

# A Rapid Closed Filter-based System for Mononuclear Cell Isolation from Whole Blood

PALL Life Sciences

Frank Igoe, Nelson Moreira, Todd Sanderson, Erika Marsilio, Lisa Bradbury, Pall Corporation

## Abstract

The differentiation and regenerative potential of adult-derived stem cells in whole blood, cord blood, and bone marrow holds great therapeutic promise. As research continues, there is a clear need for robust cell processing tools for the enrichment of blood cell subpopulations to support routine laboratory and clinical use.

Pall Life Sciences has developed a filter-based technology for the enrichment of mononuclear cells (MNC) from whole blood (WB). Previously reported (ISCT 2006) WB MNC recoveries (typically >75%) are extended in a further characterization of the Pall filter harvest system.

WB volume testing demonstrates good recovery and viability using 20-120 ml of WB. When 20, 40, 80, and 120 ml volumes are processed with the filter harvest system, MNC yields from 44-190 x 10<sup>6</sup> (94-68%) are seen. Percent recovery of granulocytes decreases rapidly with increasing WB volume, resulting in greater enrichment of MNC with higher WB volumes. As expected, the number of red blood cells (RBC) is approximately equal for all volumes from 20-120 ml. Additionally, there is no significant difference in the performance of the filter harvest system, as assessed by MNC recovery and WBC viability, when using fresh vs. ~24 hour WB for the filtration. MNC recovery from WB collected into different anti-coagulants (ACD, EDTA, Na Citrate, Li Heparin, Na Heparin) shows no significant differences. Together, these data demonstrate the robust performance of the Pall filter harvest system on a wide range of WB samples.



Given the results presented here, we believe that the advantages of a rapid (8-15 minute processing time), filter based, easy-to-use system for the enrichment of MNCs have great potential for clinical and research environments. With minimal sample manipulation during processing, there is a significant reduction in the risk of contamination and user-to-user variation. Thus, the Pall filter-based system has the potential to advance the technology for enriching rare or abundant mononuclear cells for routine use in therapeutic applications or cell-based studies.

## Materials and Methods

### HUMAN WHOLE BLOOD SAMPLES:

- For most experiments, fresh units of whole human blood collected in CPDA-1 anticoagulant in a blood bag are held at ambient temperature until use, unless otherwise noted.
- For anticoagulant experiments, WB from single donors is collected into 5 or 6 tubes (10 ml WB/tube) for each anticoagulant tested. Prior to filter harvest, WB from tubes containing each anticoagulant is pooled.
- Experiments are performed within 3 hours of blood collection, except for experiments using 24 hr WB. In these cases, the fresh sample is stored at 4°C overnight. Prior to filtration, chilled WB is allowed to equilibrate to room temperature for several hours.

### FILTER HARVEST FOR MONONUCLEAR CELL ENRICHMENT (8-15 MIN TOTAL PROCESSING TIME/SAMPLE):

- A syringe is used to transfer WB from blood bag to filtration set (50 ml unless otherwise noted).
- The WB is filtered by gravity flow (filtration time for 50 ml ranges from 3-8 min).
- Cells are recovered by back-flushing the filters (i.e., reversing the direction of the flow) with 20 ml sterile Cell Harvest solution.

**CELL COUNTS AND 3-PART DIFFERENTIALS** are generated on a Cell-Dyn\* 1800 hematology analyzer (Abbott Labs) following standard protocols. Triplicate measurements are averaged for the concentrations of RBCs, granulocytes, monocytes and other similarly-sized cells), lymphocytes, and platelets. Percent recoveries for each sub-population are determined by calculating the number of cells before and after the preparation using the following formula:

$$\frac{\text{Concentration of cells after enrichment procedure} \times \text{volume}}{\text{Concentration of cells before enrichment procedure} \times \text{WB volume}} \times 100$$

Total number of MNC is determined by the following formula:

$$(\text{Concentration of lymphocyte} + \text{monocytes}) \times \text{volume}$$

### VIABILITY DETERMINATION:

Percent viability of WBCs in the starting material and after filter harvest is determined by dye exclusion using propidium iodide.

