Upscaling T Cell Expansion in VueLife® "HP" Series Bags: From 50-HP to 1000-HP Bags

VueLife® "HP" Series Bags are Saint-Gobain Life Sciences' "high-permeability" FEP bags for *in vitro* bioprocessing applications. Applications include the culture of highly concentrated immune cells, such as T or NK cells that require higher gas exchange rates for efficient culture and/or expansion *in vitro*.

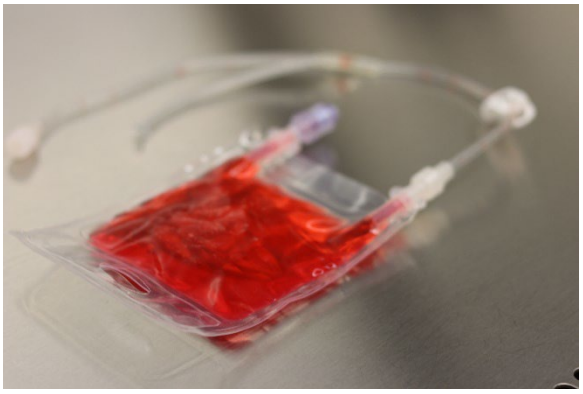

Figure 1. VueLife® "HP" Series Bag

The novel "HP" FEP laminate provides elevated oxygen and carbon dioxide permeability to the container, thereby introducing further enhanced gas exchange capability to the VueLife® product line. The fluid contact-layer remains the same material as used in the VueLife® "C" Series range, harnessing the superior material properties of FEP.

EXPANSION OF T CELLS IN VUELIFE® "HP" SERIES BAGS

The aim of this study was to compare the performance of VueLife® "HP" Series bags to their "C" Series counterparts. T cells were phenotyped before and after culture by flow cytometry to identify any impact of the *in vitro* culture protocol on surface marker expression and to characterize the composition of the produced T cell population.

Further, culture media parameters like pCO_2, pO_2, pH, glucose and lactate were measured to better understand the cells' metabolic activity and changes in the culture microenvironment in the different culture containers and conditions.

To maintain efficient cell expansion in log-phase, we increased culture volumes and transferred cultures from smaller to larger bags via sterile connection (welding). We up-scaled cultures from an initial 50 mL to 2 L in 10 days of culture.

RESULTS

Following purification of $CD4^+/CD8^+$ T cells from apheresis products, cells were resuspended in OpTmizer™ medium and seeded into VueLife® 50-C bags ("C" Series), and 50-HP bags ("HP" Series) in 50mL at a starting concentration of $1x10^6$ cells/mL.

For efficient expansion in VueLife® bags, T cell concentration was maintained at $<1x10^6$ cells/mL by addition of fresh culture medium. We found that dilution of T cell cultures to concentrations of $0.3\text{-}0.5x10^6$ cells/mL was a reliable range to maintain robust cell proliferation in our conditions.

If the culture volume exceeded the recommended fill volume for each container, the culture was transferred to a new bag via sterile connection using the PVC tubing attached to each bag and using gravity to drain the source bag into the recipient bag.

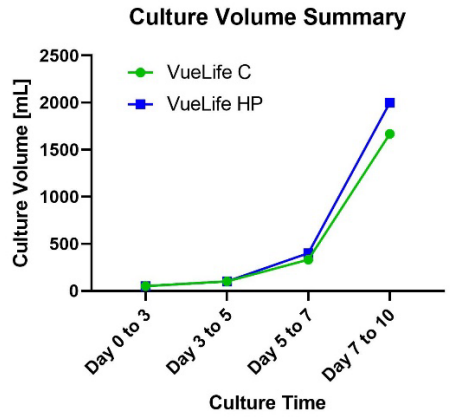

Figure 2. Summary of culture volumes used to culture T cells in VueLife® "C" and "HP" bags.

As illustrated in Figure 2, T cells cultured in VueLife® "HP" bags were typically transferred from 50-HP bags to 200-HP bags at day 5, and from 200-HP bags to 1000-HP bags at day 7 of culture. In addition, cells could be split from one 50-HP bag into multiple 200-HP bags, etc. for efficient upscaling of cultures to produce desired final cell numbers.

