# Isolating Human Peripheral Blood Mononuclear Cells from Buffy Coats *via* High Throughput Immunomagnetic Bead Separation

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## Abstract

Peripheral blood mononuclear cells (PBMCs) are a heterogeneous population of monocytes and lymphocytes. Cryopreserved PBMCs have stable viability in long-term storage, making them an ideal cell type for many downstream research purposes, including flow cytometry, immunoassays, and genome sequencing. Typically, PBMCs are isolated *via* density gradient centrifugation, however, it is a low-throughput workflow that is difficult and costly to scale. This article presents a high-throughput workflow using a magnetic bead-based PBMC isolation method that is quick to implement. Total cell concentration, viability, and population distribution with PBMCs obtained using density gradient isolation were compared, and cell viability and proportion of cell types were comparable for both techniques. Isolated PBMCs demonstrated over 70% viability up to 9 days after blood collection, although yield decreased by half after 5 days compared to PBMCs processed within 24 h of collection. In summary, this article describes a PBMC protocol that utilizes a bead-based approach to adapt to a high throughput workflow and demonstrates that both manual and automated bead-based methods can increase processing capacity and provide flexibility for various budgets.

## Introduction

Peripheral blood mononuclear cell (PBMC) isolation is a technique that separates and isolates lymphocytes and monocytes from other whole blood constituents. PBMCs are a versatile cell type used for numerous applications including, but not limited to, immunotherapy, vaccine development, target or biomarker identification, and antibody/ small molecule drug development[1,2]. These cells may be isolated from healthy or diseased individuals and can be used immediately in downstream processes or cryopreserved for future research[3]. In some instances, the downstream purpose is known, while in others, as is common in biobanking, PBMCs are isolated and stored for future unspecified applications[4].

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Density gradient centrifugation is the traditional technique for isolating PBMCs[5,6,7] from whole blood, utilizing the differential separation of the constituent cell types based on cell density during centrifugation. While there may be some variation in this method, whole blood is generally diluted with phosphate-buffered saline (PBS), layered over a density gradient medium in a specialized or standard centrifuge tube, and then spun. Four distinct layers result: the top plasma layer is enriched with platelets, a thin PBMC layer is above the density gradient medium, and finally, the bottom layer consists of red blood cells (RBCs) and granulocytes. Although this method has previously been termed 'the gold standard'[8], there are limitations for scale-up, such as lengthy processing time, centrifuge capacity, difficulty in aliquoting other blood products (i.e., plasma and RBCs), and being laborious to automate. While automation is possible for this method[9], it does require comprehensive programming of a liquid handler (with a centrifugation module to be fully automated) and would remain a lengthy process.

Henceforth, an alternate workflow that uses immunomagnetic bead separation with either an eight-holder magnet for manual processing or an instrument for fully automated processing is presented. This method utilizes an antibody cocktail that is added to cells and binds unwanted cell populations, in this case, platelets, granulocytes, and RBCs. These unwanted populations are subsequently removed by magnetic separation, leaving monocyte and lymphocyte populations in the negative fraction that are ready for downstream processing[10]. This negative selection method is faster than positive selection methods which require additional steps to remove the antibody and magnetic bead complex from the PBMCs. Negative selection is additionally advantageous as it has been described as a way to preserve cell functionality[11,12].

## Protocol

Quality control blood specimens and in-house generated data (such as cell count, viability and age of specimen at processing) were obtained from consented research studies at the NSW Health Statewide Biobank, Royal Prince Alfred Hospital (HREC approval: 2019/ETH06776). Processed (i.e., plasma depleted), unscreened, and de-identified adult human blood samples were collected in EDTA tubes. These quality control blood specimens were processed less than 72 h after collection and were used for PBMC isolation experiments comparing the density gradient and bead-based methods. For the density gradient separation method used in the representative results, see the procedure in **Supplementary File 1**. The details of the reagents and the equipment used for this study are listed in the **Table of Materials**.

### 1. Whole blood preparation

1. Gently invert 10 mL whole blood tubes (coated with ethylenediaminetetraacetic acid [EDTA], acid-citrate-dextrose [ACD], or lithium heparin) 10 times at room temperature.
2. Optional: If whole blood is to be stored, aliquot into cryotubes for -80 °C storage.
3. Centrifuge the tubes using a swinging-bucket rotor at 800 *x g* for 10 min at 22 °C with the brake ON (9 acceleration/ 9 deceleration).

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## 2. Buffy coat collection

1. After centrifugation, place the samples in a biological safety cabinet and check for the three distinct layers (as shown in **Figure 1**).
   NOTE: RBCs are found in the lower dark red layer of the centrifuge tube. A white opaque layer containing white blood cells and platelets is located above the RBC layer, known as the buffy coat. The top yellow layer contains plasma.
2. Optional: If plasma is to be stored, aliquot plasma into cryotubes for -80 °C storage.
3. Pipette 1 mL of buffy coat (starting material) from a 10 mL blood tube and transfer to the tube specified and labeled in step 3.1 and step 4.1 for the manual and automated methods, respectively. Swirl the buffy coat (white opaque layer) using the pipette tip and collect the buffy coat by aspirating.
   NOTE: Less than 100 µL of plasma and RBCs are acceptable during aspiration. If multiple blood tubes are collected for one participant, buffy coats can be pooled; however, this may affect platelet concentration[13].
4. For manual processing, proceed to section 3. For automated processing, proceed to section 4.
5. Optional: If RBCs are to be stored, aliquot RBCs into cryotubes for -80 °C storage.

## 3. PBMC purification - Manual method

NOTE: Up to 8 samples per magnet holder can be processed by a single operator.

