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Application Note

Automated CAR-T vial filling using Larkem® V40

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Celleo's Larkem V40 is a closed system for automated GMP vial filling in Grade C environments. To aseptically fill up to 40 vials in parallel, Larkem evacuates air from the closed Single-Use Set incorporating the vials, before displacing the vacuum with fluid. The manifolded vials are automatically sealed and separated, providing industry-standard vials for subsequent use. CCRM Nordic used a pre-production version of the Larkem V40 for this study.

- **Biological integrity maintained:** Larkem® V40 delivers equivalent viability, cell concentration, phenotype and potency compared with manual operations.
- **Enhanced vial fill accuracy:** Larkem® V40 provides increased volume filling accuracy compared to manual vial filling.
- **High-throughput and rapid vial filling:** Larkem® V40 enables rapid, automated, and closed filling of up to 40 cryovials in Grade C/D environments.

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This application note explores the Larkem® V40 compared to manual pipetting for the vialing of chimeric antigen receptor T (CAR-T) cells at the end of a 5-day process, analysing cell count, viability, potency, phenotype, and vial filling accuracy. The evaluative research included two donor samples that were processed using both the CliniMACS Prodigy® and G-Rex® systems.

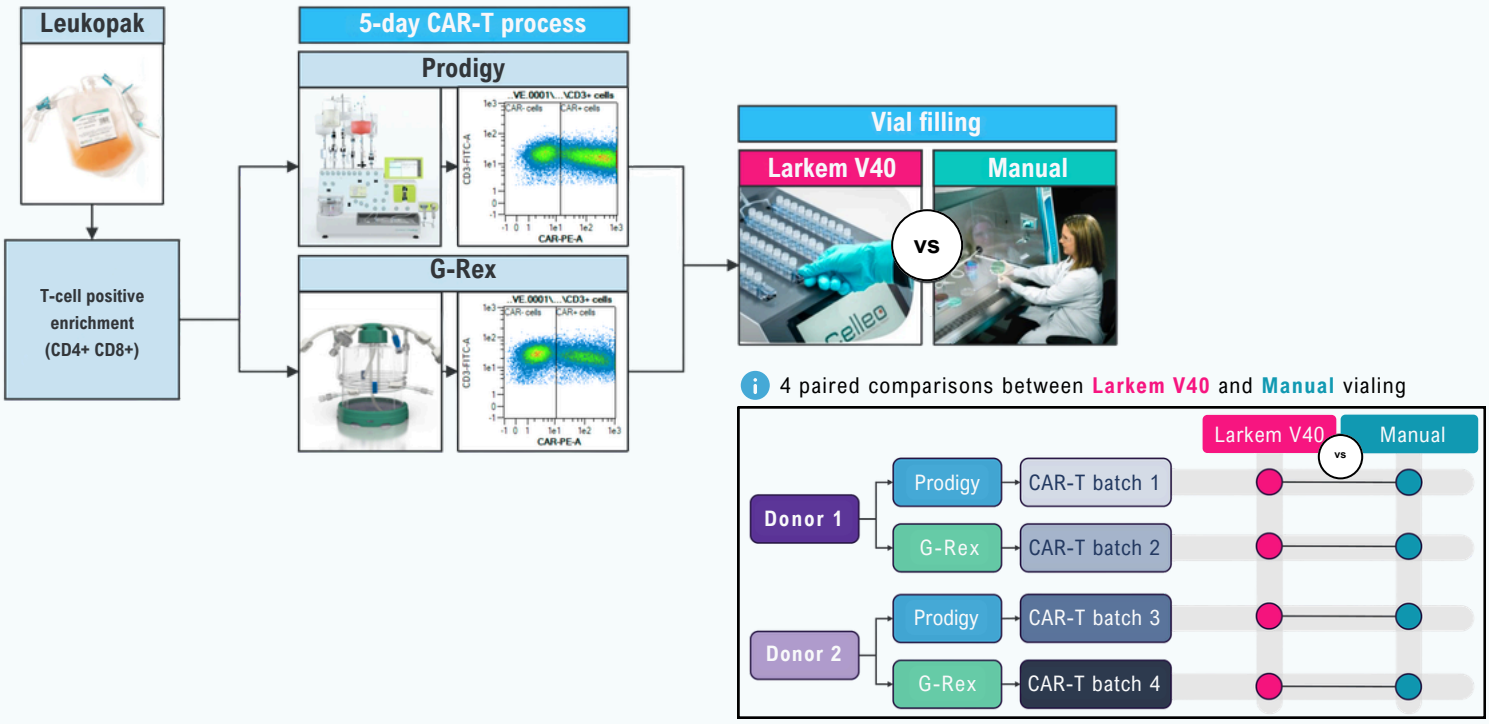
Introduction

CAR-T therapy is redefining modern medicine, particularly in blood cancers. The transformative treatment reprograms a patient's immune cells to recognise and attack cancer. Today, multiple CAR-T therapies are commercially approved, including Carvykti (Legend Biotech / Janssen – Johnson & Johnson), Breyanzi (Bristol Myers Squibb), Kymriah (Novartis), and Yescarta (Kite) [1,2], with their proven efficacy driving growing adoption of CAR-T as a treatment option.

Despite the science proving to be life-changing for patients, the manufacturing of CAR-T therapies remains expensive and complex, with manual, open processing in Grade A/B environments making production highly resource-intensive while increasing product variability and sterility risk, potentially leading to batch failure and the loss of critical patient doses.