This added flexibility provides users to adjust cultures based on the performance of the individual cell product, which may differ in rate and capability of expansion depending on the donor's health and potential treatment strategy prior to donation.

In addition, VueLife® bags can be fully customized to accommodate the desired scale and connectivity needs. Please contact us for further details.

T Cell Yield and Concentrations

T cell cultures were sampled on day 3, 5 and 7 and harvested at day 10 of culture to assess cell count and viability. As shown in Figure 3, we found that "HP" bags can support expansion of T cells up to ~150 fold in 10 days of culture, producing a mean of $7.4x10^9$ cells (± $0.8x10^9$ cells) in n=3 runs. This yielded ~2x the expansion seen in "C" bags.

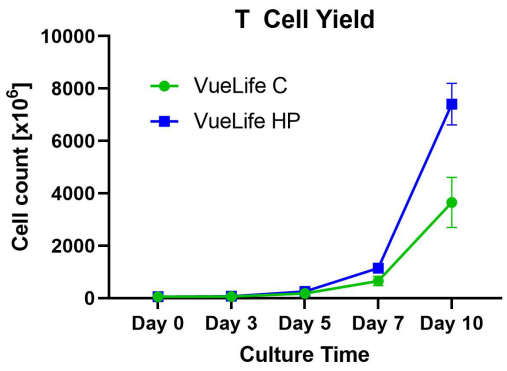

Figure 3. Absolute T cell count in VueLife® "C" and "HP" culture containers at the indicated culture period. Shown are mean ± SD values of n=3 independent experiments.

This benefit in cell expansion was a direct result of the "HP" laminate used when comparing the T cell yield in "HP" bags to their "C" Series counterparts, as both bags were filled and processed according to the same protocol by the same operator. The main difference in T cell performance was driven by the different cell proliferation rates, as shown in Figure 4.

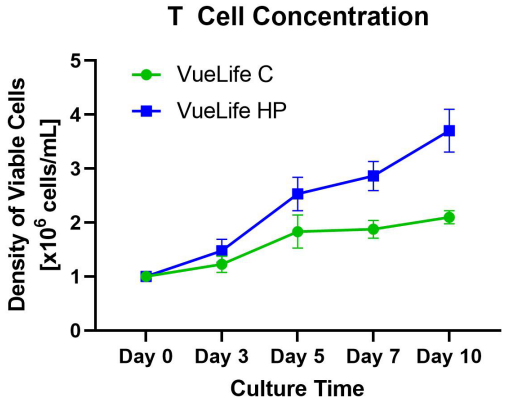

Figure 4. Concentrations of viable T cells in VueLife® "C" and "HP" culture containers at the indicated culture period. Shown are mean ± SD values of n=3 independent experiments.

Based on our cell culture experience *in vitro* and computational modeling of cell cultures *in silico*, we recommend using the nominal fill volume for each bag as indicated, as we found gas exchange rates and microenvironmental factors to be optimal at this volume. In addition, we have seen positive results in our T cell cultures when bags

were filled with volumes ranging from ½ to 2-fold of the nominal volumes. However, this result was specific to this study, and users are encouraged to perform their own validation studies to investigate optimal fill volumes and procedures for their specific applications.

T Cell Viability and Activation

Importantly, cell viability was found to be consistently above 90% throughout culture in all VueLife® containers (Figure 5), which indicates robust cell health during the entire expansion period.

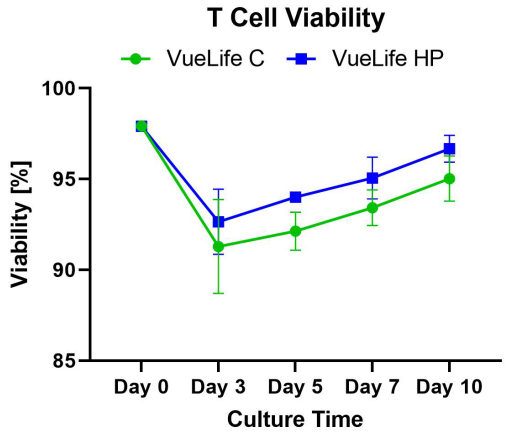

Figure 5. T cell viability in VueLife® "C" and "HP" culture containers at the indicated culture period. Shown are mean ± SD values of n=3 independent experiments.

