A NOVEL WHOLE BLOOD CRYOPRESERVATION METHOD FOR FLOW CYTOMETRY BASED LEUCOCYTE IMMUNOPHENOTYPING

Xiaochen Guo, QinLei Gu, Mingdong Fu, Chengyu Li, Ziqiang Hou, Rongrong Zhai, Xuemei Zhang, Bo Wei, Jinpeng Li, Xiaoying Jin, Lan Li
WuXi AppTec, 1336 WuZhong Avenue, Suzhou, Jiangsu, China

Poster Number: Day2-P25
Want a copy? Questions?

NOVEL ASPECTS

Cryopreservation of cynomolgus monkey and mini pig whole blood fit the "3R" principle, as this practice refines the use of leftover blood from single blood draw and reduces the frequency of blood draw for reanalysis and regular immunophenotyping (IPT) analysis.

In some cases, whole blood sample from the exact animals are preferred but no longer available due to experimental design at the time of retrospect analysis. This method preserves the blood and allows direct comparison of new samples with retrospect samples with the potential for multi-site whole blood flow analysis.

INTRODUCTION

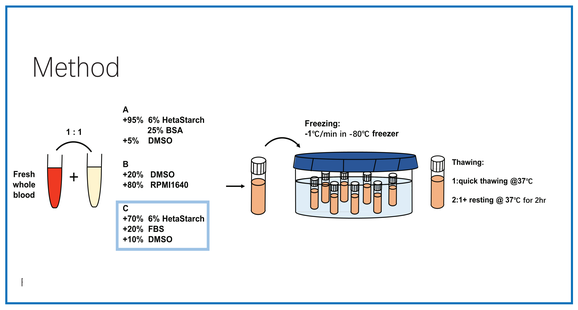
Accurate characterization of whole blood requires sample to be acquired <24hr after blood collection [1]. However, fresh whole blood samples are not always readily accessible. The '3R' principle also encourages the refined use of leftover blood and reduced frequency of blood draw. Although reliable results have been reported from frozen peripheral blood mononuclear cells (PBMC) in multi-center studies [2], PBMC isolation process is time consuming and limited by the access to centrifuge equipment and trained staff members when processing large cohorts of whole blood samples. Whole blood cryopreservation method has been studied in the past [3-5]. However, most of them focused on human whole blood preservation.

In this study, we aimed to develop one practical whole blood cryopreservation method for cynomolgus monkeys and hopefully will also work for minipigs, beagle dogs and rats, animal species commonly used for IPT analysis in our bioanalysis facility.

METHOD

Stained samples were treated with FACS lysing solution (BD Biosciences) according to the manufacture's protocol to remove RBCs. IPT analysis were performed on BD LSRFortessa™ at about 48hrs, 7days, 14days and 1 month post frozen. Data were analyzed with Diva software (BD).

Figure 1. Schematic representation of attempted frozen and thawing method.



RESULTS

Cynomolgus monkeys, minipigs and beagle dogs showed ~90% viability after cryopreservation. Representative data showed gating strategy for cynomolgus monkeys, minipigs and beagle dogs subpopulations

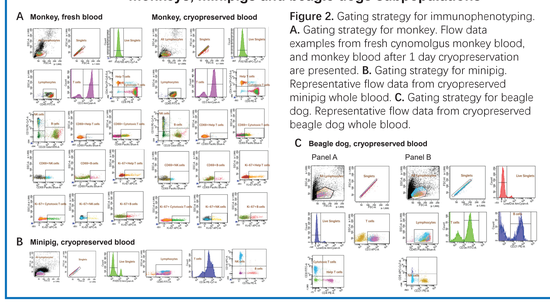


Figure 2. Gating strategy for immunophenotyping. **A.** Gating strategy for monkey. Flow data examples from fresh cynomolgus monkey blood, and monkey blood after 1 day cryopreservation are presented. **B.** Gating strategy for minipig. Representative flow data from cryopreserved minipig whole blood. **C.** Gating strategy for beagle dog. Representative flow data from cryopreserved beagle dog whole blood.

The combination of formula C (recipe see Figure 1) + quick thawing steps provides better stability after whole blood recovery.

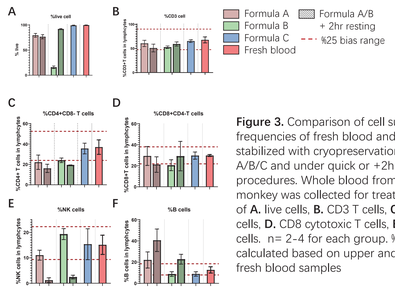


Figure 3. Comparison of cell subpopulation frequencies of fresh blood and blood samples stabilized with cryopreservation reagent formula A/B/C and under quick or +2hr resting thawing procedures. Whole blood from cynomolgus monkey was collected for treatment. Percentages of **A.** live cells, **B.** CD3 T cells, **C.** CD4 Helper T cells, **D.** CD8 cytotoxic T cells, **E.** NK cells, and **F.** B cells. n= 2-4 for each group. %25 bias were calculated based on upper and lower limit of fresh blood samples

Preserved rat blood showed inconsistent cell viability from different runs. Cell coagulation was observed during run1 and run3.