New technology advancements focused on automating and closing the workflow are enabling efficient and scalable production. Larkem® V40 addresses a critical bottleneck in the CAR-T workflow by replacing a manual, open container filling process with closed, automated operation, reducing contamination risk, lowering cost and enabling scalable production outside high-grade cleanrooms. This improves throughput and consistency while reducing batch failure and increasing successful patient dose delivery.

Experiment overview



Methods

CAR-T manufacture process

T-cells were isolated ($CD4^+CD8^+$) from fresh leukopaks using CliniMACS Prodigy® from Miltenyi Biotec. This was performed for two healthy donors, from each donor, $100x10^6$ T-cells were seeded in TexMACS™ GMP Medium (Miltenyi Biotec) with 150 IU/mL IL-2 (ProPak™ Recombinant Human IL-2 GMP Protein, Bio-Techne) into either the CliniMACS Prodigy® or into G-Rex® 100M-CS (Wilson Wolf) and activated with T cell TransACT™ (Miltenyi Biotec). On Day 1 cells were transduced with an anti-CD19 CAR lentivirus (MOI:2.5). On Day 3, G-Rex® media was topped up to 1 L; in the CliniMACS Prodigy® a culture wash and feed to 250 mL was performed and agitation was initiated. After 5 days cells were harvested. G-Rex® cultures were washed 3x using Sefia Select™ S-Wash (Cytiva). For CliniMACS Prodigy® cultures, no additional washes were performed. For both platforms, formulation was performed with Sefia Select™ using the ReadySelect application. For each donor, four input bags from each culture system were formulated in 1:1 CryoStor® CS10 (STEMCELL Technologies): PBS. One bag was used for automated vialing using Larkem® V40 (20x vials per run), while a second bag was used for manual vialing into Nunc™ 1.8 mL internally threaded vials (20x vials, Thermo Fisher Scientific). The remaining two drug product bags were kept untouched, and all samples cryopreserved in a VIA Freeze™ Quad controlled rate freezer (Cytiva). Total contact time with DMSO was < 30 minutes. Samples were placed into long-term cryogenic storage. Additional in-process tests were performed, including donor profiling and CAR transduction (not reported in this application note).

Fill volume accuracy and precision

Cryovials were thawed for 5-6 minutes in a bead bath and weighed in a precision balance (precision of 0.01 g). After counting, cryovials were emptied with a suction pump and the empty vial was weighed again in the same precision balance. Liquid weight was calculated as the difference between full cryovial weight and empty cryovial weight. Weight was converted to volume (mL) by multiplying the liquid weight by the density 1:1 PBS-EDTA and CS10 solution (1.035 g/mL).