The diameter of T cells is typically used as an indicator for the cell's activation status. To achieve rapid proliferation *in vitro*, CD3/CD28 Dynabeads™ are often used in clinical-scale manufacturing to specifically bind to the T cell surface receptors and trigger cell proliferation. This effect is limited in its duration, and overstimulation of the cells can result in T cell exhaustion. In our study, T cell diameter increased after the first 3 days of culture, and then gradually decreased until harvest (Figure 6), indicating consistent T cell activation.

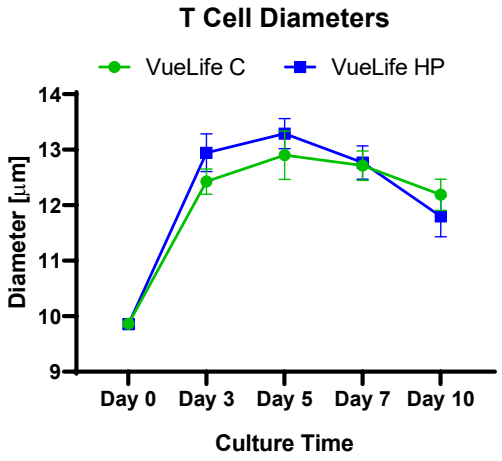

Figure 6. Diameter of T cells in VueLife® "C" and "HP" culture containers at the indicated culture period. Shown are mean ± SD values of n=3 independent experiments.

T Cell Phenotype and Characterization of Subsets

In addition to the quantity of cells produced, the quality plays a vital role in ensuring the efficacy and potency of the administered cell product. We analyzed T cells at the start of culture and after harvest (day 10) by flow cytometry for the expression of canonical T cell surface markers. As shown in Figure 7, T cells expanded in VueLife® "C" and "HP" bags were highly comparable in their phenotype, as shown in the expression levels of CD3, CD4 and CD8.

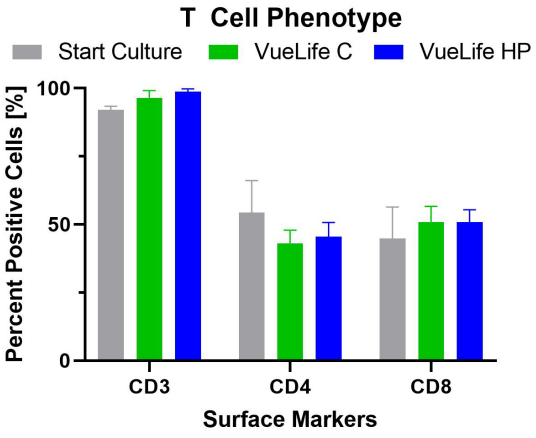

Figure 7. T cell surface profile at the start (day 0) and end (day 10) of culture in VueLife® "C" and "HP" culture containers. Shown are mean ± SD values of n=3 independent experiments.

