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Assessment of the LOVO device for final harvest of novel cell therapies: A Production Assistance for Cellular Therapies (PACT) multicenter study

Laarni Ibenana[1],

Robert Anderson[1],

Adriana. Brooks[4],

Cheryl Cox[3],

Adrian Gee[2],

Margaret Gilbert[2],

Joshua M. Hare[4],

Linda Kelley[3],

Aisha Khan[4],

Natalia Lapteva[2],

Aaron Orozco[2],

David Styers[1],

Darin Sumstad[5],

Ibekwe Ugochi[2],

David H. McKenna[5]

[1]The Emmes Company, LLC, Rockville, MD

[2]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX

[3]Moffitt Cancer Center, Tampa, FL

[4]Interdisciplinary Stem Cell Institute, Miller School of Medicine, University of Miami, Miami, FL

[5]Molecular and Cellular Therapeutics, University of Minnesota, Saint Paul, MN

Abstract

Introduction: The final harvest and/or wash of a cell therapy product is an important step in manufacturing, as viable cell recovery is certainly critical to the overall success of a cell therapy. Most harvest/wash approaches in the clinical lab involve centrifugation which can lead to loss of cells and decreased viability of the final product. Here we report a multicenter assessment of

Corresponding Author: David H. McKenna, mcken020@umn.edu, Molecular & Cellular Therapeutics, 1900 Fitch Avenue, Saint Paul, MN 55108.

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the LOVO Cell Processing System (Fresenius Kabi), a cell processing device that uses a spinning filtration membrane instead of centrifugation.

Materials and Methods: Four NIH Production Assistance for Cellular Therapies (PACT) cell processing facilities (CPFs) assessed the LOVO Cell Processing System for final harvest and/or wash of the following three different cell products: activated T cells (ATCs), tumor-infiltrating lymphocytes (TILs), and bone marrow-derived mesenchymal stromal cells (MSCs). Each site compared their current in-house, routinely used method of final cell harvest and/or wash to that of the LOVO.

Results: Final harvest and/or wash of ATCs, TILs, and MSCs using the LOVO resulted in satisfactory cell viability and recovery with some substantial improvement over the CPFs in-house methods. Processing time was variable among cell types/facilities.

Discussion: The LOVO Cell Processing System provides an alternative to centrifuge-based technologies. The system employs a spinning membrane filter, exposing cells to g-forces minimally as compared to centrifugation, and is automated and closed. This small multicenter study demonstrated the ability of the LOVO to yield satisfactory cell viability and recovery for T cells and MSCs.

Keywords

cell culture; harvest; recovery; Viability; T cells; mesenchymal stromal cells

Introduction

As the cell therapy field continues to grow, so too does the need for optimized, automated devices for the various steps in cell manufacturing. In addition to media and reagent reduction, the final harvest and/or wash of a cell therapy product is an important step in manufacturing to assure viable cell recovery which is certainly critical to the overall success of a cell therapy. Most harvest/wash approaches in the clinical lab involve centrifugation [1–3], and depending upon the volume of the cell culture, this could lead to a prolonged process and consequent detrimental effects on the cells. An alternative technology utilizing a spinning filtration membrane was available; however, this instrument was removed from the market in 2010 [4].

The LOVO Cell Processing System (LOVO) (Fresenius Kabi, Bad Homburg, Germany) was introduced in 2014. This instrument is a benchtop cell processor (see Figure 1.a.) that automates the often open-system, labor- and time-intensive tasks of washing and concentrating cell therapy products. Using spinning filtration membrane technology (see Figure 1.b.), the LOVO removes supernatant and cells or particles <4 μm in size allowing for cell concentration without the need for centrifugation while reducing the need for manual re-suspension, as is typically required following centrifugation. The LOVO, as such, eliminates the known detrimental effects of centrifugation and, perhaps more importantly, that of resuspension [5]. It is also a closed system, minimizing the possibility of compromised sterility, while also allowing for standardization through automation. Here we report a multicenter independent assessment of the LOVO for final harvest and wash of cell culture along with a single center post-thaw/wash evaluation.

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Materials and Methods

This study was performed under the NIH (NHLBI)-sponsored Production Assistance for Cellular Therapies (PACT) contract mechanism. Four PACT manufacturing centers participated in the study: Baylor College of Medicine, Center for Cell and Gene Therapy; University of Miami Interdisciplinary Stem Cell Institute Cellular Manufacturing Program; University of Minnesota, Molecular and Cellular Therapeutics; and Moffitt Cancer Center. The PACT Coordinating Center, The Emmes Company, LLC, Rockville, MD, oversaw data collection and analysis. Each site chose a cell type to assess and compared their current in-house, routinely used method of final cell harvest and/or wash to that of the LOVO. Baylor College of Medicine and Moffitt Cancer Center evaluated the LOVO for harvest and wash of activated T cells (ATCs) and tumor-infiltrating lymphocytes (TILs), respectively. Minnesota and Miami both assessed the LOVO with bone marrow-derived mesenchymal stromal cells (MSCs) – large volume harvest (Minnesota) and post-thaw DMSO removal (Miami). The focus of the study was on processing time and cell viability and recovery. Two centers looked at additional testing, including residual cytokine levels (Baylor) and post-thaw short-term stability and cytokine expression (Miami).

Baylor College of Medicine

Peripheral blood mononuclear cells (PBMCs) were purified from healthy donor buffy coats (Gulf Coast Blood Center, Houston, TX) by Ficoll (Lymphoprep, Axis Shield, Oslo, Norway) centrifugation. ATCs were prepared by plating 5-10x10^6 PBMCs at 0.5x10^6 cells/mL into 24-well plates pre-coated with 1 microgram/mL of anti-CD3 (OKT3 clone) and anti-CD28 (BD BioSciences, San Diego, CA) monoclonal antibodies (mAbs). Cells were cultured in 45% v/v/v advanced RPMI 1640 (GIBCO Life Technologies Corporation Grand Island, NY), 45% v/v/v Click's medium (Irvine Scientific, Santa Ana, CA), 2 mM GlutaMAX TM-I (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum (FBS) (HyClone, Thermo Scientific, Logan UT) in the presence of 10 ng/mL IL-7 and 100 ng/mL of IL-15 (R&D Systems, Minneapolis, MN). T cell cultures were expanded in T-75 flasks (Corning Inc., Corning, NY) and then in two G-Rex100 devices (Wilson Wolf Manufacturing, Saint Paul, MN).