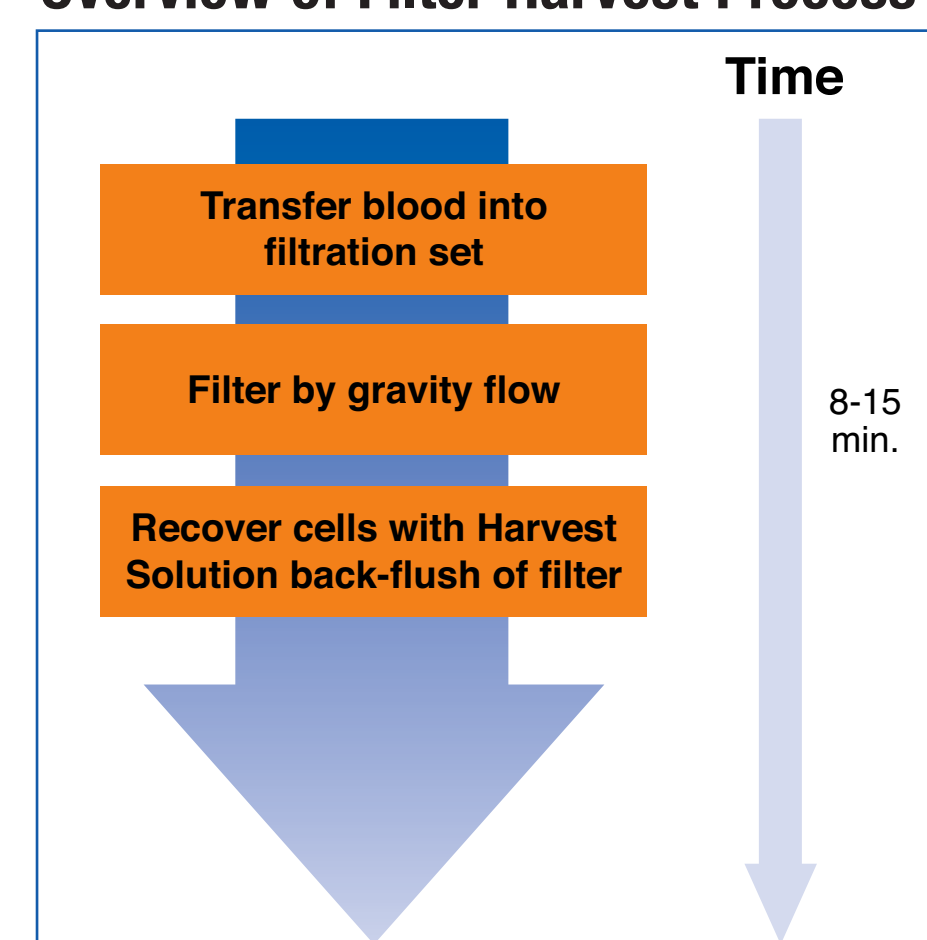
- 50 ul of cell containing samples, WB or filter harvest samples (FH), are incubated in 1ml 1x H-lyse buffer (to lyse RBCs, R&D Systems) for 18 minutes at RT.
- 10 ul of propidium iodide (1 mg/ml in PBS, Sigma) is added to each tube (except no stain controls) and incubated an additional 2 minutes at RT.
- The cells are pelleted by centrifugation at 500 x g for 5 minutes; then the supernatant is removed. The cells are resuspended in 1 ml PBS for analysis.
- Percent viability is determined by calculating the percentage of unstained cells vs. total cells as determined by flow cytometry (BD FACS Calibur).

## Results

### Protocol Comparison (Fig. 1)

- The filter harvest method takes about 8-15 minutes, from start to finish, to process ~50-120 ml of whole blood. This method has been successful for whole blood volumes of 20-120 ml.
- No special training or equipment is required for the MNC filter harvest method. It is simple to use.
- The system is essentially closed; thus, there are minimal concerns regarding contamination of sample during this process.