Cell viability and concentration

Cryovials were thawed for 5-6 minutes in a bead bath before assessment of cell count using NC-202 cell counter (two counts per sample, averaged).

Cell phenotype

Cell suspensions were stained with the StainExpress™ CAR T Transduction Cocktail (Miltenyi Biotec) in addition to an antibody against the G4S-linker segment of the CAR construct (for detection on the cell surface). Data shows the frequency (%) of G4S+ (CAR+) events within live, singlet, CD45+, CD3+ events. (Method refers to Figure 4A).

Cell suspensions were stained with fluorochrome-labeled antibodies against CD45RA, CD4, CD3, CD62L, CD45RO, CD8, the G4S-linker domain of the CAR construct, and a live/dead cell dye. Data shows the frequency (%) of CD62L+ CD45RO+ (Central Memory) events within live, singlet, CD45+, CD3+, G4S+ (CAR+) events. (Method refers to Figure 4B).

All samples were analyzed using a MACSQuant® Analyzer 10 Flow Cytometer (Miltenyi Biotec) using the automated gating package included in the CAR T Cell Express Mode package.

Cell potency

Rested Anti-CD19 CAR-T cells were co-cultured with CellTrace™ Violet (CTV)-labelled target Jeko-1 cells (CD19+) for approximately 18 hours. Each condition was assessed in technical duplicates. Number of CTV+ (target) events were counted by flow cytometry and cytotoxicity calculated relative to wells containing target cells only. (Method for CAR-T cytotoxic functionality).

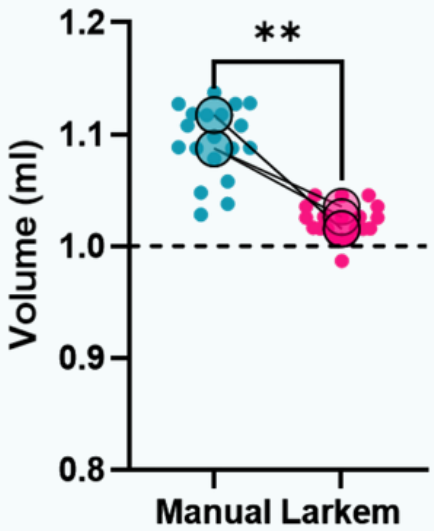
Rested Anti-CD19 CAR-T cells were co-cultured with target Jeko-1 cells (CD19+) for approximately 18 hours. Each condition was assessed in technical duplicates. Culture supernatants were harvested and secretion of Granzyme B, IFNg, Perforin, and TNFa was assessed by multiplexed automated ELISA using the Ella™ platform (Bio-Techne). (Method for secretion of effector cytokines).

Results and discussion

Larkem® V40 improves fill volume accuracy and precision

Larkem® V40 delivers enhanced vial fill volume accuracy and precision compared to manual pipetting. This demonstrates improved process control for consistency and accurate vial filling. (*Figure 1*)

Figure 1



	Manual	Larkem V40
Number of cryovials	20	20
Mean volume (ml)	1.10	1.03
Std. Deviation (ml)	0.05	0.01
Coefficient of variation	4.4%	1.3%

Figure 1. *Large dots represent the median value for the 5 cryotubes analyzed per CAR-T batch (2 donors x 2 platforms) and are between manual and Larkem® V40 vialing. Data was shown to have normal distribution with a Shapiro-Wilk test, and the two groups were compared with a Paired t test. P value = 0.0092.*

Equivalent cell viability

Larkem® V40 provides equivalent cell viability compared with manual pipetting, highlighting that Larkem® V40 serves as an effective aliquoting system, and that the inherent exposure to tubing, shear stress in fluid pathway, and heal-seal-separate mechanisms to fill vials, do not impact cell health in vials. (*Figure 2*)