Four independent experiments were carried out. ATCs were harvested on days 13 to 21 of culture. For each experiment cultures from two G-Rex100 devices were pooled, and viable cells were counted using a hemocytometer and the trypan blue exclusion method. Pooled cells were split into two fractions. One fraction was washed manually with three wash buffer exchanges, which is routinely used in the facility for formulation of clinical products. Wash buffer consisted of Hank's Balanced Salt Solution without Ca_2+ Mg_2+ (HBSS, Gibco, Paisley UK) with added 5% Human Serum Albumin (HSA) (25% Flexbumin, Baxalta US Inc, Round Lake, IL). Supernatants were aspirated after each centrifugation (total 4 centrifugations) at 400xG for 5 mins. After the last wash, cells were re-suspended in 50 mL of wash buffer and counted. Cells were centrifuged, counted in a hemocytometer, viability assessed by the trypan blue exclusion method and formulated at 1x10^7 cells/mL in the wash buffer. The sterility of the cells was tested by inoculation of 2-3 mL of cells into BD BACTEC Standard/10 Aerobic/F, Lytic/10 Anaerobic/F and Myco/F bottles (Dun

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Laoghaire, Ireland). A sample of cells was taken for IL-15 enzyme-linked immunosorbent assay (ELISA) and flow cytometry for viability using 7-AAD (BD Biosciences, San Diego, CA) staining and analysis for CD3, CD4, and CD8 (BD BioSciences, San Jose, CA).

The other fraction of cells was harvested and washed using the LOVO Cell Processing System (software version 3.0). LOVO disposable kits were modified pre-procedure to allow Source and Wash Solution luer connections to be made in the biosafety cabinet. All four experiments were performed using a six-wash-cycle protocol, with a 90% supernatant wash-out programmed for each cycle. For each procedure, a 1 L bag of wash buffer was used as the wash solution. To target a final product volume of 50 mL, the settings for the first procedure included an 80 mL final In-Process bag dilution volume and a 2 mL final IP Bottom Port Rinse. After the final product volume requirement was adjusted to 78 mL, the settings for the second, third, and fourth procedures were edited to use a 300 mL final In-Process bag dilution and a 10 mL rinse. After LOVO washes the cells were harvested and counted. Cells were transferred into conical tubes centrifuged and re-suspended at $1x10^7$ cells/mL, and samples of cells were taken for IL-15 ELISA, sterility with BD BACTEC bottles, and flow cytometry, as described above.

Human IL-15 was measured in pooled culture supernatant and washed cells using IL-15 ELISA microplate kit (R&D Systems, Minneapolis, MN) as described by manufacturer. The limit of detection was 3.9 pg/mL. Figure 2.a outlines the experiments performed at Baylor.

Moffitt Cancer Center

Tumor-infiltrating lymphocytes (TIL) were isolated as previously described [6]. Briefly, de-identified surgically resected melanoma tumor samples were minced into 4–6 mm^3 fragments and placed in culture with RPMI-1640 with L-glutamine (Gibco/Invitrogen, Grand Island, NY) and 10% human AB (hAB) serum (Gemini Bio-Products, West Sacramento, CA) containing 6000 IU/mL interleukin-2 (IL-2) (Novartis, San Diego, CA). Fragments were monitored for growth every 2–3 days for up to 5 weeks. Cells were split when they became 90% confluent. The fastest-growing TIL from individual fragments were assessed for tumor reactivity by overnight co-culture with autologous, HLA-matched and HLA-mismatched tumor cells at a 1:1 ratio. Interferon (IFN)-γ in culture supernatants was measured by enzyme-linked immunosorbent assay. TIL were determined to be reactive if HLA-matched tumor co-culture yielded at least 200 pg/mL of IFN-γ and was at least 2-fold higher compared with medium alone or HLA-mismatched tumor co-culture. The highest IFN-γ-producing TIL numbering 10^7 were selected, pooled and cryopreserved for subsequent rapid expansion.

Three independent rapid expansion experiments were carried out using pooled pre-rapid expansion TIL from the experiment above. Thawed cells were cultured at $5x10^6$ cells into each of two G-Rex®100M-CS flasks (Wilson Wolf, St. Paul, MN) in 400mL media containing AIM V (Gibco/Invitrogen, Grand Island, NY), RPMI-1640 with L-glutamine and 10% hAB serum. Cells were cultured at a 1:200 ratio with irradiated allogeneic peripheral blood mononuclear cell feeder cells, 6000 IU/mL IL-2 and 30 ng/mL OKT3 antibody (Miltenyi Biotech, Bergisch Gladbach, Germany). Flasks were monitored for the next 14 days and split as needed to maintain the TIL concentration at 10^6 cells/mL. At day 14

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approximately 3L of cells were harvested using an Acacia pump with attached Acacia Pump Chamber Set (MPS Medical, Brea, CA) into a 3L transfer bag (Charter Medical, Winston-Salem, NC). Samples were taken for cell counts and viability. The cell suspension was equally divided into 2L transfer bags, each containing approximately 1.5L with a total of 10^9 cells, for subsequent volume reduction and wash utilizing either the Cell Saver® 5+ Autologous Blood Recovery System (Haemonetics Corporation, Boston, MA) or LOVO Cell Processing System (software version 3.0).