1. Label 3 x 5 mL polystyrene tubes with letters A-C.
   NOTE: Use sequential numbering if performing more than one sample, i.e., 1A, 1B, 1C, 2A, 2B, 2C.
2. Add 60 µL of 0.1 M EDTA (for a final EDTA concentration of 6 mM, pH 8.0, see **Supplementary File 2** for the recipe) to tube A containing the buffy coat transferred in step 2.3.
3. Add 50 µL of the cocktail mix tube (see **Table of Materials**) to tube A, mix by pipetting up and down at least 3 times, and incubate for 5 min at room temperature.
4. Add 890 µL of PBS to tube A and mix by pipetting up and down at least 3 times.
5. Vortex the magnetic bead tube (see **Table of Materials**) for 30 s.
6. Add 50 µL of the magnetic beads to tube A and mix by pipetting up and down at least 3 times.
7. Immediately place tube A into the magnet stand and incubate for 5 min at room temperature.
8. Carefully pipette the enriched cell suspension into tube B, collecting the clear fraction with no/minimal (<100 µL) RBCs for optimal PBMC recovery.
   NOTE: Do not disturb the beads bound to the magnet. A transfer pipette is recommended.
9. Remove tube A from the magnet stand and dispose.
10. Add 50 µL magnetic beads to cell suspension in tube B and mix by pipetting up and down at least 3 times.
11. Immediately place tube B into the magnet stand and incubate for 5 min at room temperature.
12. Carefully pipette the enriched cell suspension into tube C, collecting only the clear fraction.
    NOTE: Do not disturb the beads bound to the magnet. A transfer pipette is recommended.

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13. Remove tube B from the magnet stand and dispose.
14. Immediately place tube C into the magnet stand and incubate for 5 min at room temperature.
15. Carefully pipette the enriched cell suspension into a labeled centrifuge tube and top up to 2 mL with PBS.
16. Transfer 50 µL of the cell suspension to a sample cup of the automated cell counter. For a 1:10 dilution cell count, add 450 µL of PBS. Proceed to section 5 for cell counting steps.

## 4. PBMC purification - Automated method

NOTE*:* Up to 4 samples can be processed by a single automated PBMC instrument. Multiple instruments can be run in parallel by a single operator.

1. Label 2 x 14 mL tubes with letters A and B, 1 x 50 mL centrifuge tube with letter C, and 1 x 50 mL centrifuge tube with "waste".
   NOTE: Only one waste container is required for every run on the automated PBMC instrument. Use sequential numbering if performing more than one sample, i.e., 1A, 1B, 1C, 2A, 2B, 2C.
2. Add 60 µL of 0.1 M EDTA (for a final EDTA concentration of 6 mM) to tube A containing the buffy coat transferred in step 2.3.
3. Vortex the magnetic bead tube (see **Table of Materials**) for 30 s.
4. Turn on the automated PBMC instrument by switching the power on at the front of the instrument.
5. On the automated PBMC instrument home screen, select **profile** and select the desired protocol.
   NOTE: **EasySep Direct Human PBMC isolation 19654 - buffy coat** was the protocol profile selected for this PBMC isolation. See the manufacture's standard procedure for this profile in protocol report[14].
6. Enter the starting volume (the amount of buffy coat aspirated into tube A), and repeat for each sample.
7. Select all the quadrants that will use the same reagent kit.
   NOTE: If the same protocol is used for more than one quadrant, the automated PBMC instrument will suggest using the same reagent kit for all quadrants[14].
8. Load the instrument's carousel with labeled consumables, filter tips and buffer container. The reagent kit containing the magnetic bead tube and cocktail mix tube are to be loaded into quadrant 1.
   NOTE: The instrument will ask if the user wishes to use 1 reagent kit for all quadrants. Select 'yes' and highlight all quadrants using the reagent kit.
9. Once loading is complete, remove lids from consumables and reagents and select run on the instrument screen.
10. When the run is complete, select **unload** and remove samples (containing PBMC suspension labeled in tube C) from the instrument's carousel. Store the magnetic bead tube, cocktail mix tube, and buffer container at 4 °C. Discard tubes labeled as A, B, and waste (see step 4.1) and filter tips.
    NOTE: No top-up with PBS is necessary for the automated method, as the PBMC suspension produces a final volume of 7 mL.
11. Transfer 500 µL of the cells to a sample cup for a no-dilution cell count. Proceed to section 5 for cell counting.

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### 5. Cell counting

1. Perform cell counting and viability using the trypan blue dye exclusion method following the manufacturer's instructions[15].
   NOTE: For the author's internal processes, cell analysis is performed using an automated cell counter with the following settings to acquire the PBMCs.
   Dilution factor = 10 (for manual protocol, if cell suspension is in a low volume) or 1 (for automated protocol)
   Cell type: PBMC
   Concentration range = 5 x $10^4$ to 1 x $10^7$ cells/mL
   Size range (diameter) = 8 µm to 50 µm
   Number of Images = 50

### 6. Cryopreservation preparation

1. Centrifuge (using a swinging bucket rotor) the sample tubes at 300 *x g* for 8 min at 22 °C with the brake ON (9 acceleration/ 9 deceleration).
2. After centrifugation, place the samples back in the biological safety cabinet.
3. Using a pipette, carefully remove the supernatant. A small amount of supernatant can be left with the PBMC pellet to ensure it is not disturbed.
4. Resuspend the pellet in 3 mL of cold cryopreservation medium. Slowly and gently mix the suspension up and down until homogenous.
   NOTE: The volume of the cyropreservation medium can be adjusted based on the desired final PBMC concentration.
5. Dispense 1 mL of the resuspended PBMC into a pre-assigned cryotube and place it inside a control rate freezing container for a minimum of 4 h at -80 °C.
   NOTE: The volume of PBMCs aliquoted to each cryotube can be adjusted based on the investigator's preference.
6. After storing cryotubes at -80 °C for a minimum of 4 h, transfer the cryotubes to a liquid nitrogen vapor phase tank for long-term storage.