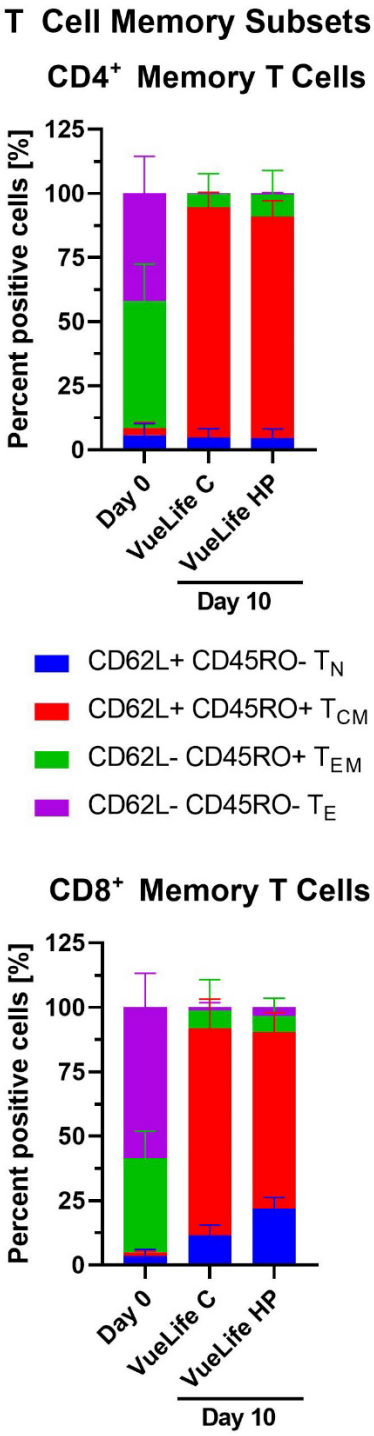

Figure 8. Phenotype of Memory T cell subpopulations before and after culture in VueLife® "C" and "HP" bags. Shown are mean ± SD values of percent positive cells [%] expressing the indicated marker(s) relative to unstained controls. Abbreviations: T_N: Naïve T Cell; T_{CM}: Central Memory T Cell; T_{EM}: Effector Memory T cell; T_E Effector T Cell

When comparing the produced $CD4^+$ and $CD8^+$ T cell subpopulations harvested from VueLife® "C" and "HP" containers, we found that the majority of T cells expressed a Central Memory T Cell phenotype (T_{CM}; Figure 8), regardless of the bag material used. In addition, all cultures were able to retain a comparable fraction of naïve T cells (T_N), which can give rise to a plethora of downstream T cell subtypes following stimulation with antigen. Within the characterized memory T cell subpopulations, T cells with a more naïve and central memory phenotype have been found to be beneficial in the production of CAR-T cells, shown by long-term engraftment and higher overall efficacy (refs page 7).

Cell Culture Microenvironment

Cells are highly responsive to mechanical and biochemical cues presented to them via the culture microenvironment, which is the foundation of the cell-surface interaction studies performed by Saint-Gobain Life Sciences to better understand this interplay of factors. The microenvironment presented to the cells, considered to be a "living drug," will impact their health and potency as demonstrated by the myriad of clinical trials investigating different routes of manufacture, formulation, and administration.

In our in-house cell culture studies, we routinely analyze and quantify the levels of gases, i.e. partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2), and metabolic factors (glucose, lactate) present in culture media at the start of culture and as the cultures progress.

As shown in Figure 9, the concentrations of pO_2 followed a similar trend in VueLife® "C" and "HP" bags during the first 7 days of culture, but then diverged in the final 3 days of culture. In VueLife® "C" bags, oxygen levels remained constant, while "HP" bags showed increasing pO_2 levels, which is a direct result of the improved gas permeability provided by the "HP" laminate.

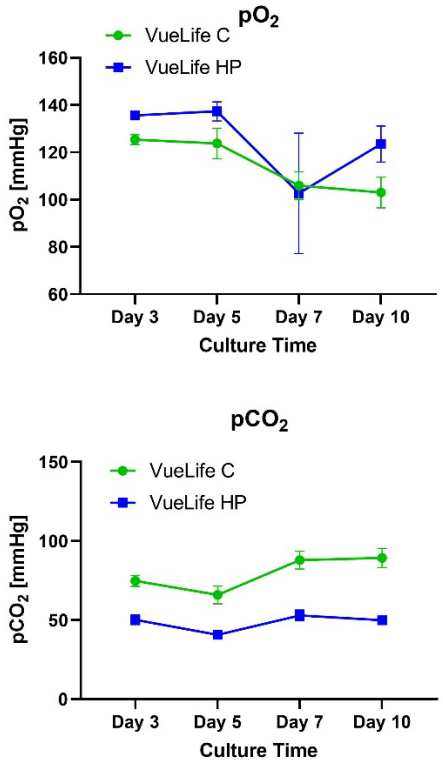

Figure 9. Partial pressure of oxygen (pO2) and carbon dioxide (pCO2) in VueLife® "C" and "HP" bags during culture. Shown are mean ± SD values of n=3 independent experiments.