The Cell Saver® 5+ program used a draw rate of 250 mL/min and centrifuge speed of 4800 rpm for volume reduction. Wash buffer consisted of 1L Plasmalyte A (Corning Life-Sciences, Durham, NC) with 1% human serum albumin (HSA) (Grifols, Clayton, NC) and 300 IU/mL interleukin-2 (IL-2) (Novartis, San Diego, CA). After concentration, the washed product was collected in a 600 mL transfer bag (TerumoBCT, Lakewood,CO). The collection bowl was rinsed with 60 mL and added to the final product bag. The final product volume for all three experiments averaged 298 ± 37 mL consisting of 6.2 ± 1.3x10^9 total cells at an average concentration of 20.4 ± 3.1x10^6 cells/mL.

The LOVO Cell Processing System disposable kits were used unmodified. All experiments were performed using a two-cycle wash/concentration protocol with a 93% supernatant wash-out programmed for each wash cycle. Wash buffer consisted of 3L Plasmalyte A with 1% HSA. Settings for the procedures included a 608 mL final In-Process bag dilution volume and 10 mL final IP Bottom Port Rinse. Final product volume of 165 mL at media suspension rate of 150mL/min was targeted yielding 8 ± 1.8x10^9 at an average concentration of 48.4 ± 11.8x10^6 cells/mL.

Quality control testing was performed on a sample from the final product of each concentration/wash method. Cell count and viability were evaluated on a K2 Cellometer (Nexcelom, Lawrence, MA) using acridine orange/propidium iodide (AO/PI) dyes. The percentages of CD3, CD4, and CD8 expressed were measured with a FACanto, 8 Color flow cytometer (Beckman Dickenson, San Jose, CA) (FITC-CD3, PE-CD4, APC-CD8). IFN-y expression was measured using the R & D Human ELISA Kit (R&D Systems, Minneapolis, MN). Microbial testing included Gram stain, EndoSafe Endotoxin Test (Charles River, Charleston, SC) and a 14-day sterility test with BacTAlert (bioMereixu Inc, Durham, NC). Figure 2.b outlines the experiments performed at Moffitt.

University of Minnesota

MSCs were isolated from marrow as previously described [7]. Briefly, enrichment of the mononuclear cell (MNC) fraction of a marrow sample was accomplished using an automated separation method (Cobe 2991) involving ficoll hypaque density gradient medium, specific gravity 1.077 g/dl (Ficoll Paque Premium, GE Healthcare). A wash with Hank's Balanced Salt Solution (without phenol red, calcium or magnesium) followed. MNCs were seeded at 1.0-1.5x10^5 cells/cm^2 in αMEM with 16.5% fetal bovine serum (FBS) and GlutaMax (1%, 200 mM) at a media depth of 1.6 mm in an appropriately sized T-flask and placed in a 5% CO_2/37°C incubator. On days one and two, non-adherent cells were removed, and media was changed. Fresh media was exchanged every 2-4 days until 70-90% confluence. MSCs were then detached with TrypLE Select (Thermo Fisher Scientific,

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Waltham, MA) and cryopreserved in a high serum cryosolution (80% FBS/20%DMSO) at 10% final DMSO concentration (FBS: 48%; αMEM: 42%) as passage 1 (P1) cells.

For these experiments, P1 MSCs were then thawed and inoculated into a cell factory at 40-50 viable cells/cm^2. Media was exchanged every 2-4 days. At 70-90% confluence, cells were detached with TrypLE Select, quenched with media, and pooled. Samples were taken for cell count (SysmexXS-1000i, Kobe, Japan) and viability (AO/PI). The cells were split with half processed on the LOVO Cell Processing System (software version 3.0), the other half on the Cobe 2991 Cell Processor (Terumo BCT, Lakewood, CO).

The LOVO disposable kits were not modified for the experiments. However, to facilitate the simultaneous harvest from multiple source bags, the bags of cells were connected to each other using a manifold. An intermediate conical bag was then added between the manifold and the LOVO source lead to capture any air introduced by unequal draining of the bags during processing. Finally, due to the size of the culture, larger bags were used for waste. All experiments were performed using a four-wash cycle protocol with supernatant wash-out programmed for each wash cycle (94%, 50%, 67%, and 93%, respectively). The kit prime and first cycle utilized cell culture media with a switch to CryoStor buffer (BioLife Solutions, Bothell, WA) for the remaining cycles. Final product target volume was 100 mL and the media suspension rate was 100 mL/min.

The Cobe 2991 processing set was installed for the volume reduction procedure as per the manufacturer's instructions. Transfer bags containing the cells were attached to the processing set. Settings were as follows: centrifuge speed: 1500 rpm; super out rate: 450 mL/minute; minimum agitate time: N/A; super out volume: 600 mL. Cells were volume reduced at 1500 rpm (310 x g) for 5 minutes per spin. Following volume reduction, the product was washed three times at 1500 rpm for 5 minutes/spin with CryoStor buffer. At the end of each wash, supernatant was expelled into the waste bag. The final product was then diluted to 2-20 million cells/mL, post-processing samples were removed for cell count and viability (AO/PI), and the final product was cryopreserved at 1-10 million cells/mL in 10% DMSO (final concentration). Figure 2.c outlines the experiments performed at Minnesota.

University of Miami

Bone marrow (BM) aspirate from the iliac crest was collected aseptically from healthy human donors (n=3). The entire volume of BM was processed following a Lymphocyte Separation Medium (LSM) (Corning/Mediatech, Manassa, VA) based separation protocol (specific gravity 1.077 g/dl) in order to isolate the mononuclear cell (MNC) fraction. The BM aspirate was diluted in an equal volume, 1:1 ratio, with Plasma-Lyte A (Baxter, Deerfield, IL) Separately a Leucosep 50 mL conical tube (Greiner Bio-One, Monroe, NC) was prepared by adding 15 mL of the LSM and centrifuged (400 × g, 5 min) so the media would penetrate the filter and deposit in the bottom of the tube. At this point approximately 30mls of the diluted BM aspirate were overlaid into the Leucosep conical tube and centrifuged (800 × g, 30 min). The resulting fraction of mononuclear cells was recovered and washed twice in wash media composed of Plasma-Lyte A and 1% final concentration of human serum albumin (HSA) (25% Flexbumin, Baxalta US Inc, Round Lake, IL).