### 7. Statistical data analysis

1. Representative result data was analyzed using statistical and graphing software as specified in **Supplementary File 3**.

## Representative Results

The proportions of lymphocytes, monocytes, neutrophils, eosinophils, and basophils pre- (i.e., in whole blood) and post-PBMC isolation were measured when PBMCs were isolated using the bead-based or density gradient separation method. Proportions of lymphocytes and monocytes were significantly enriched by both methods (**Figure 2A,B**). Additionally, proportions of neutrophils, eosinophils, and basophils (i.e., granulocytes) were significantly decreased in PBMCs isolated by the bead-based method (**Figure 2C-F**). There were no significant differences in the proportions of lymphocytes, monocytes, neutrophils, eosinophils, basophils, and granulocytes between the density gradient and bead-based methods (**Figure 2**). Percentage recovery was additionally calculated for the cell types listed above (see **Supplementary Figure 1**). Mean cell viabilities were over 95% for both methods, and were not significantly different (**Figure 3A**). PBMCs were also counted using a size range (diameter) of 8 µm to 50 µm, where the density gradient

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separation method produced approximately two times higher total cell counts (**Figure 3B**).

Next, the automated bead-based method was compared to the manual method. No significant differences were identified for cell viability (**Figure 4A**) or total cell counts (**Figure 4B**) using the manual or automated method. The time to process these 8 samples was compared, including hands-off time requiring no human intervention. The total processing time of 8 samples was 43 min *vs*. 57 min for the manual *vs*. the automated method. The automated method included 35 min of hands-off processing (**Figure 4C**).

The bead-based workflow of PBMC isolation (with whole blood and plasma aliquoting) was piloted over 9 months, whereby 1410 PBMC samples were isolated from unscreened human blood using the manual platform for the first 820 participants and then using the automated method for the next 590 participants. Processing was performed either on the same day or up to 10 days post-collection as per the study acceptance criteria, with delays in processing largely due to sample transit times. With either the manual or automated method, PBMCs processed within 24 h of the collection had the highest viability and recovery (**Figure 5**). Mean cell viability was >90% for PBMCs processed within 5 days and >70% for PBMCs processed within 10 days (**Figure 5A**). Mean PBMC yields decreased by 50% after 5 days compared to specimens processed within 24 h (**Figure 5B**).

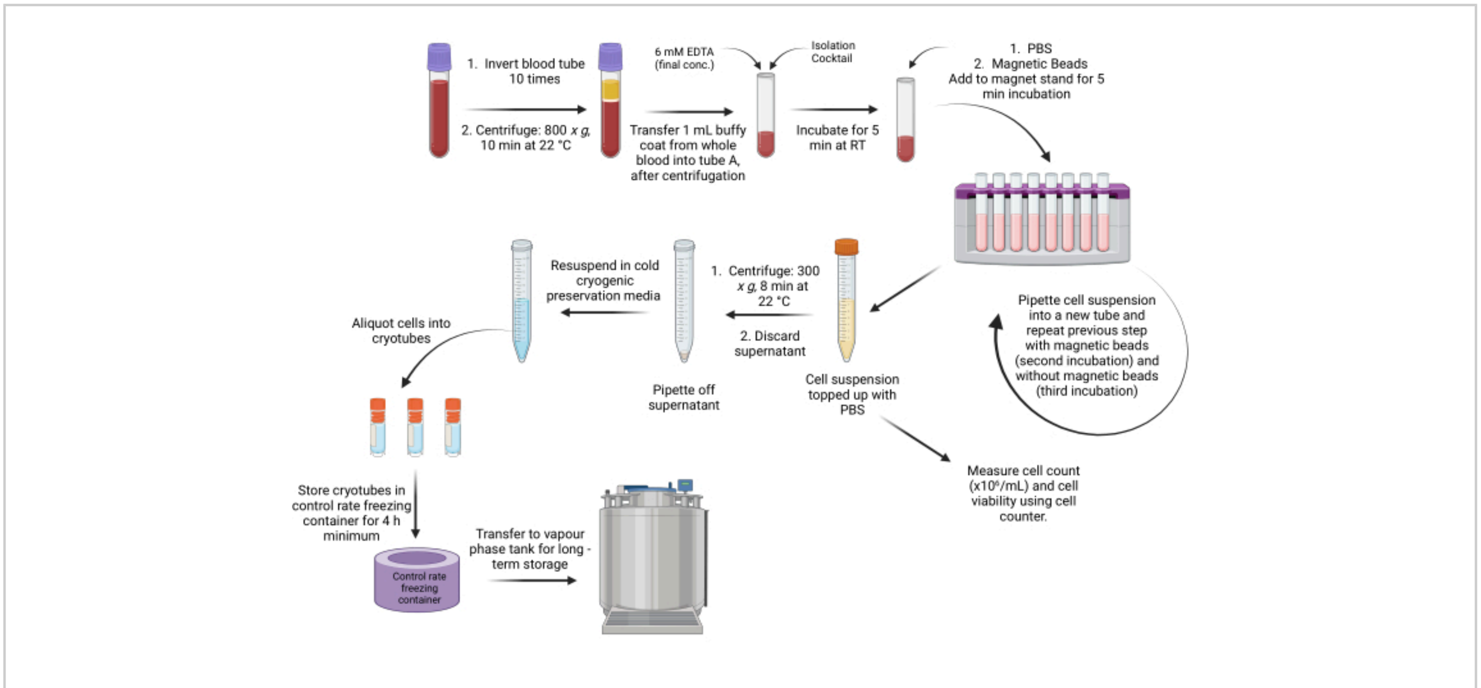


**Figure 1: Schematic workflow of the bead-based PBMC isolation protocol from buffy coat.** Please click here to view a larger version of this figure.

