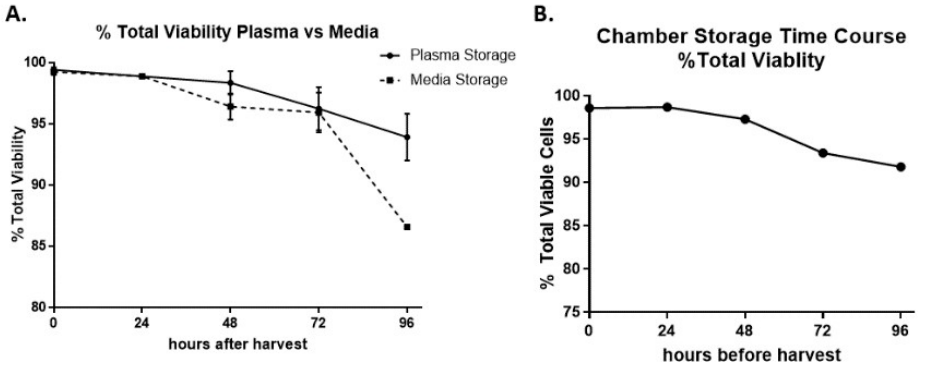

Figure 2: Total viability of LRS chamber cells under different storage conditions. **A.** Cells were harvested from LRS chambers with either 100% donor plasma (solid line) or culture media containing 10% serum (dotted line) and stored at 4°C for up to 5 days. At the times indicated, total viability was assessed by flow cytometry and 7-AAD staining (multiple experiments). **B.** LRS chambers were stored unharvested at 4°C. Cells were harvested from stored chambers at times indicated and assessed for viability by flow cytometry and 7-AAD staining (single experiment)

Recommended LRS Chamber Harvest Protocol

Materials

1 Ring stand with clamp
1 50 mL conical tube
conical tube rack
70% ethanol or isopropanol
scissors
1 ***blunt-end*** 18G needle 20 mL syringe
40 mL of Harvesting Media: PBS or culture media supplemented with BSA, HSA, or serum
Hemocytometer or automated cell counter

Cells inside LRS chambers are sterile. To maintain sterility, perform all steps inside a biological safety cabinet, practice sterile technique and use only sterile supplies and media.

Figure 3: Mounted LRS Chamber. Chamber mounted, drained of contents, and washed with 100% plasma.

Protocol

1. Mount LRS chamber on a ring stand with clamp, wide side down (Figure 3). Position tubing over a closed 50 mL conical tube sitting inside a tube rack.
2. Spray LRS chamber with alcohol and allow to dry
3. Open 50 mL tube and snip the <u>bottom</u> tubing but leave ~½ inch of slack (Figure 3). Repeat for the top tubing, which will break the vacuum and the chamber will start dripping into the open tube.
4. For best results, allow the contents of the chamber (~10 mL) to drip by gravity into the tube (takes ~10 min)*. **If this is going very slowly or has stopped, take the syringe and blunt-end needle and inject air into the top tubing to resume drip.*
5. Fill syringe up with 20 mL of Harvesting Media and inject into the top of the chamber**. Allow all media to drip into the tube.
   ***Move the tubing in a circle while slowly injecting the Harvesting Media to help clear out blood from the sides of the chamber.*
6. Repeat with another 20 mL of Harvesting Media for a total volume of ~50 mL of LRS eluate + Harvesting Media.
7. Fill syringe with air and push air through the chamber to expel any remaining volume. The chamber should be cleared of most blood and cells (Figure 3), although trace amounts may remain.
8. Cap tube and gently mix by inverting.
9. Remove a sample for counting and proceed to downstream research applications.

Warning

This product is composed of human-derived materials. Always wear appropriate personal protective equipment when handling this product and treat it as potentially infectious, using Universal Precautions, regardless of the results of infectious disease testing.

Limitations and Publications

This product is for research use only and not for use in humans, for further manufacture, or resale. Nothing produced directly from this product may be sold. When publishing scientific results obtained using this product, acknowledge supplier as Bio-Sharing.org.