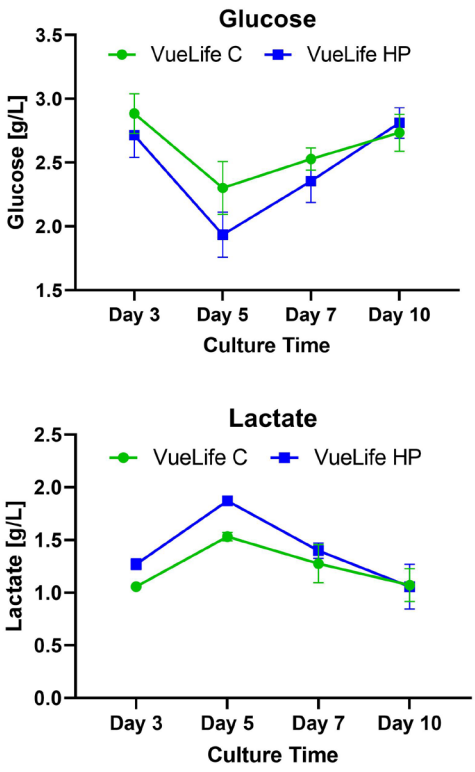

Figure 10. Concentrations of glucose and lactate measured in VueLife® "C" and "HP bags during culture. Shown are mean ± SD values of n=3 independent experiments.

When comparing pCO_2 levels (Figure 9), VueLife® "C" bags showed higher levels of pCO_2 during culture, with concentrations ~2 fold that of their "HP" counterparts. These data points are in agreement with the material properties used to construct the "C" and "HP" Series bags, respectively, in which the "HP" laminate provides a CO_2 transmission rate of ~16000 cc/m^2-day at 25°C vs: ~4000 cc/m^2-day for "C" bags at 25°C.

The two main metabolic factors we quantify throughout culture to delineate the type of biochemistry used to generate ATP in cells are glucose and lactate. As summarized in Figure 10, *glucose levels could be maintained at consistent levels in VueLife® cultures. Concentrations of lactate correlated inversely with glucose levels, as expected.*

Finally, both CO_2 and lactate will impact overall pH levels in the buffered culture media, depicted in Figure 11. In VueLife® bag cultures, pH levels remained constant in media for T cells cultured in "C" bags and increased in "HP" bags. This further highlights the positive impact of the "HP" laminate on the homeostasis of the cells' microenvironment engineered in VueLife® "HP" bags.

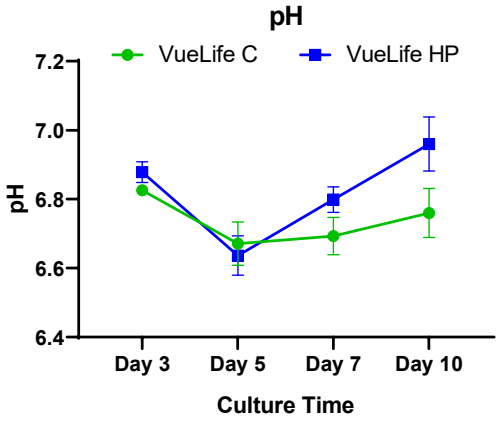

Figure 11. pH levels in VueLife® "C" and "HP" bags during culture. Shown are mean ± SD values of n=3 independent experiments.

MATERIALS AND METHODS

See also separate Technical Bulletin: "Protocol for T Cells in VueLife® "HP" Series 50-HP to 200-HP to 1000-HP Bags".

List of Materials

Product	Manufacturer	Catalog #
50-HP bag	Saint-Gobain	50-HP
200-HP bag	Saint-Gobain	200-HP
1000-HP bag	Saint-Gobain	1000-HP
50-C bag	Saint-Gobain	50-C
197-C bag	Saint-Gobain	197-C
750-C1 bag	Saint-Gobain	750-C1
OpTmizer™ media bag	Gibco	A10221-03
OpTmizer™ supplement	Gibco	A10484-02
L-Glutamine	Gibco	25030-081
Human IL-2 IS	Miltenyi Biotec	130-097-748
Dynabeads™ CD3/CD28	Gibco	11132D
Leukopak	Miltenyi Biotec	150-000-452
StraightFrom® Leukopak® CD4/CD8 MicroBead Kit	Miltenyi Biotec	130-122-352
Running buffer	Miltenyi Biotec	130-091-221
PBS	Gibco	14190-144

VueLife® Series bags

This study was conducted using Saint-Gobain Life Sciences' VueLife® "HP" Series and "C" Series bags.