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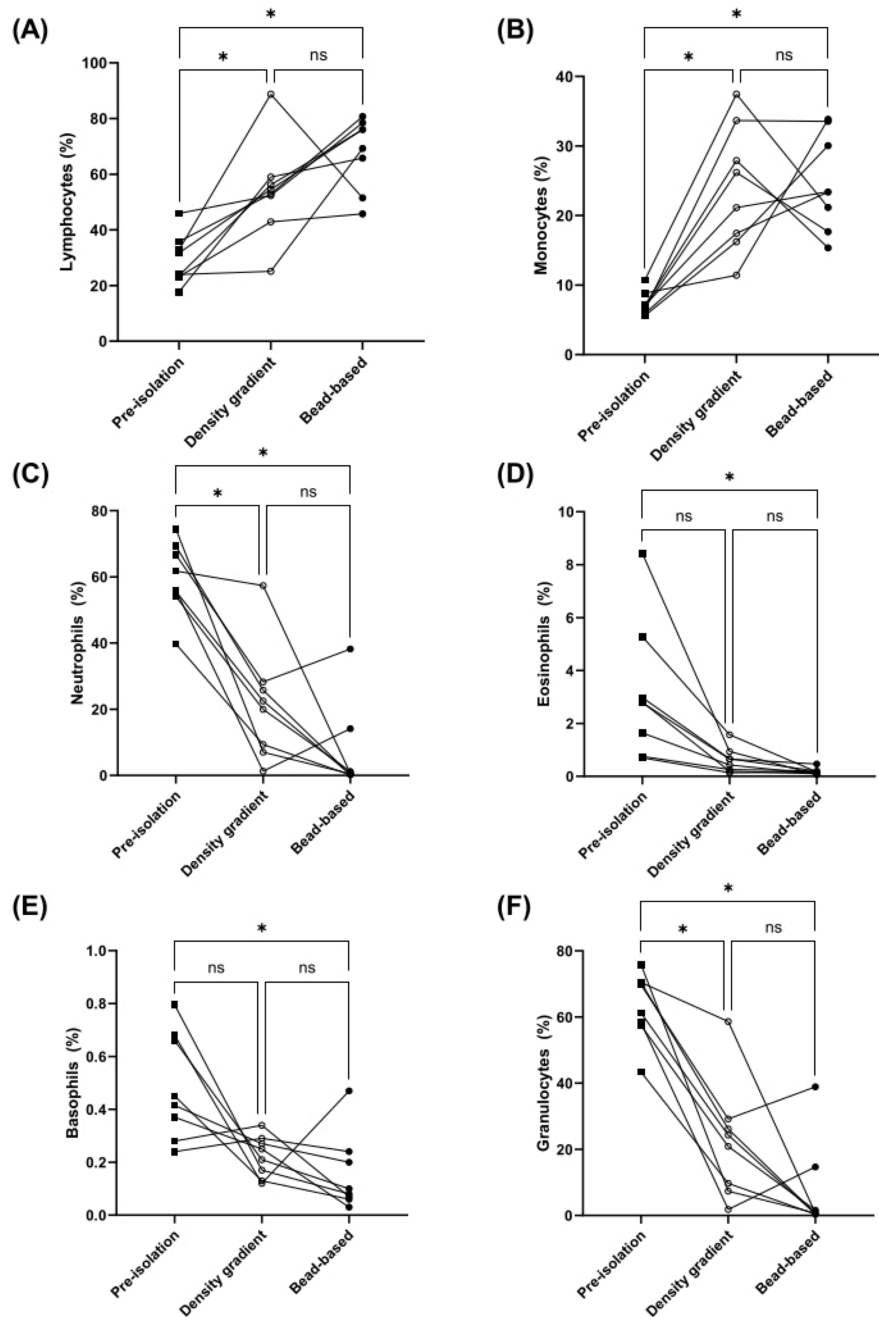


**Figure 2: Proportions of lymphocytes, monocytes, neutrophils, eosinophils, basophils, and granulocytes in whole blood pre- and post-PBMC isolation by density gradient and bead-based methods.** Percentage of lymphocytes (**A**), monocytes (**B**), neutrophils (**C**), eosinophils (**D**), basophils (**E**), and granulocytes (**F**) in matched whole blood pre-PBMC isolation (squares) and in PBMCs post isolation *via* the density gradient (open circles) or bead-based (closed circles) method

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(*n* = 8). ns = not significant, and *$p < 0.05$ as determined by One-Way ANOVA (A and B) or Friedman's test (**C-F**). Please click here to view a larger version of this figure.

