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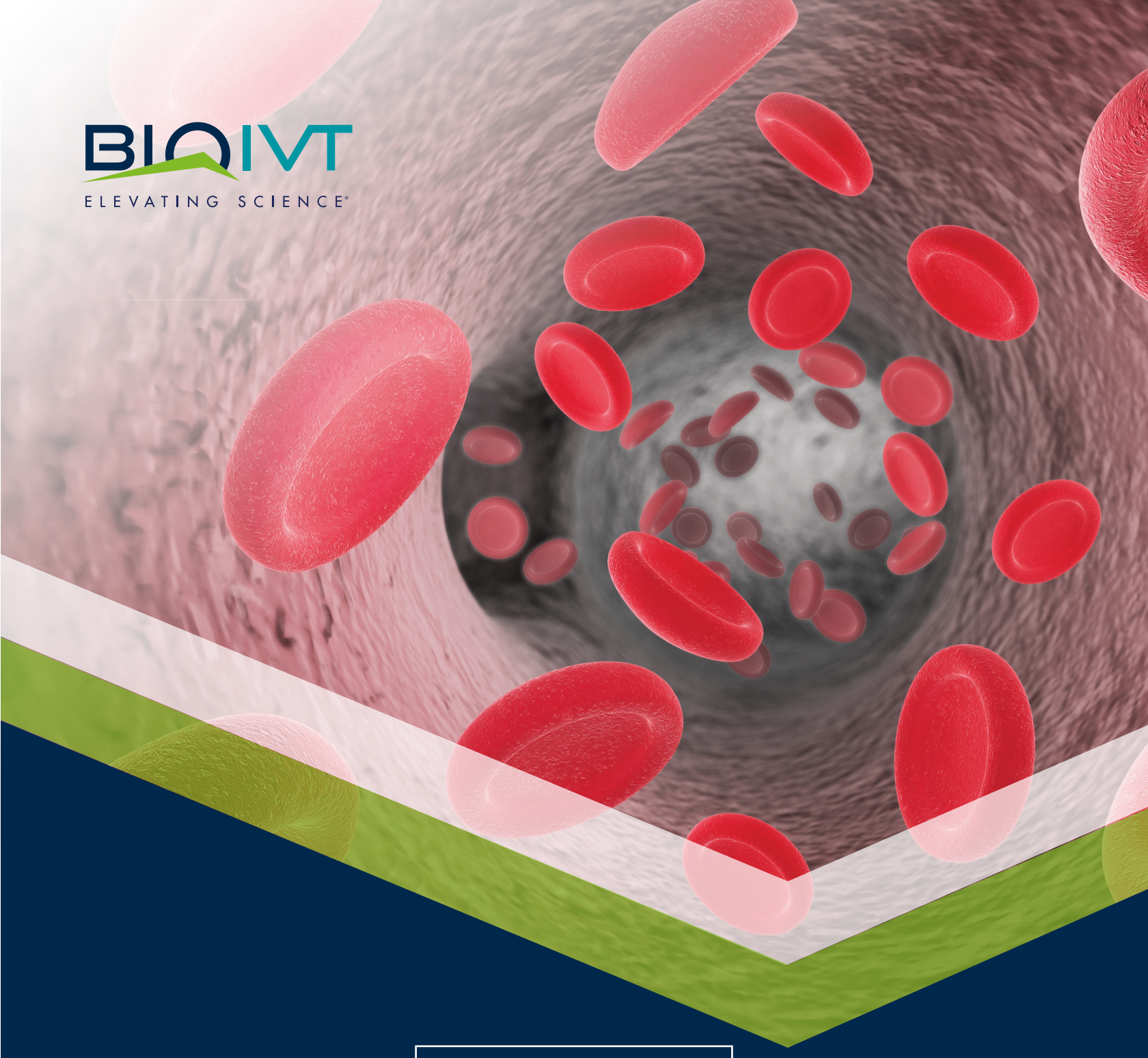


WHITE PAPER

Red Blood Cell Contaminants:

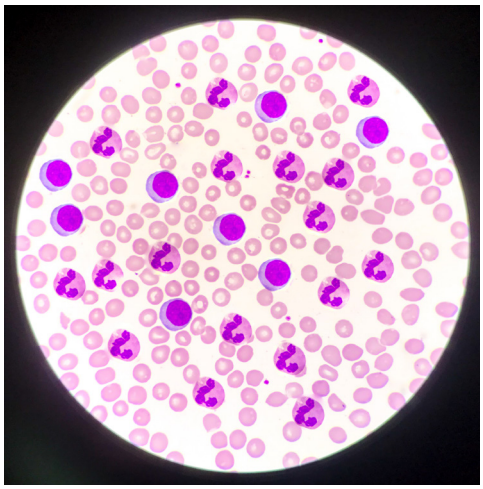
Upstream Mitigation for Downstream Success

Introduction

The field of cell therapy has rapidly expanded in the last decade, spearheaded by the many successes in chimeric antigen receptor (CAR) T-cell therapies for leukemias and lymphomas. The research and development (R&D) of these life-saving therapies begins with starting material from a healthy donor: a source for peripheral blood mononuclear cells (PBMCs). Starting material cell populations can either be heterogenous – such as whole blood, Leukocyte Reduction System (LRS) cones or plasma-suspended buffy coats – or homogenous – such as apheresis products or isolated immune cell subsets. With these heterogenous cell populations comes the risk of red blood cell (RBC) contamination which can have deleterious effects on downstream cell cultures. While this heterogenous starting material tends to be cheaper than its purified counterparts, are these upfront savings preserved throughout the R&D process?

Sources of Contamination

Red blood cell (RBC) contamination comes from a variety of sources. For instance, manual isolation of the PBMC layer from whole blood using density gradient media requires a deft operator, thoroughly trained with established SOPs. Still, variability in patient starting material may yield a thin PBMC layer, potentially increasing the risk of contamination. Even partially fractionated sources of PBMCs, such as LRS cones, have shown substantial RBC contamination, often as many as $6x10^{10}$ RBCs per cone!



Additional methods, such as RBC lysis buffers and washing steps, can help reduce contamination from heterogenous starting materials. However, these methods add to the total reagent and labor costs.

Downstream Impact

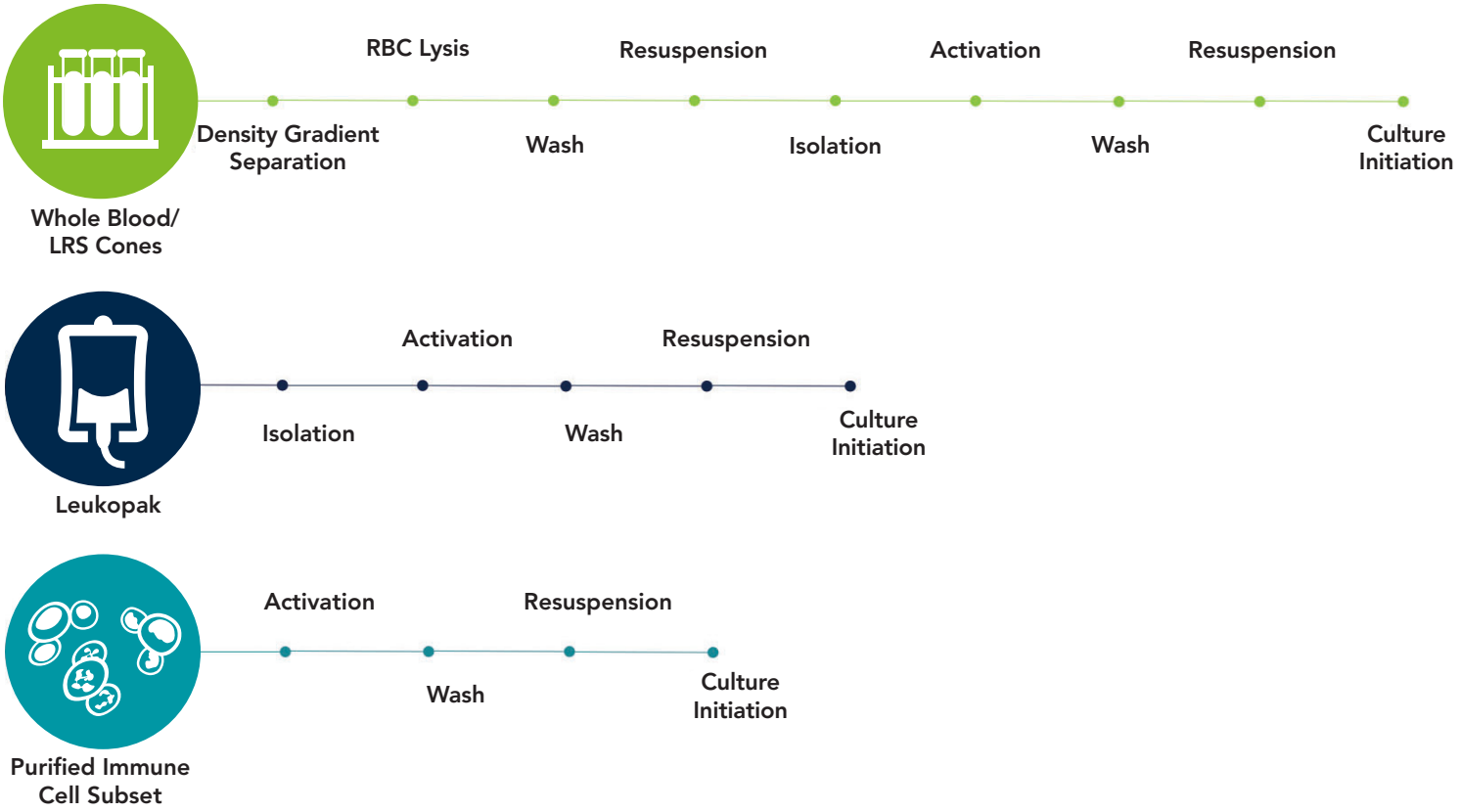
Once PBMCs are isolated, typical cell therapy processes require downstream activation and expansion. Here, the impetus is to reach a therapeutic dose in the fastest time possible to reduce cost and turnaround time for the patient. It is also in these steps where RBC contamination has the greatest impact.