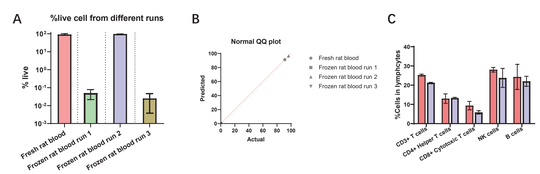


Figure 5. Cell frequencies of fresh and cryopreserved rat whole blood. Cryopreserved rat whole blood was stored at -80℃ for 2 days. Percentages of **A.** live cells from different runs. **B.** QQ plot for different runs. **C.** CD3+T cells, NK cells and B cells from rat were presented. CD45+ lymphocytes gate was used as parental gate. n=2-4 for each group.

Cryopreserved cynomolgus monkey blood showed stable T/B/NK cell population after 36-day storage at -80ºC. Ki-67 population were not preserved after freezing and thawing.

However, percentages of smaller population, such as activated T/B/NK cells was no longer reliable when stored for 24 days or longer.

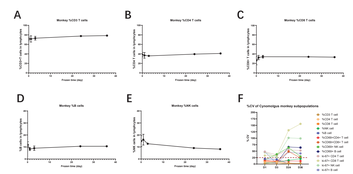


Figure 7. Cell frequencies of fresh and cryopreserved cynomolgus monkey whole blood. CD45+ lymphocytes gate was used as parental gate. Cryopreserved cynomolgus whole blood was stored at -80℃ for 1, 3, 24 and 30 days. Percentages of **A.** CD3+ T cells, **B.** CD4+T cells. **C.** CD8+T cells, **D.** CD16-CD20+ B cells, **E.** CD16+CD20- NK cells and **F.** %CV of **A.-E.** , CD69+ helper/ cytotoxic T/ B/ NK cells, and ki-67 helper/ cytotoxic T/ B/ NK cells were presented. Red line suggests 25% CV. n= 1-4 for each group

Cryopreserved minipig blood showed reduced subpopulation stability after 2-week storage in a -80ºC freezer.

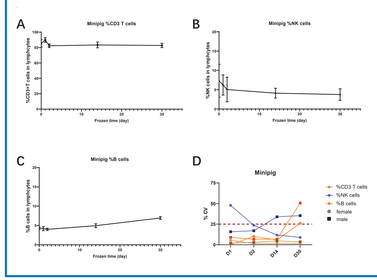


Figure 4. Cell frequencies of fresh and cryopreserved minipig whole blood. CD45+ lymphocytes gate was used as parental gate. Cryopreserved minipig whole blood was stored at -80℃ for 1, 2, 14 and 30 days. Percentages of **A.** CD3+ T cells. **B.** CD16+CD79a- NK cells. **C.** CD16-CD79a+ B cells. **D.** %CV of CD3 T cells, NK cells and B cells from male and female minipig were presented. Red line suggests 25% CV. n= 2-4 for each group

Cryopreserved beagle dog blood showed reduced B cell percentage after short storage at -80ºC

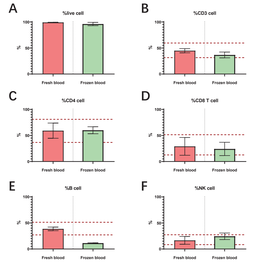


Figure 6. Cell frequencies of fresh and cryopreserved beagle dog whole blood. Cryopreserved beagle dog whole blood was stored at -80℃ for 2 days. Percentages of **A.** live cells, **B.** CD3+ T cells. **C.** CD4+T cells, **D.** CD8+ T cells, **E.** CD21+ B cells and **F.** CD5-CD21- NK cells were presented. For **B.** & **E.-F.** , CD45+ lymphocytes gate was used as parental gate. For **C.** & **D.** CD3+ T cells gate was used as parental gate. n=2-4 for each group. ±%25 bias (red line) were calculated based on upper and lower limit of fresh blood samples.

CONCLUSION

- Monkeys, minipigs and beagle dogs showed ~90% viability after cryopreservation.
- Cryopreserved beagle dog blood showed reduced B cell percentage after short storage at -80° C.
- The combination of formula C (recipe see Figure 1) + quick thawing steps provides better stability after whole blood recovery.
- Cryopreserved minipig blood showed reduced subpopulation stability after 2-week storage in a -80° C freezer.
- Preserved rat blood showed inconsistent cell viability from different runs, due to cell coagulation after thawing.
- Cryopreserved cynomolgus monkey blood showed stable T/B/NK cell population after 36-day storage at -80° C. Ki-67 population were not preserved after freezing and thawing.
- However, percentages of smaller population, such as activated T/B/NK cells was no longer reliable when stored for 24 days or longer.

REFERENCES

[1] Diks et al. (2019). Impact of blood storage and sample handling on quality of high dimensional flow cytometric data in multicenter clinical research. Journal of Immunological Methods, 475, 112616.
[2] Finak et al. (2016). Standardizing Flow Cytometry Immunophenotyping Analysis from the Human ImmunoPhenotyping Consortium. Scientific Reports, 6(1), 20686.
[3] Braudeau et al. (2021). An easy and reliable whole blood freezing method for flow cytometry immuno-phenotyping and functional analyses. Cytometry Part B: Clinical Cytometry, 100(6), 652–665 .
[4] Nguyen et al. (2023). A comprehensive assessment of four whole blood stabilizers for flow-cytometric analysis of leukocyte populations. Cytometry Part A, 103(4), 313–324.
[5] Serra et al. (2022). Comparison of Whole Blood Cryopreservation Methods for Extensive Flow Cytometry Immunophenotyping. Cells, 11(9), 1527.

药明康德
WuXi AppTec