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The BM cells were cultured in tissue treated T-175 (Cellstar,Greiner Bio-One, Cellstar, Monroe, NC) flasks maintained at 37°C in a humidified atmosphere containing 5% CO_2. The complete culture medium, consisting of alpha-MEM (Life Technologies/ Gibco, Carlsbad, CA) supplemented with 20% fetal bovine serum (FBS) (Fisher Scientific/Hyclone, Pittsburgh, PA) was replenished every three to four days. Once the combined cell culture reached approximately 80-90% confluency, the cells were harvested and re-plated in tissue treated T-175 flasks. After two passages, the majority of non-adherent cells were removed during the medium exchanges. The cell cultures were then harvested and cryopreserved in 20 mL of cryopreservation medium containing 85% Hespan (BBraun Medical, Irvine, CA), 10% FBS and 5% DMSO (Mylan, Rockford, IL) at cell density of $5x10^6$/mL (i.e., 100 x 10^6 MSCs per bag). Additional samples were taken prior to cryopreservation for testing, i.e. cell count and viability, using trypan blue Solution, 0.4% (Thermo Fisher/Gibco, Waltham, MA), flow cytometry, sterility, endotoxin, mycoplasma.

For each of the three experiments one bag of MSCs was thawed using a 37°C water bath to be washed via the “in-house” method. This method involved the addition of 20 mL of wash medium followed by mixing and transfer to a 50 mL conical tube. The bag was rinsed with an additional 10 mL of wash medium and added to the tube. The cells were centrifuged at 500 g for 10 minutes at room temperature and re-suspended with 50 mL of wash medium for further analysis and testing.

Another bag of the same product was thawed in the 37°C water bath and underwent the wash procedure using the LOVO Cell Processing System (version 3.0). For all three experiments the standard wash cycle protocol was followed using 500mL of the same wash media described above. The retentate (or final product) volume requirement was set to 80 mL. The use of the LOVO cell processing disposable kit with Bags Access was slightly modified by using a plasma transfer set with spike and male luer adapter. Upon conclusion of the automated washes, the cells were collected and transferred into conical tubes. Cells were counted with crystal violet using a hemocytometer. The viability was determined using trypan blue. Samples from the supernatants were collected for sterility and endotoxin testing.

Additional cell samples were collected for product stability, cytokine and flow cytometry analysis. For each wash method the obtained cells were analyzed for expression of CD45 and CD105 (Beckman Coulter, cat# IM0782U, 61R-cd105cHUPE), and DAPI was used as viability dye. The isotype controls and stained samples were analyzed using Cytoflex Flow Cytometer (Beckman Coulter). Additionally, 10 x 10^6 MSCs were plated into T-175 flasks for cytokine analysis. Flasks were maintained in culture for four days at 37°C incubator with 5% CO_2. After this period the supernatant culture medium was collected and stored at −20°C until the day of analysis. Cytokine expression was analyzed using ELISA kits for MCP1, IL6, TIMP2, IL8, VEGF, and PDGF [SimpleStep ELISA® Kits (Abcam, Human MCP1 ELISA Kit cat # ab179886, Human IL6 ELISA Kit cat# ab178013, Human TIMP2 ELISA Kit cat # ab188395, Human IL8 ELISA Kit cat# ab214030, Human VEGF ELISA Kit cat # ab222510, Human PDGF BB ELISA Kit Cat # ab184860)] as per manufacturer instructions. The diluted samples were incubated with the antibody cocktail on the coated

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plate for one hour. The wells were rinsed and then incubated with substrates for 20 minutes. Stop solution was then added and the plates were read immediately.

The remainder of the cell suspensions were equally divided into four portions and kept at 4°C in the refrigerator for post-thaw stability testing. Samples from each experiment arm were removed from the refrigerator at 2-, 4-, 6-, and 8- hours post-thaw for cell count and viability. Figure 2.d outlines the experiments performed at Miami.

Results

See Table 1 for summary of cell volume, cell recovery, viability, viable cell recovery, and wash time for all experiments at all sites.

Baylor College of Medicine

With T cells, the manual method of four washes required less time (average of 60 minutes), as compared to the LOVO process (six washes) which averaged 104 minutes (range 85 min to 122 min, n=4). Viable cell recovery was 91.1±7.6% for manual washes and 90.4±18.1% for LOVO washes. In the first experiment the final product volume was set to 50 mL for LOVO and resulted in the lowest viable cell recovery (63.2% for the LOVO compared to 81.3% for the manual method). The low value was thought to be related to the target end volume (50 mL) and following adjustment to a higher volume of 78 mL, LOVO viable recoveries improved to 99.4±18.1%.

Cell phenotype ($CD3^+$, $\%CD3^+CD8^+$, $\%CD3^+CD4^+$) before and after harvest/wash was not significantly affected by procedure used. The frequency of $CD3^+CD4^+$ T cells was significantly different pre- and post-manual method. However, this is not biologically significant and is likely attributable to small sample size (n=4).

Both arms in the first three experiments maintained sterility. However, the sterility sample for the manual method only in the fourth experiment yielded a positive result (Ralstonia picketti in the aerobic BACTEC bottle). The sample was resubmitted and tested negative after two weeks of incubation, suggesting the initial positive result may be attributable to contamination during inoculation of the bottle.

The average IL-15 concentration in culture supernatants was 167.3 ng/mL (range 38.6-420 ng/mL, n=4); this was reduced by ~1000 fold to 25.2±9.5 pg/mL after the 6^{th} wash on the LOVO and to 4.4±8.8 pg/mL by manual washes. In the first experiment we measured IL-15 concentration in the last wash sample, which was 17.53 pg/mL for manually-processed cells and 20.8 pg/mL for LOVO-processed cells. In experiments 2, 3 and 4 we evaluated IL-15 concentration in the last wash sample and in cells formulated at $1x10^7$ cells/mL. After additional manual wash IL-15 concentration decreased from 27 pg/mL in the last wash sample to below 3.9 pg/mL for formulated cells washed on LOVO and was not detectable for all manually processed samples. See Table 2.