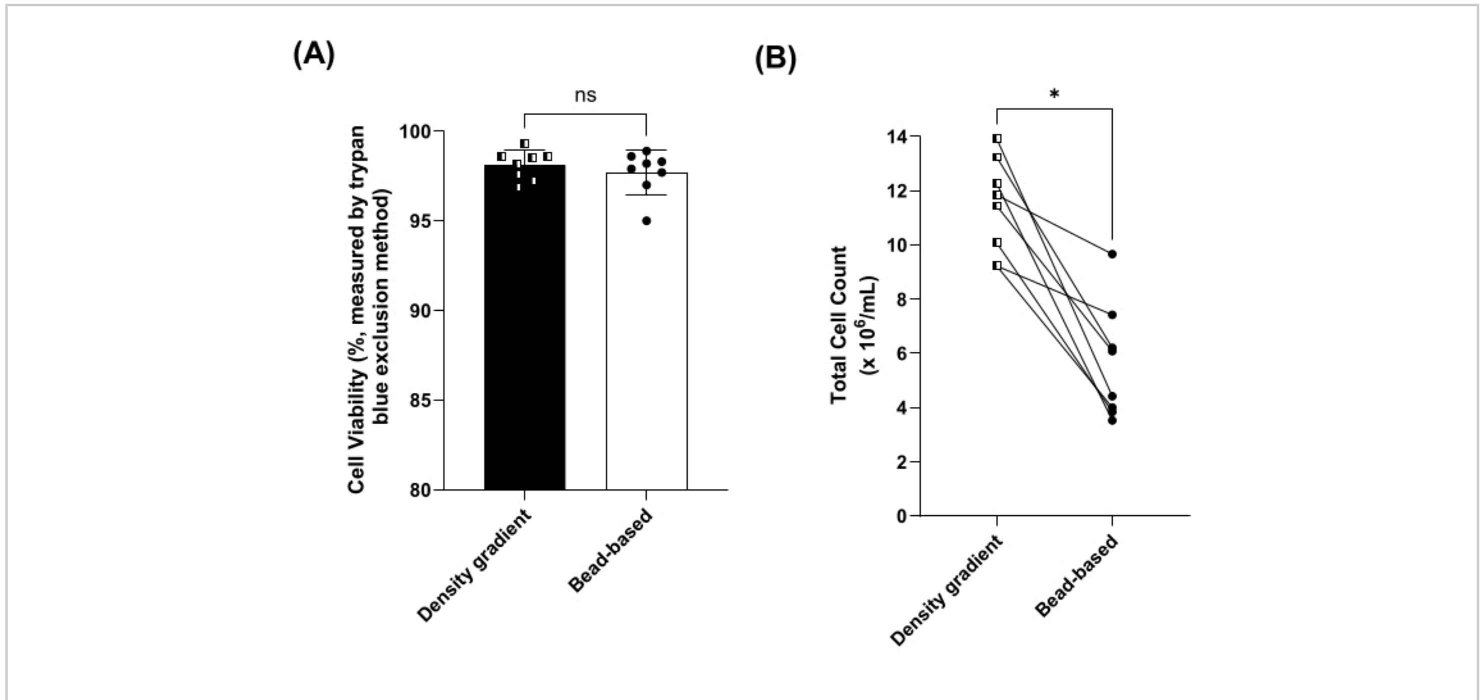


**Figure 3: Viability and total cell count of PBMC isolated by density gradient and bead-based methods.** (**A**) Mean (± SD) cell viability for samples processed using density gradient (squares, dark bar) *vs*. bead-based (circles, open bar) methods for 8 paired samples. ns = not significant by paired *t-test*. (**B**) Total cell count for samples processed using density gradient (squares, dark bar) *vs*. bead-based (circles, open bar) methods for 8 paired samples. *$p < 0.05$ determined using a paired *t-test*. Please click here to view a larger version of this figure.

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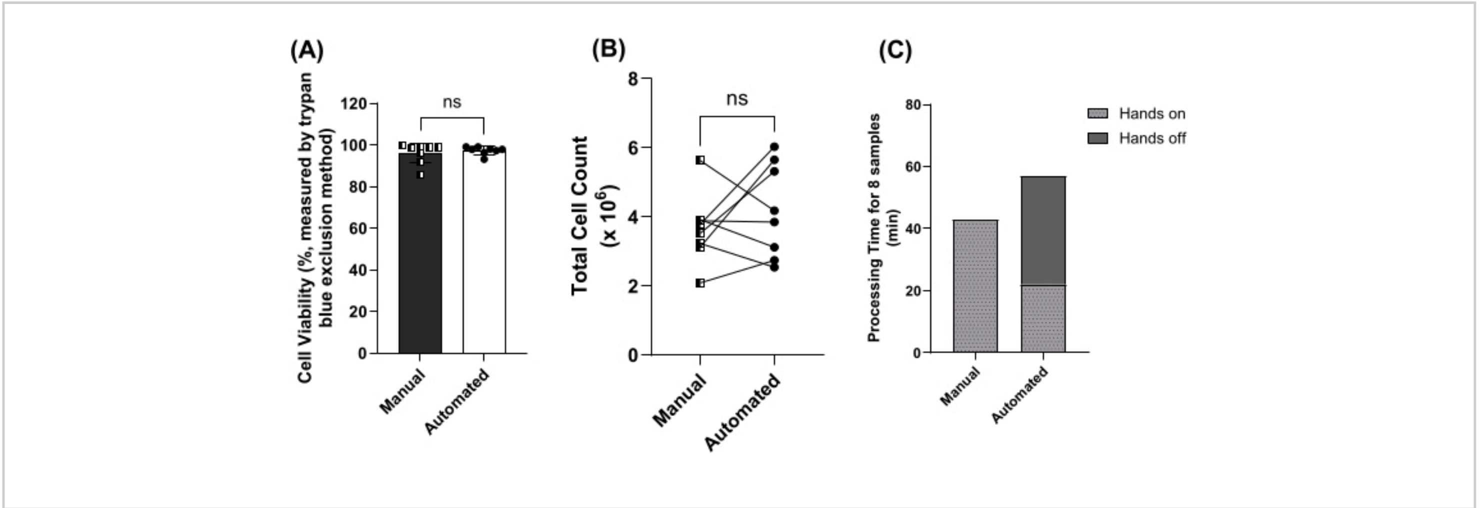


**Figure 4: Comparison of manual and automated bead-based methods.** (**A**) Mean (± SD) cell viability for samples processed using manual (squares, dark bar) *vs*. automated (circles, open bar) bead-based methods for 8 paired samples. ns = not significant by Mann-Whitney test. (**B**) Total cell count for samples processed using manual (squares) *vs*. automated (circles) bead-based methods for 8 paired samples. ns = not significant by paired *t-test*. (**C**) Processing time for 8 samples processed using manual *vs*. automated methods, including hands-on (light bar) and hands-off time periods (dark bar). Please click here to view a larger version of this figure.

