

Abstract

Peripheral blood mononuclear cells (PBMCs) are widely used in biomedical research to support pharmacokinetic/pharmacodynamic endpoints in drug discovery and development. However, PBMC isolation from non-human primates (NHP) can be problematic. The variation of mean corpuscular volume (MCV) in red blood cells, the time taken, and the skills of the technician to avoid contamination of red blood cells can all affect PBMCs isolation. In this study, we compared two different methods of PBMC isolation: 50mL tubes with density gradient insert with 90% density gradient and standard 50 mL tubes with 90% density gradient, both using whole blood collected from eight female *Macaca fascicularis*, between 8-11 and 14-21 years old. Using these two methods in a series of experiments, cell viability and cell recovery were measured using an automated cell counter and cell purity was measured using a hematology analyzer. A lipid profile was performed on all samples using a chemistry analyzer to investigate a possible relationship between the amount of lipid in samples and higher PBMC contamination. Statistical differences in parameter measured in PBMCs between methods were determined using a paired two-tailed Student test. Results showed that cell viability (P value= 0.02) and cell recovery (P value = 0.01) were remarkably better with 50mL tubes with density gradient insert than with standard 50 mL tubes. Red blood cell contamination of the final PBMC preparation was significantly reduced; however, the removal of platelets and reticulocyte were similar between both methods. Moreover, blood samples that had above normal total protein showed higher PBMC contamination with red blood cells in both 50mL tubes with density gradient insert and standard 50mL tubes. Blood samples that had above normal total protein also showed lower PBMC contamination with reticulocytes using the standard 50mL tube isolation method and higher contamination with reticulocyte using the 50mL tubes with density gradient insert method. This needs further investigation. The results from these experiments indicate that the 50mL tubes with density gradient insert method offered several advantages over the standard 50mL tube isolation method.

Introduction

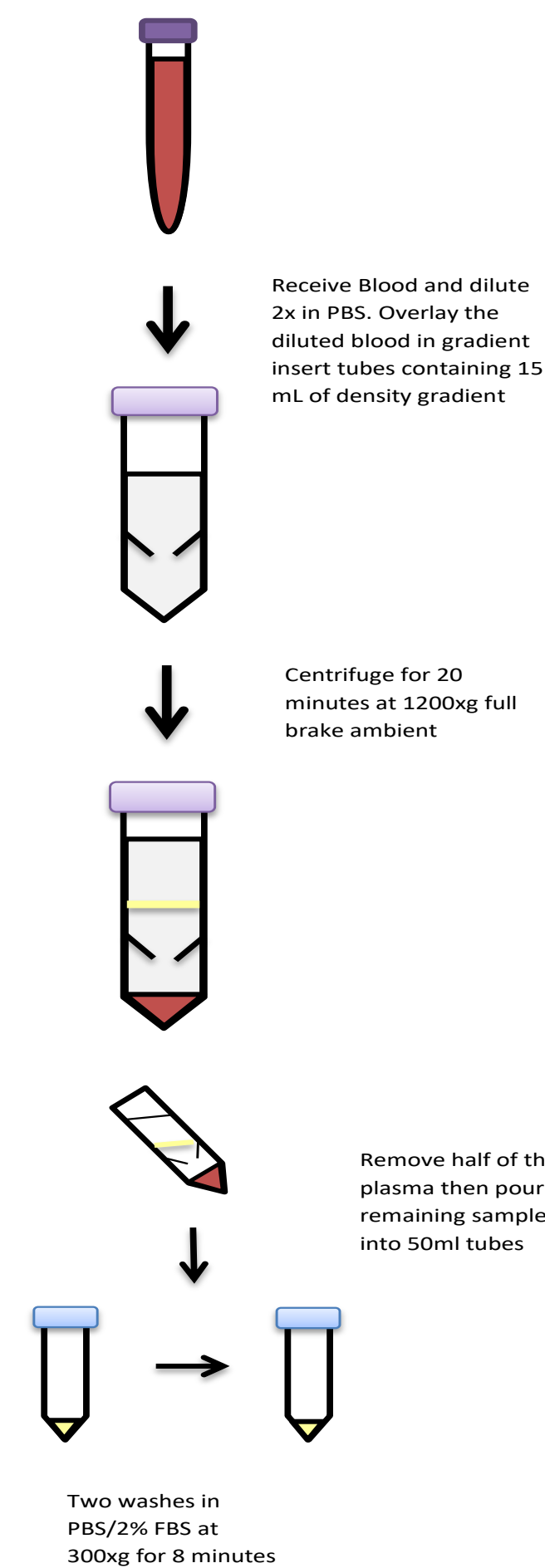
Non-human primates are extensively used in biomedical research because of their genetic, physiological, behavioral, and neurological similarities to humans. *Macaca fascicularis* are used as animal models for drug discovery, development, and toxicology/safety. Moreover, these animals are used for vaccine development for HIV/AIDS based on immunological similarity to humans. Finally, non-human primates have been used for the study of hepatitis B virus as the virus is naturally occurring and transmissible only in this population of macaques and because they develop a similar immune response observed in humans. PBMCs are used in studies for pharmacokinetics/pharmacodynamics, RNA sequencing, flow cytometry and other assays that provide information for the creation of new therapies or improve existing ones. In this study we have used whole blood collected from *Macaca fascicularis* and isolated PBMCs by two different common methods to conclude which would be more advantageous in biomedical research.