Figure 2

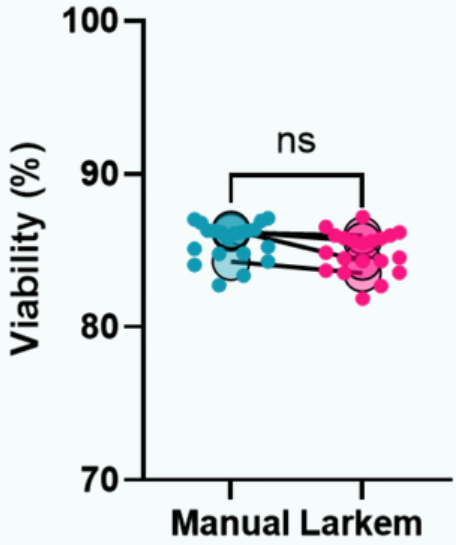


Figure 2: *Small dots are values for individual cryotubes analyzed (5 per CAR-T batch/vialing method). Large dots are the median value for the 5 cryotubes analyzed and are paired for each CAR-T batch between manual and Larkem® V40 vialing. Data was shown to have normal distribution with a Shapiro-Wilk test, and the two groups were compared with a Paired t test. ns = non-significant.*

Comparable cell concentration

Viable cells/mL is comparable between Larkem® V40 and manual pipetting. This highlights that Larkem® V40 enables gentle operation that does not affect cell concentration in vials. (*Figure 3*)

Figure 3

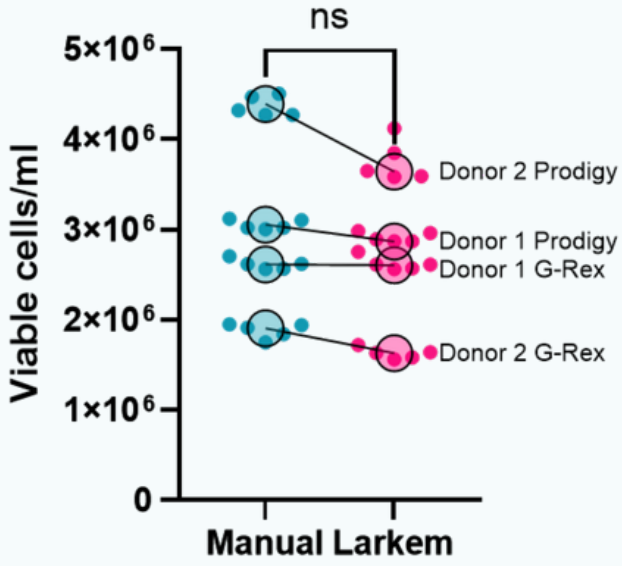


Figure 3: *Small dots are values for individual cryotubes analyzed (5 per CAR-T batch/vialing method). Large dots are the median value for the 5 cryotubes analyzed and are paired for each CAR-T batch between manual and Larkem® V40 vialing. Data was shown to have normal distribution with a Shapiro-Wilk test, and the two groups were compared with a Paired t test. ns = non-significant.*

Equivalent cell phenotype

Larkem® V40 delivers equivalent CAR-T cell phenotype compared to manual vialing both pre- and post-cryopreservation, demonstrating preservation of cell biology throughout the filling process. (*Figure 4*)

Cell potency maintained

Cytotoxic functionality of CAR-T cells vialed using Larkem® V40 is equal to manual controls and secretion of effector cytokines by Larkem® V40 vialed CAR-T cells is equivalent to manually vialed CAR-T at a 4:1 effector-to-target (E:T) ratio. (*Figure 5*)

Figure 4 (A)