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Moffitt Cancer Center

Total and viable TIL cell recoveries were greater using the LOVO compared to the Cell Saver 5 cell washing device. Total cell recovery was 80.6±11.7% versus 71.1±12.5 on the LOVO versus Cell Saver 5; total viable cell recovery was 78.3±13.2% versus 66.7±10.1 on the LOVO versus Cell Saver 5; and viability of the final washed cell product was comparable on the LOVO versus the Cell Saver 5, 89.4 ±5.0% and 86.7±9.9%, respectively. The volume of the final cell product was consistently 165 ml with the LOVO device; whereas, the volume of the Cell Saver 5 product was almost double at approximately 300 ml. The LOVO cell washing method was significantly longer in duration requiring 41 versus 17 minutes on the Cell Saver 5. The phenotype of the TIL product, largely CD3+CD8+, did not change significantly after washing using either device, and all products met lot release criteria for safety, purity and sterility testing (data not shown).

University of Minnesota

The in-house method for moderate (2-5 L range) volume MSC culture harvest and wash involving the Cobe 2991 Cell Processor required an average of 90 minutes (range: 60-150 minutes) for completion, while the harvest and wash with the LOVO Cell Processing System was complete on average at 96 minutes (range: 57-120 min). Viability, total cell recovery, and viable cell recovery were 89.3%, 76.7%, and 70.3%, respectively, on the Cobe 2991, while results on the LOVO were 94.3%, 82.3%, and 80.0%. Average final volume was 123 mL (91-154 mL) with the Cobe 2991 and 69 mL (68-71 mL) with the LOVO.

University of Miami

LOVO provided an expedited method (average of 40-45 minutes) as compared to the manual method (average of 100 minutes) for washing MSCs. Viability, total cell recovery, and viable cell recovery were 89.2%, 90.9%, and 81.6%, respectively, using in-house manual method, while results on the LOVO were 91.7%, 85.4%, and 80.9%, respectively. Because Miami was studying low volume frozen MSC product and assessment of a wash and not a culture harvest, short-term post-thaw and wash stability was included. Analysis at t=0 and 8hrs post-thaw and wash showed cell loss with both methods with cell recovery of 70.07% for LOVO and 78.60% with the in-house method (data not shown). Flow cytometry analysis was as expected of MSCs and was unremarkable (data not shown).

The ratio of change in cytokine expression was calculated for LOVO as compared to the in-house wash method. All cytokines except IL-8 showed a 1.34 average fold increase with the LOVO method. IL-8 showed a decrease by 0.16 fold. There was no expression of PDGF observed for either method (data not shown). Analysis is limited by the samples size (n=3). Cells obtained from both wash methods met the release criteria for sterility (negative for aerobic, anaerobic and fungal), mycoplasma (negative), and endotoxin (</=5EU/mL).

Discussion

With the increase in complexity and the substantial investment of time and funding in novel cell therapy development and manufacturing, the importance of final harvest and/or wash is certainly apparent. Assuring optimal cell viability and recovery on the final day of

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processing increases the likelihood of high product quality and potency and also reduces the variables related to the product in an early phase investigational trial. Efficient removal of culture media and reagents may also be a priority. Most approaches to harvest and wash involve centrifugation, and depending upon the size of cell culture, this process can be protracted. Additionally, cell viability and recovery may be lower than desired due to prolonged physical stress and the limitations of the technology. The LOVO provides an alternative to centrifugation-based technology, utilizing a spinning membrane filtration technology which significantly reduces the potentially harmful cell product exposure to g-forces and pelletization. This multicenter NHLBI-sponsored PACT study evaluated the use of the LOVO for harvest and wash of T cells [ATCs (Baylor College of Medicine) and TILs (Moffitt Cancer Center)] and large volume harvest and wash (Minnesota) and post-thaw wash (Miami) of bone marrow-derived MSCs.

In general cell recovery, viability, and hence, the viable cell recovery, were equivalent or better with the LOVO as compared to the in-house method. Viability and recovery with harvest/wash of TIL (Moffitt) was slightly better with the LOVO Cell Processing System. Viability and recovery with harvest/wash of MSCs (Minnesota) was substantially better with the LOVO Cell Processing System, and with Baylor's experiment #1 removed as an outlier, the LOVO performed better than their in-house method. The experiments performed by the Miami lab were different from those of the other facilities in that they involved a small volume (20 mL) cryopreserved product with 100 million total cells and a wash-only procedure. While the viability results for the MSCs from Miami were slightly better with the LOVO, the small volume product may have played a role in the lower cell recovery.

It is often important to demonstrate removal of certain media components or reagents for patient safety or other regulatory concerns. Baylor included an assessment of post-harvest/wash IL-15 concentration and found that both the in-house and LOVO methods substantially removed the cytokine (i.e., approximately 1000-fold reduction). While adding a manual wash to the end of the LOVO process may obviate some of the benefits of having a fully automated, closed system approach, the Baylor lab was able to further reduce the IL-15 concentration to not detectable or nearly not detectable with such an approach.

It is best practice for a busy cell processing laboratory to institute processes and employ technologies that improve on expenditure of time and effort. The LOVO improved on processing time for the washing of MSCs (Miami). Processing by the other sites was slightly (Minnesota) to substantially (Moffitt and Baylor) prolonged with the LOVO, though both in-house and LOVO methods at Moffitt were brief. LOVO processing time could likely be further optimized. However, in this study with an increase in time for processing on the LOVO at some sites, the pros and cons (e.g., cost of device and disposables, ease of use, etc.) of each system must be weighed to determine the best approach. As the LOVO is an automated system, it can allow for standardization of methods, consistency of final product, and, more than likely, a decrease in processing errors can be expected. Additionally, the device allows for entering and tracking kit/consumables for each run as well as generation of a corresponding report if using the companion DXT software. These are certainly advantages over manual methods.