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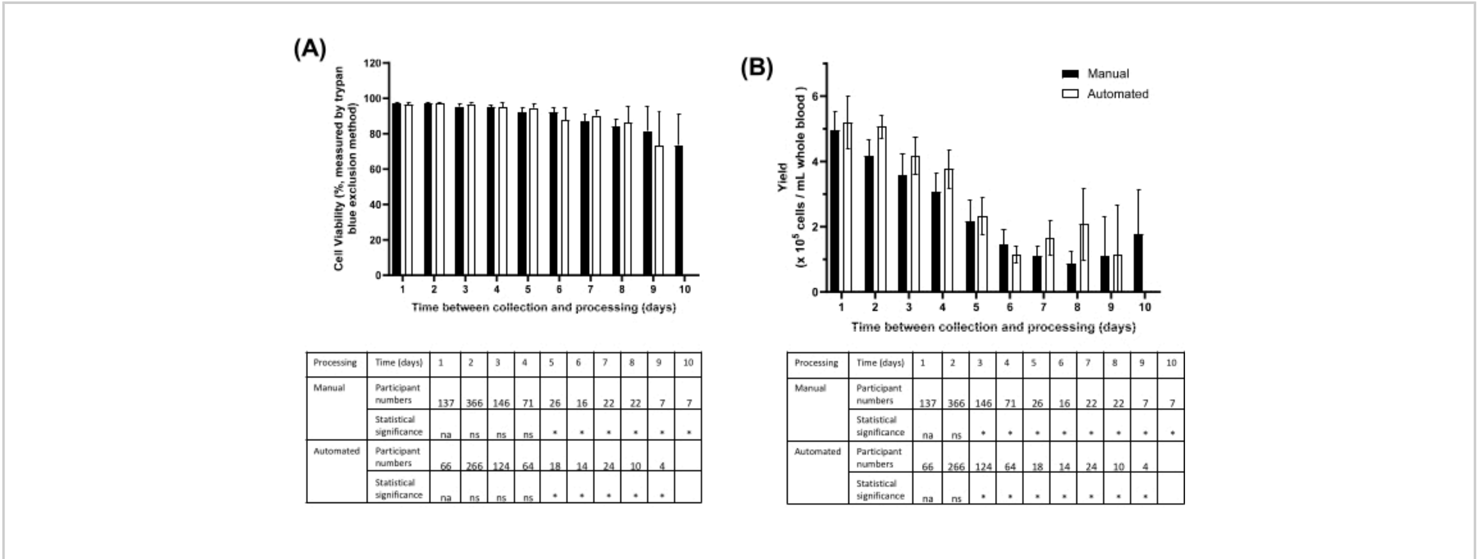


(A)

| Processing | Time (days) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Manual | Participant numbers | 137 | 366 | 146 | 71 | 26 | 16 | 22 | 22 | 7 | 7 |
| | Statistical significance | na | ns | ns | ns | * | * | * | * | * | * |
| Automated | Participant numbers | 66 | 266 | 124 | 64 | 18 | 14 | 24 | 10 | 4 | |
| | Statistical significance | na | ns | ns | ns | * | * | * | * | * | |

(B)

| Processing | Time (days) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Manual | Participant numbers | 137 | 366 | 146 | 71 | 26 | 16 | 22 | 22 | 7 | 7 |
| | Statistical significance | na | ns | * | * | * | * | * | * | * | * |
| Automated | Participant numbers | 66 | 266 | 124 | 64 | 18 | 14 | 24 | 10 | 4 | |
| | Statistical significance | na | ns | * | * | * | * | * | * | * | |

**Figure 5: Viability and yield of PBMC isolated from the bead-based method either manually or using automation according to the time between sample collection and PBMC storage.** (**A**) Mean (± 95% CI) cell viability for participant samples processed using manual (dark bar) *vs*. automated (open bar) bead-based methods. Table detailing participant numbers. ns = not significant. na = not applicable. *$p < 0.05$ by Tukey's multiple comparison's test. (**B**) Total cell count for participant samples processed using manual (dark bar) *vs*. automated (open bar) bead-based methods. Table detailing participant numbers. ns = not significant. na = not applicable. *$p < 0.05$ by Tukey's multiple comparison's test. No value for Automated Day 10 due to the replicate number being lower than 3. Please click here to view a larger version of this figure.

**Supplementary Figure 1: Figure of differential cell percentage recovery with the density gradient and bead-based methods. The percentage was calculated using absolute cell counts pre-isolation in whole blood and post-isolation cell suspension using the density gradient method (squares, dark bar) and bead-based method (circles, open bar) (*n* = 8). ns = not significant, and *$p$ < 0.05 as determined by unpaired *t-test*. Please click here to download this File.**

**Supplementary File 1: Density gradient protocol. Please click here to download this File.**

**Supplementary File 2: Preparation of 0.1 M EDTA, pH 8.0 in PBS. Please click here to download this File.**

**Supplementary File 3: Detailed procedures performed to generate the representative data. Please click here to download this File.**

## Discussion

Human PBMCs are versatile cell types used for numerous assays; however, isolation throughput is often a limitation in many laboratories, including biobanks[16]. Previously, the NSW Health Statewide Biobank isolated PBMCs using the density gradient method. Automation was investigated for the density gradient separation method to increase processing capacity, but barriers to implementation were identified, including (i) the cost of a liquid handler with a centrifugation unit to be fully automated, with the added requirement for a HEPA unit to produce a sterile product, (ii) trained personnel

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for programming, and (iii) time required for protocol testing. Therefore, this study explored alternative methods and identified the commercially obtained Human PBMC kit that could be used for manual and automated processing. Sterility is ensured as the equipment required for processing fits within a standard (length of 1.2 m) biological safety cabinet. This paper details modifications made to the manufacturer's recommended protocol[14], to increase efficiency and lower reagent costs without sacrificing quality. Additionally, the manufacturer's protocol was extended to detail steps to biobank specimens for future research, including whole blood (step 1.2), plasma (step 2.2), and RBC (step 2.5) aliquoting from the original blood tube, as well as cell counting and cryopreservation.