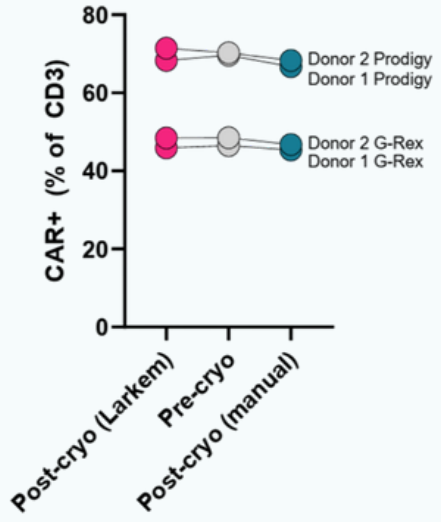


Figure 4 (B)

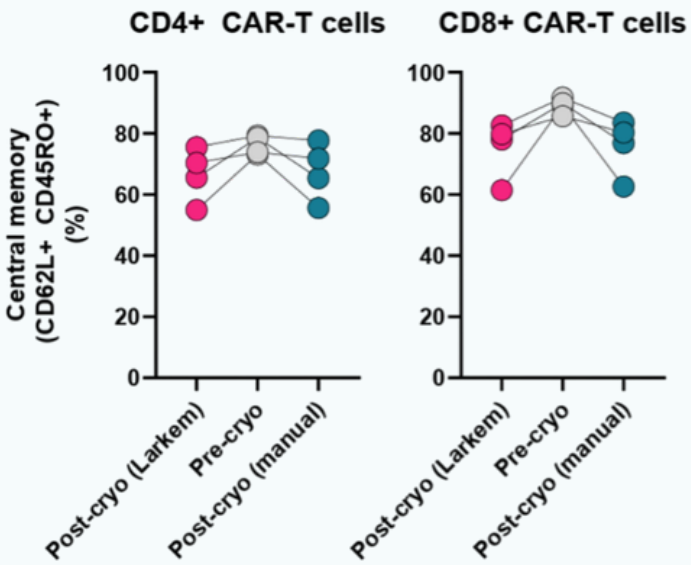


Figure 4: *(A) Data shows the frequency (%) of G4S+ (CAR+) events within live, singlet, CD45+, CD3+ events, both pre- and post-cryopreservation. (B) Data shows the frequency (%) of CD62L+ CD45RO+ events within live, singlet, CD45+, CD3+, G4S+ (CAR+) events, both pre- and post-cryopreservation.*

Figure 5 (A)

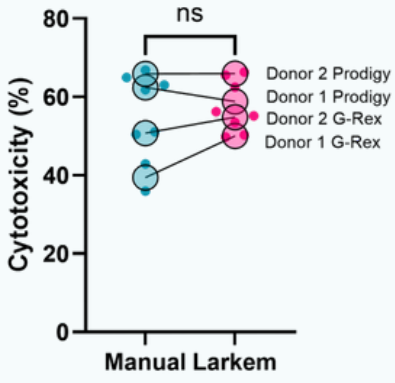


Figure 5 (B)

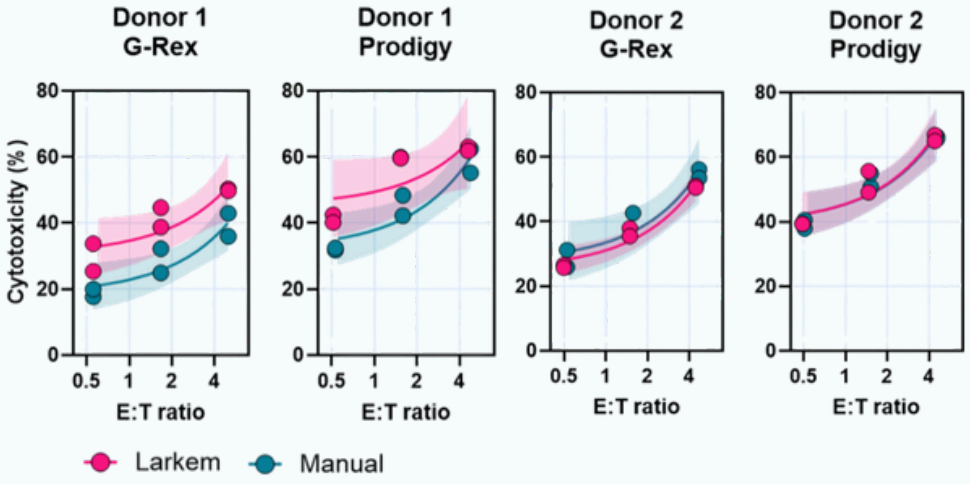


Figure 5 (C)