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Another advantage of devices such as the LOVO is the closed system and, with that, the decreased likelihood of loss of integrity and sterility concerns. One in-house experiment at Baylor did show growth of Gram-negative bacilli, Ralstonia pickettii, a soil bacterium and an emerging nosocomial pathogen reported in contaminated medical solutions, including saline, sterile water, as well as disinfectants. A repeat sampling yielded no growth after two weeks, suggesting the original positive was perhaps due to contamination at inoculation.

In summary the LOVO Cell Processing System provides an alternative to centrifuge-based technologies. The system employs a spinning membrane filter and is automated and closed. Cell exposure time to the filter is minimal, particularly as compared to the time spent spinning by centrifugation with the in-house methods (i.e., manual, Cobe 2991, CellSaver 5+). This small multicenter study demonstrated the ability of the LOVO to yield satisfactory cell viability and recovery for T cells and MSCs. The ‘gentler’ approach to harvest and wash may have additional impact on cell function, though this remains to be demonstrated.

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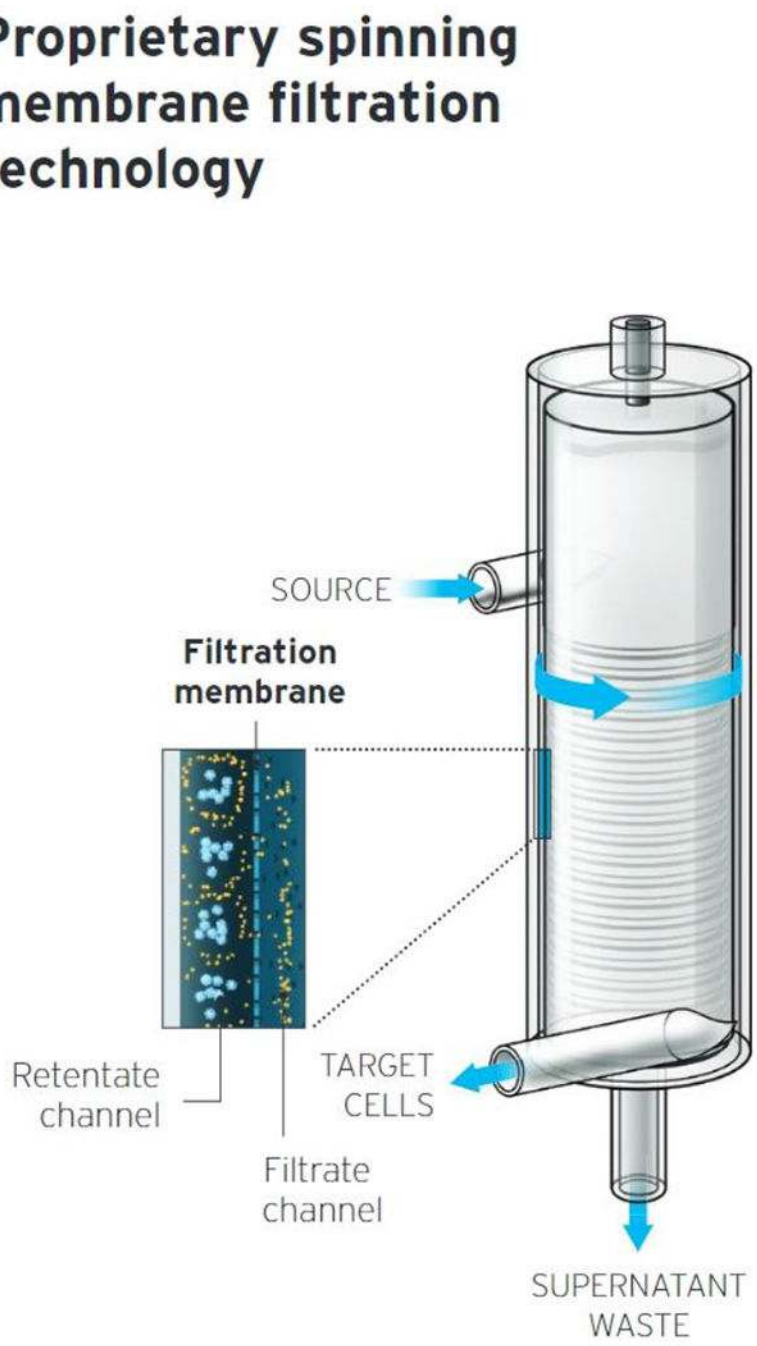


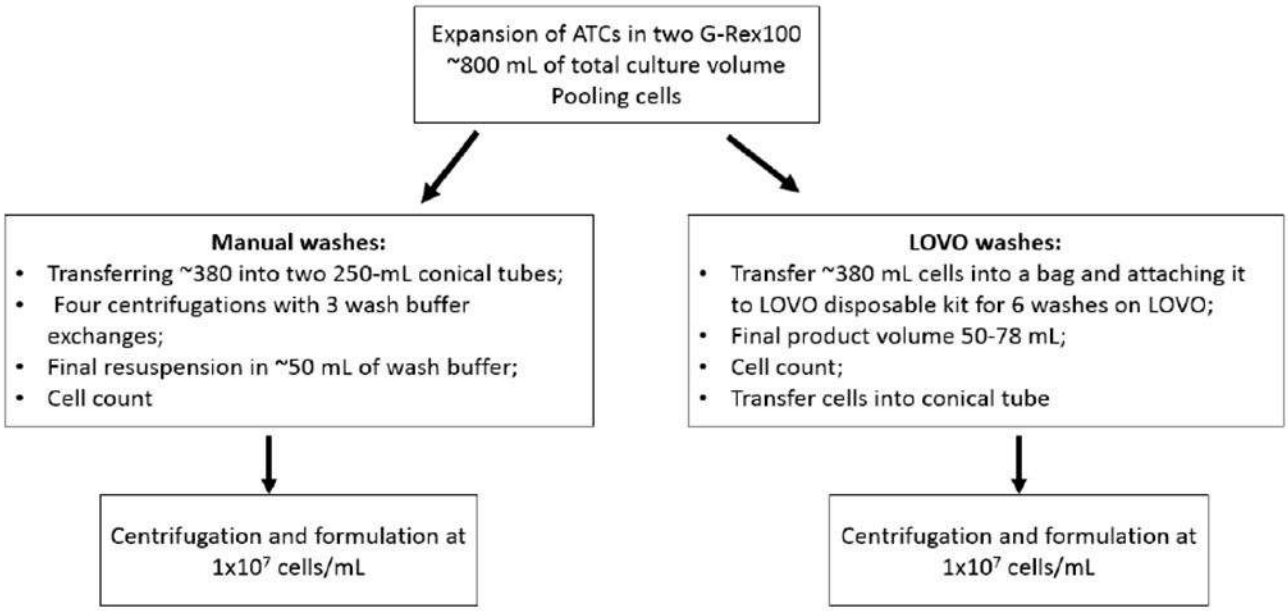
Figure 1.