In this study, three PBMC isolation protocols were compared: density gradient separation, manual, and automated bead-based isolation. Modifications to the manufacturer's manual bead-based protocol were made, including the removal of buffy coat dilution prior to PBMC isolation, and elimination of the requirement for break OFF for centrifugations, allowing investigators to follow an adaptable, cost-effective, and high throughput PBMC isolation protocol. First, eight matched whole blood samples were used to isolate PBMCs to compare the density gradient separation and manual bead-based technique. Importantly, cell population distributions, cell viability, and recovery of PBMCs were not significantly different between the two methods compared, as shown in **Figure 2A-F**, **Figure 3A**, and **Supplementary Figure 1**, respectively. In the representative data, cell counts were higher for the density gradient separation method using the trypan blue exclusion method but not when a hematology cell analyzer was used. The PBMC cell type settings on the cell counter employed a cell diameter range of 8-50 µm, and therefore, counts will include PBMCs as well as granulocytes (approximately 12-15 µm in diameter) when using the trypan blue exclusion method[17]. While the hematological cell counter offered higher specificity than the trypan blue exclusion method, some recovery calculations were higher than 100%, reflecting the instrument's margin of error (see **Supplementary Figure 1**). It is, therefore, recommended that investigators apply a combination of cell counting techniques when comparing the yields from PBMC isolation protocols, as most techniques do not offer both specific and highly sensitive differential cell counts. Further, assays were not performed to compare the functional activity of PBMCs yielded from either technique, which is a limitation of our analysis.

Next, the manual and automated bead-based methods were compared, and no significant differences between PBMC yield or viability were identified from 8 matched samples (**Figure 4A,B**). Cell populations were not individually compared, as the same antibody isolation cocktail was used for both methods. Importantly, the hands-on time to process 8 samples was reduced from 43 min to 22 min using the automated protocol (**Figure 4C**). While throughput, prevention of technician burnout, and consistency of specimen processing are ensured using automation, the cost of reagents and consumables are significantly higher at 3-4 fold more than the manual bead-based method. This is after making modifications to the manufacture's protocol to use 1 mL of buffy coat (from a whole blood volume of 10 mL) instead of the recommended range of 2-5 mL (from a whole blood volume of 10 mL and/or greater). If budget is a limitation, opting for the manual method can reduce processing time by ~25% while maintaining reagent and consumable costs comparable to those of the density separation method. It is recommended to process no more than 8 samples at a time per technician to ensure adequate

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staggering of samples (~30 s/sample) within the 5 min incubation periods (steps 3.7, 3.11, and 3.14).

In both the manual and automated bead-based methods, the removal of the buffy coat is a critical step to ensure optimal PBMC isolation. It is important to note that a buffy coat rather than whole blood is used in this protocol as the volume of reagents are based on starting material volumes[10]. Effectively removing the entire buffy coat volume can be technically challenging. Initially, this method detailed removing 0.5 mL buffy coat, however it was increased to 1 mL to improve recovery. To ensure adequate and consistent recovery of buffy coat, detailing this process is important in protocol documentation and training. It is recommended to swirl a pipette tip whilst aspirating the buffy coat while taking care not to aspirate too many RBCs from the layer below (step 2.3). It is important not to saturate the antibody cocktail that binds unwanted cells (i.e., granulocytes and red blood cells), which can impact yield and purity[10]. To minimize RBCs collected during the buffy coat extraction, the pipette tip should be inbetween the plasma and buffy coat layer. The volume of the buffy coat can be increased from 1 mL; however, care must be taken as described above to ensure that no more than 10% of the collected volume contains RBCs. Alternatively, an automated liquid handler can be used to collect buffy coats consistently[18]. However, the dedicated hours required to calibrate and troubleshoot liquid handling instrument protocols, especially considering the expense, may not be feasible for most laboratories.

The transition and application of the automation bead-based protocol was essential given the NSW Health Statewide Biobank's aim to process 23,000 PBMCs over the next 3 years. Here, it was demonstrated that PBMCs can be isolated from ACD tubes up to 9 days from collection with mean viabilities >70%. While yields were optimal 24 h post-collection, processing within this timeframe is not always feasible as samples may need to be transported. It was shown that PBMCs isolated with either the manual or automated bead-based method may have yields of >3 x $10^5$ cells/mL whole blood when isolated within 4 days of collection and >1 x $10^5$ cells/mL whole blood when isolated within 10 days of collection. Low numbers for samples over 5 days are noted as a limitation of this analysis. Further, data was not separated based on participant ages, genders, and clinical history, as this information was not made available. Although a comparative analysis is needed to examine the effects of delays in processing times for both methods, it has previously been reported that delays in PBMC isolation using the density gradient method decrease cell quality and significantly increase RBC contamination[19,20]. In addition, granulocyte proportions increase if processing is delayed, and hence, specimens of similar "ages" should be grouped for downstream analyses[20,21,22]. Of note, this experiment was performed using blood anticoagulated with acid citric dextrose (i.e., trisodium citrate, citric acid, and dextrose); however, yield and/or proportion of cell types may vary if other anticoagulants are used[23]; therefore investigators are advised to choose an appropriate anticoagulant based on the intended downstream PBMC analyses.