T cell isolation from Leukopak™

$CD4^+/CD8^+$ cells were isolated from fresh Leukopak™ apharesis products (Miltenyi Biotec) from healthy donors using Miltenyi Biotec's StraightFrom® CD4/CD8 MicroBead Kits and the MultiMACS™ Cell24 Separator Plus instrument.

T cell activation and expansion

Isolated $CD4^+/CD8^+$ T cells were resuspended in OpTmizer® media supplemented with L-Glutamine (200µM/mM, Gibco) and IL-2 (200 IU/mL, Miltenyi Biotec), and activated using GibcoTM CTSTM DynabeadsTM CD3/CD28 at a bead-to-cell ratio of 1:1. T cells were expanded for a culture period of 10-12 days and then harvested for characterization by flow cytometry.

For efficient expansion in VueLife® bags, T cell concentration was maintained at $<1x10^6$ cells/mL by addition of fresh culture medium. We found that dilution of T cell cultures to concentrations of $0.3\text{-}0.5x10^6$ cells/mL was a reliable range to maintain robust cell proliferation in our conditions.

The culture bags were visually examined every 2-3 days, cell aggregates dispersed by manually homogenizing the culture medium, and culture samples collected to determine cell growth and medium composition.

Characterization of T cells

Beads were removed from cell samples prior to analysis using the DynaMag®. Cell counts and viability were determined using the Vi-CELL™ Cell Viability Analyzer (Beckman Coulter Inc.). This system utilizes the Trypan Blue dye exclusion method for quantification of viable cells in the measured cell population and provides results for mean cell size. The surface marker profile of

generated T cell subsets was assessed via a BD LSR II flow cytometer (Becton Dickinson and Company) at the Flow Cytometry Core Facility, University of Massachusetts Medical School in Worcester, MA. The acquired data was analyzed using FlowJo v10 (FlowJo, LLC).

Media Composition Analysis

Cell culture media samples were analyzed using the Vi-CELL MetaFLEX™ bioanalyte analyzer (Beckman Coulter, Inc.) to evaluate pH, partial pressure levels of oxygen and carbon dioxide (pO_2 and pCO_2), glucose, lactate and electrolytes.

CONCLUSIONS

The results of this study highlight the improved performance of Saint-Gobain Life Sciences' novel VueLife® "HP" Series Bags for the expansion of human T cells towards clinical use in Cell and Gene Therapy applications.

Figure 12. Saint-Gobain Life Sciences' VueLife® bags are fully closed, single-use containers that can be customized to suit the specific application and processing requirements.

The protocol and results described here are intended to provide an example to illustrate the performance of VueLife® "HP" bags with the products and culture parameters used in this setting. Many variables can affect T cell expansion, including starting cell populations (T cell subsets), cytokines and growth factors. Thus, users will need to investigate the culture conditions that will provide the optimal result with their specific protocols.

Please contact us if you have any questions related to the information presented here. We are continuously working to improve the use of our products.

REFERENCES:

1. Wang, Xiuli et al. "Comparison of naïve and central memory derived $CD8^+$ effector cell engraftment fitness and function following adoptive transfer." *Oncoimmunology* vol. 5,1 e1072671. 20 Aug. 2015, doi:10.1080/2162402X.2015.1072671 https://pubmed.ncbi.nlm.nih.gov/26942092/
2. Caccamo N, Joosten SA, Ottenhoff THM and Dieli F (2018) Atypical Human Effector/Memory CD4+ T Cells With a Naive-Like Phenotype. Front. Immunol. 9:2832. doi: 10.3389/fimmu.2018.02832 https://www.frontiersin.org/articles/10.3389/fimmu.2018.02832/full

ABOUT

SAINT-GOBAIN

Saint-Gobain Life Sciences is proud to take part in providing solutions for a multitude of cell therapy applications while collaborating with customers and industry partners to develop custom disposables, often for integration into automated systems. Through our material science expertise as well as our deep experience in bringing manufacturing technologies to scale, we are uniquely positioned to offer solutions to the numerous challenges faced by cell therapy manufacturers today.