PBMCs must be properly enumerated to determine the appropriate concentration of activation and isolation reagents. Calculating cell density is also a critical determinant for the long-term health of a cell culture. According to Fesnak et al., "Red blood cells and platelets can make accurate lymphocyte enumeration difficult and/or confound flow cytometry."[1] Inaccurate enumeration methods may therefore impact activation kinetics, isolation purity and expansion times if the cell culture is overly diluted.

Expansion must be sustained throughout the duration of a culture, so growth factors such as cytokines are frequently supplemented to cell culture media. In T-cell based therapies, "RBCs suppress T-cell responsiveness through a mechanism requiring cell-cell contact. IL-2 synthesis is suppressed by RBC-exposed T-cells, but addition of exogenous IL-2 does not rescue proliferative capabilities," according to Long et al. [2] This reduced proliferation can negatively impact expansion times, adding to additional days of culture and the associated labor costs therein. Furthermore, IL-2 supplementation may be wasted in completed cell culture mediums, resulting in unused materials.

Open and manual operations to reduce RBC contamination increases the number of operator touchpoints, thus increasing the risk of microbial contamination. It may take several days of culture expansion for these errors to be detected, resulting in wasted supplies and effort. Equivalent RBC depletion methods exist in closed, automated technologies that substantially mitigate microbial contamination. However, the consumables for these devices are often several times more expensive than the price differential between heterogenous and homogenous starting materials.

Number of Touch-points by Starting Material



Upstream Mitigation

While the temptation exists to source PBMCs from heterogenous cell populations such as whole blood or LRS cones, any cost savings may quickly disappear due to contamination.

Leukapheresis products have long been the gold-standard for starting material within the cell therapy field. However, for R&D applications, isolated immune cell subsets can reduce your processing time and guarantee a high-level of starting material consistency.

Visit **BioIVT.com** for additional resources including protocols, on-demand webinars, how-to videos and more.

Videos

Webinars

Blogs

References

1. Fesnak, A., Lin, C., Siegel, D. L., & Maus, M. V. (2016). CAR-T Cell Therapies From the Transfusion Medicine Perspective. Transfusion medicine reviews, 30(3), 139–145. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4914456/.
2. Long, K., Meier, C., Bernard, A., Williams, D., Davenport, D., & Woodward, J. (2013). T-cell suppression by red blood cells is dependent on intact cells and is a consequence of blood bank processing. Transfusion, 54(5), 1340–1347. https://onlinelibrary.wiley.com/doi/epdf/10.1111/trf.12472

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