In summary, a protocol for PBMC isolation using magnetic beads that is adaptable to high throughput workflows is detailed to fulfill the requirements for scaling without compromising cell viability. Both the manual and automated methods can be optimized to produce specific cell concentrations by changing starting and resuspension volumes. The NSW Health Statewide Biobank has transitioned from extracting ~60 PBMCs per month using the traditional density gradient separation technique to ~300

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PBMCs per month using this automation-compatible bead-based method. The authors' next aim is to use the automated platform to process up to 1200 PBMCs per month and further compare PBMCs isolated by both magnetic bead-based (manual and automated) and density gradient techniques to guide the implementation of this technique to other laboratories with a particular focus on biobanks.

## Disclosures

The authors declare no conflicts of interest.

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## References

1. Alexovic, M. et al. Human peripheral blood mononuclear cells: A review of recent proteomic applications. *Proteomics.* **22** (15-16), e2200026 (2022).
2. Zhang, M. Huang, B. The multi-differentiation potential of peripheral blood mononuclear cells. *Stem Cell Res Ther.* **3** (6), 48 (2012).
3. Arimilli, S., Damratoski, B. E., Chen, P., Jones, B. A., Prasad, G. L. Rapid isolation of leukocyte subsets from fresh and cryopreserved peripheral blood mononuclear cells in clinical research. *Cryo Lett.* **33** (5), 376-384 (2012).
4. Kleeberger, C. A. et al. Viability and recovery of peripheral blood mononuclear cells cryopreserved for up to 12 years in a multicenter study. *Clin Diagn Lab Immunol.* **6** (1), 14-19 (1999).
5. Ulmer, A. J., Scholz, W., Ernst, M., Brandt, E., Flad, H. D. Isolation and subfractionation of human peripheral blood mononuclear cells (PBMC) by density gradient centrifugation on percoll. *Immunobiology.* **166** (3), 238-250 (1984).
6. Fuss, I. J., Kanof, M. E., Smith, P. D., Zola, H. Isolation of whole mononuclear cells from peripheral blood and cord blood. *Curr Protoc Immunol.* **7**, 711-718 (2009).
7. Gautam, A. et al. Investigating gene expression profiles of whole blood and peripheral blood mononuclear cells using multiple collection and processing methods. *PLoS One.* **14** (12), e0225137 (2019).
8. Hamot, G., Ammerlaan, W., Mathay, C., Kofanova, O., Betsou, F. Method validation for automated isolation of viable peripheral blood mononuclear cells. *Biopreserv Biobank.* **13** (3), 152-163 (2015).
9. Coppola, L. et al. Purification of viable peripheral blood mononuclear cells for biobanking using a robotized liquid handling workstation. *J Transl Med.* **17** (1), 371 (2019).
10. Stemcell Technologies. Vol. 27156, *1-4 STEMCELL TECHNOLOGIES.* (2020).
11. Hornschuh, M. et al. Negative magnetic sorting preserves the functionality of ex vivo cultivated non-adherent human monocytes. *Biology (Basel).* **11** (11), 1583 (2022).

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12. Bhattacharjee, J., Das, B., Mishra, A., Sahay, P., Upadhyay, P. Monocytes isolated by positive and negative magnetic sorting techniques show different molecular characteristics and immunophenotypic behaviour. *F1000Res.* **6**, 2045 (2017).
13. Ohlsson, S., Diedrich, B., Uhlin, M., Sandgren, P. Optimized processing for pathogen inactivation of double-dose buffy-coat platelet concentrates: Maintained *in vitro* quality over 7-day storage. *Vox Sang.* **113** (7), 611-621 (2018).
14. Stemcell Technologies. *Easysep direct human PBMC isolation kit for processing 100 mL buffy coat.* https://www.stemcell.com/products/brands/easysep-cell-separation.html (2022).
15. Beckman Coulter. *Vi-cell XR cell viability analyzer instructions for use.* https://mbcbiolabs.com/wp-content/uploads/2022/08/Beckman-Coulter-ViCell-Manual.pdf (2017).
16. Fuchs, Y. F. et al. Next-generation biobanking: Employing a robotic system for automated mononuclear cell isolation. *Biopreserv Biobank.* **21** (1), 106-110 (2023).
17. Tigner, A., Ibrahim, S. A., Murray, I. V. *Histology, white blood cell.* StatPearls Publishing, (2024).
18. Mathay, C., Ammerlaan, W., Betsou, F. Automatic buffy coat extract on: Methodology, feasibility, optimization, and validation study. *Biopreserv Biobank.* **10** (6), 543-547 (2012).
19. Golke, T. et al. Delays during PBMC isolation have a moderate effect on yield, but severly compromise cell viability. *Clin Chem Lab Med.* **60** (5), 701-706 (2022).
20. Yi, P. C. et al. Impact of delayed PBMC processing on functional and genomic assays. *J Immunol Methods.* **519**, 113514 (2023).
21. Mckenna, K. C., Beatty, K. M., Vicetti Miguel, R., Bilonick, R. A. Delayed processing of blood increases the frequency of activated CD11b+ CD15+ granulocytes which inhibit t-cell function. *J Immunol Methods.* **341** (1-2), 68-75 (2009).
22. Mallone, R. et al. Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive t-cell responses: Position statement of the t-cell workshop committee of the immunology of diabetes society. *Clin Exp Immunol.* **163** (1), 33-49 (2011).
23. Betsou, F., Gaignaux, A., Ammerlaan, W., Norris, P. J., Stone, M. Biospecimen science of blood for peripheral blood mononuclear cell (PBMC) functional applications. *Curr Pathobiol Rep.* **7** (2), 17-27 (2019).


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