a. The LOVO Cell Processing System (Fresenius Kabi, Bad Homburg, Germany).

b. Diagram of spinning membrane filtration technology.

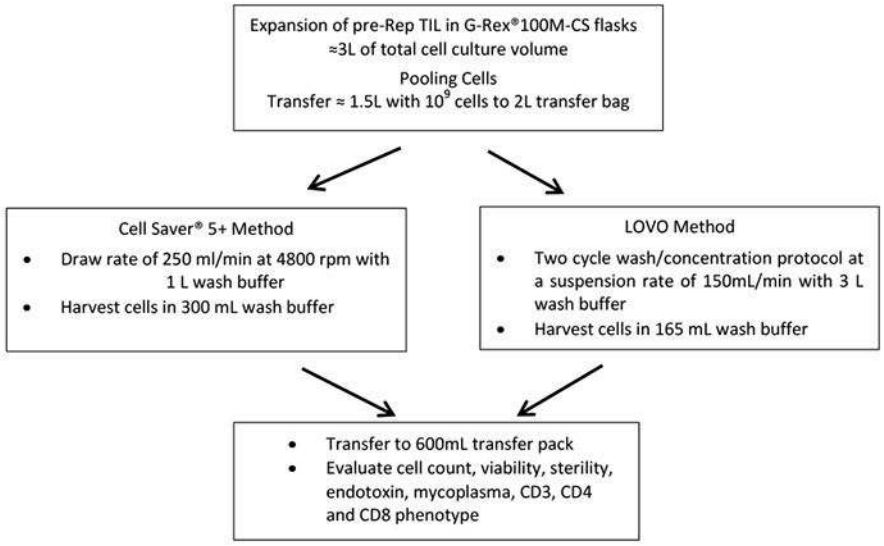
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A) Baylor