Objective

- Measure parameters to assess the best method of isolation
- Overcome the differences in concentration of density gradient among cynomolgus monkeys (*M. fascicularis*)
- Select a method that optimizes purity, viability, recovery, and throughput for standardized use on future studies

Methods

Tube with Density Gradient insert



Standard 50mL Conical Tube

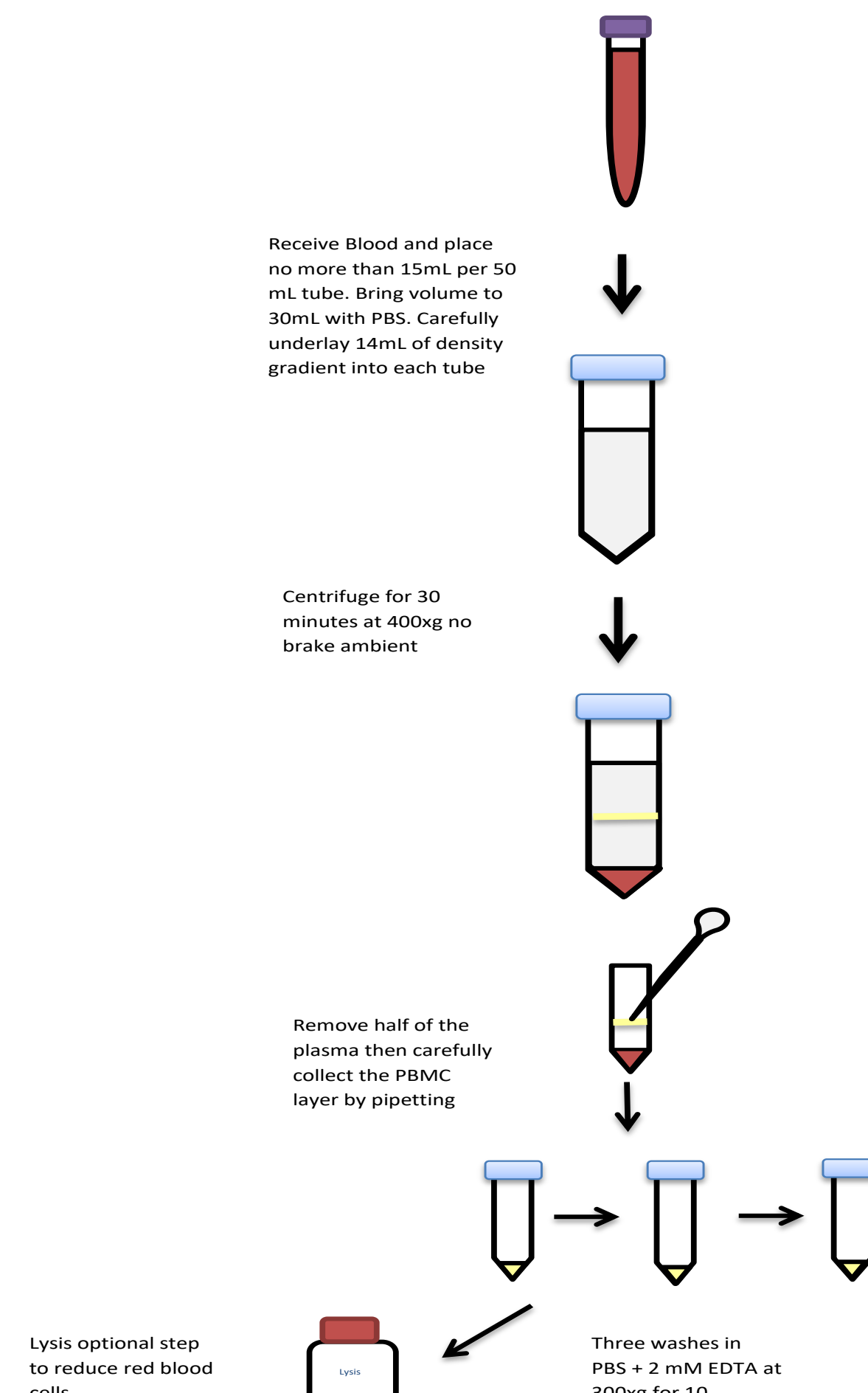
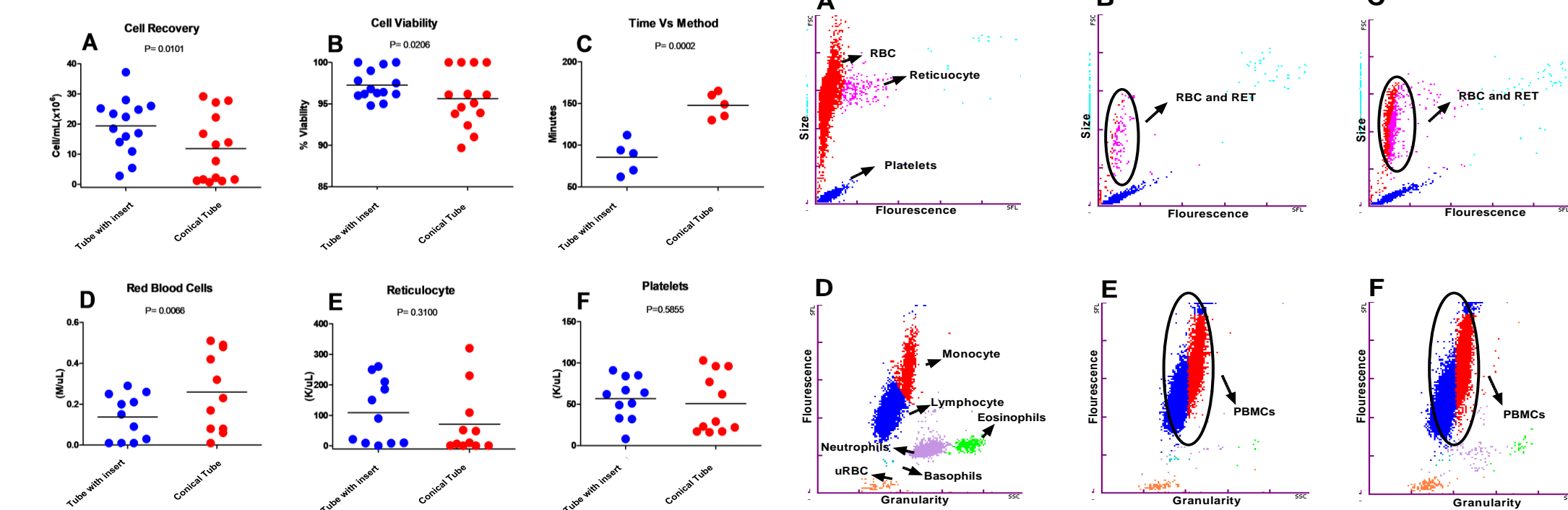


Figure 1. Comparison of two methods of PBMC isolation from whole blood from cynomolgus monkeys (*Macaca fascicularis*).

Results



Graph. 2. A. Cell recovery between methods B. Cell viability between methods C. Time used to isolated PBMCs D. Red blood cell removal from sample E. Reticulocyte removal from sample F. Platelets removal from sample

Figure 3. A. Cell blood count before PBMC isolation B. Cell blood count after isolation using the tubes with density gradient insert C. Cell blood count after isolation using conical tubes D. White blood cells before isolation E. White blood cell after isolation using tubes with density gradient insert F. White blood cells after isolation using conical tubes

Conclusions

This data demonstrates that 50mL tubes with density gradient insert with 90% density gradient offers advantages on cell viability, cell recovery, red blood cell purity, and time.

References

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