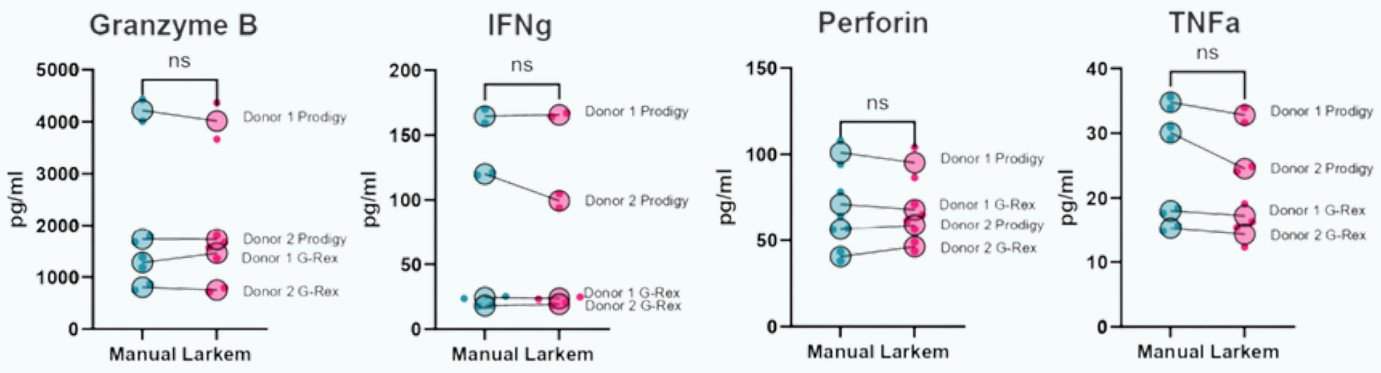


Figure 5: *(A) The average % cytotoxicity at a 4:1 effector-to-target (E:T) ratio is displayed, (B) all E:T ratios tested for each sample, (C) Culture supernatants were harvested and secretion of Granzyme B, IFNg, Perforin, and TNFa was assessed by multiplexed automated ELISA using the Ella™ platform (Bio-Techne).*

Conclusion

The research demonstrates efficient vialing of CAR-T cells using the automated, closed-system Larkem® V40. The system provides improved vial filling accuracy and precision compared to manual vialing while maintaining biological integrity, with equivalent cell count, viability, potency and phenotype.

To learn more about Larkem® V40 visit celleo.com.

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About CCRM Nordic

CCRM Nordic is a public-private partnership organization with the purpose of addressing bottlenecks in the translation and commercialization of advanced therapy medicinal products (ATMPs). It is based in Gothenburg, Sweden, supporting innovators globally. Learn more: www.ccrmnordic.se

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Celleo delivers intelligent production systems that enable efficient, scalable cell and gene therapy manufacture, with the vision of advancing transformative therapies toward population-level health impact. Our systems support leading biopharmaceuticals, CDMOs and developers of novel therapeutics across key workflows, including cell banking, drug product, QC samples, critical reagent preparation, and other emerging applications.
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Disclaimer

This application note is intended for informational purposes only and has not undergone peer review. The CAR-T processes described herein were performed as proof-of-concept studies to evaluate system performance; they are not intended for clinical use or patient treatment. CCRM Nordic and Celleo are separate and independent entities with no financial affiliation.

Contact

To discover the full research covering nucleation during cryopreservation, DMSO contact time, immune composition, differentiation and more, email pedro.velica@ccrmnordic.se, chao.sheng@ccrmnordic.se. For business related questions contact: bd@ccrmnordic.se.