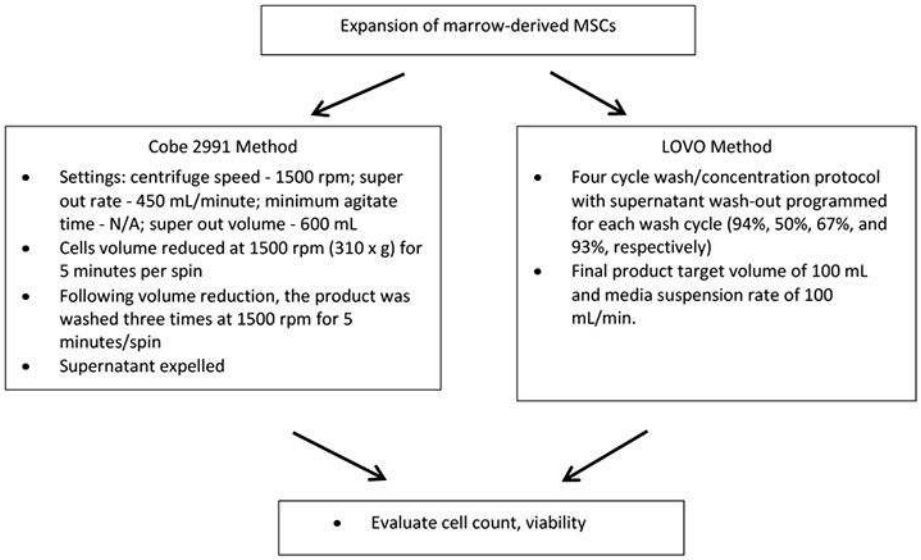


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B) Moffitt



C) Minnesota



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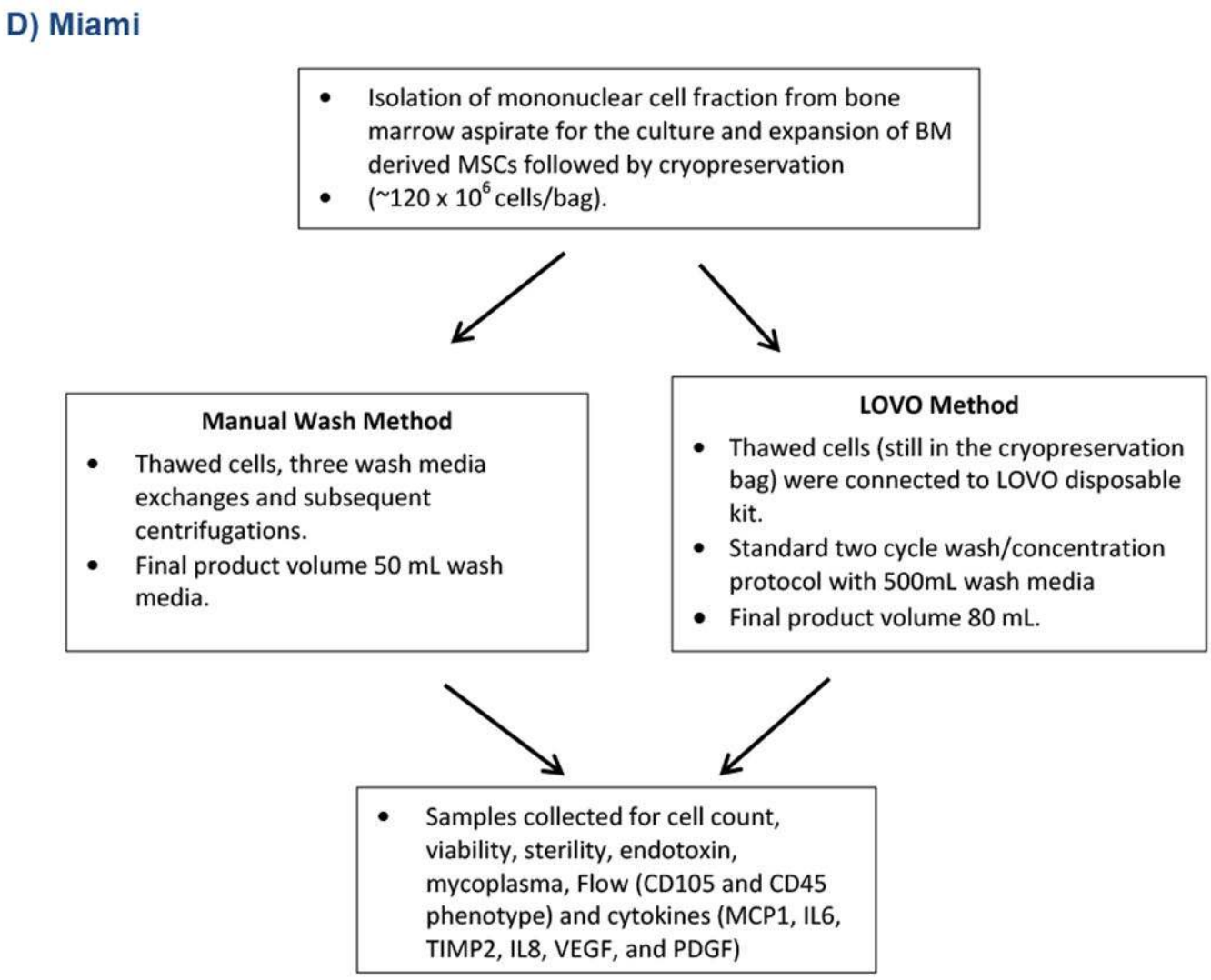


Figure 2.
Experimental design for comparison of in-house/established method versus LOVO automated procedure: A) Baylor; B) Moffitt; C) Minnesota; D) Miami.

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Table 1.

Summary of cell volume, cell recovery, viability, viable cell recovery, and wash time for all experiments at all sites.

Cell Product			Starting and Final Volume (mL)		Total Cell Recovery %		Viability %		Viable Cell Recovery %		Time (minutes)	
CPF	Sample ID	Cell Type	In-House	LOVO	In-House	LOVO	In-House	LOVO	In-House	LOVO	In-House	LOVO
BCM	1	T Lymphocytes	390/50	390/46.5	80.2	62.7	98.7	98.1	81.3	63.2	61	85
BCM	2	T Lymphocytes	350/50	350/78	85.9	95	97.8	97.9	88.9	98.3	69	122
BCM	3	T Lymphocytes	387.5/48	387.5/79	100	100	96.8	95.6	98.5	100	60	89
BCM	4	T Lymphocytes	380/50	380/78	93.1	100	98.2	95	95.5	100	60	121
		Average *	377/50	377/70	89.8	89.4	97.9	96.7	91.1	90.4	62.5	104.3
UMiami	1	MSCs	20/50	20/50	92	78.4	93	92	88.2	75.3	55	40
UMiami	2	MSCs	20/50	20/50	97.8	85.9	88.5	95	83.7	84.5	45	38
UMiami	3	MSCs	20/50	20/50	83	92	86	88	73	83	45	34
		Average *	20/50	20/50	90.9	85.4	89.2	91.7	81.6	80.9	48.3	37.3
MCC	1	TIL	1494/300	1407/165	73.4	83.2	93.8	94.5	73.6	82.7	17	42
MCC	2	TIL	1434/262	1423/165	57.6	67.8	91	89.2	55.1	63.5	17	41
MCC	3	TIL	1279/331	1283/165	82.3	90.7	75.6	84.5	71.3	88.8	17	40
		Average *	1402/298	1371/165	71.1	80.6	86.7	89.4	66.7	78.3	17	41
UMN	1	MSCs	2730/125	2878/68	71	84	83	96	60	80	60	111
UMN	2	MSCs	1906/91	1776/68	78	75	91	94	74	75	60	57
UMN	3	MSCs	5376/154	5525/71	81	88	94	93	77	85	90	120
		Average *	3337/123	3393/69	76.7	82.3	89.3	94.3	70.3	80	70	96

Note: BCM- Baylor College of Medicine; UMiami – University of Miami; MCC – Moffitt Cancer Center; UMN – University of Minnesota; MSCs – Mesenchymal stromal cells; TIL – Tumor infiltrating lymphocytes; Viable cell recovery is calculated as follows: (post-wash TNC x post-wash viability)/(pre-wash TNC x pre-wash viability).

* Averages for the starting and final volumes were rounded to the nearest whole number.

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Table 2.

(Baylor College of Medicine) IL-15 concentration and amount in ATCs before and after manual and LOVO washes.

	Before washes (ng/mL)	Before washes (ng)	Last manual wash	Formulated cells after manual washes	Last LOVO wash (pg/mL)	Last LOVO wash (ng)	Formulated cells after LOVO washes (pg/mL)
Experiment 2	28.6	10,010	Not detected	Not detected	26.63	2.1	1.46
Experiment 3	188	72,850	Not detected	Not detected	15.58	1.2	Not detected
Experiment 4	420	159,600	Not detected	Not detected	37.8	2.9	Not detected
Average ± Standard Deviation	212±197	80,820±75,113	Not applicable	Not applicable	27±11	2.1±0.09	0.5±0.8