Figure 1  
Overview of Filter Harvest Process

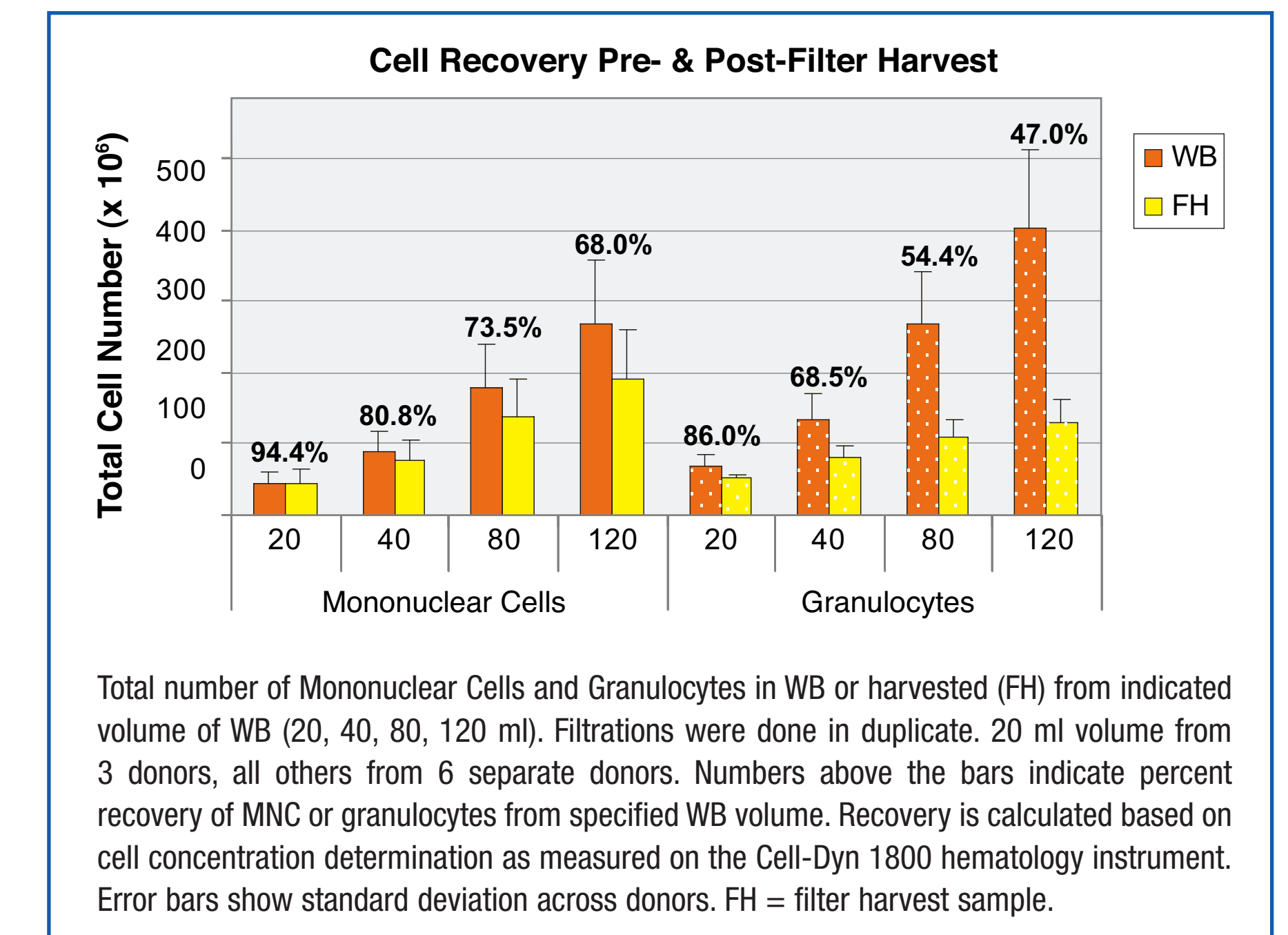


## Results (continued)

### WB Volume Testing – Evaluation of the Major Cell Populations Before and After Filter Harvest Using 20-120 ml of WB (Fig. 2, Cell-Dyn data):

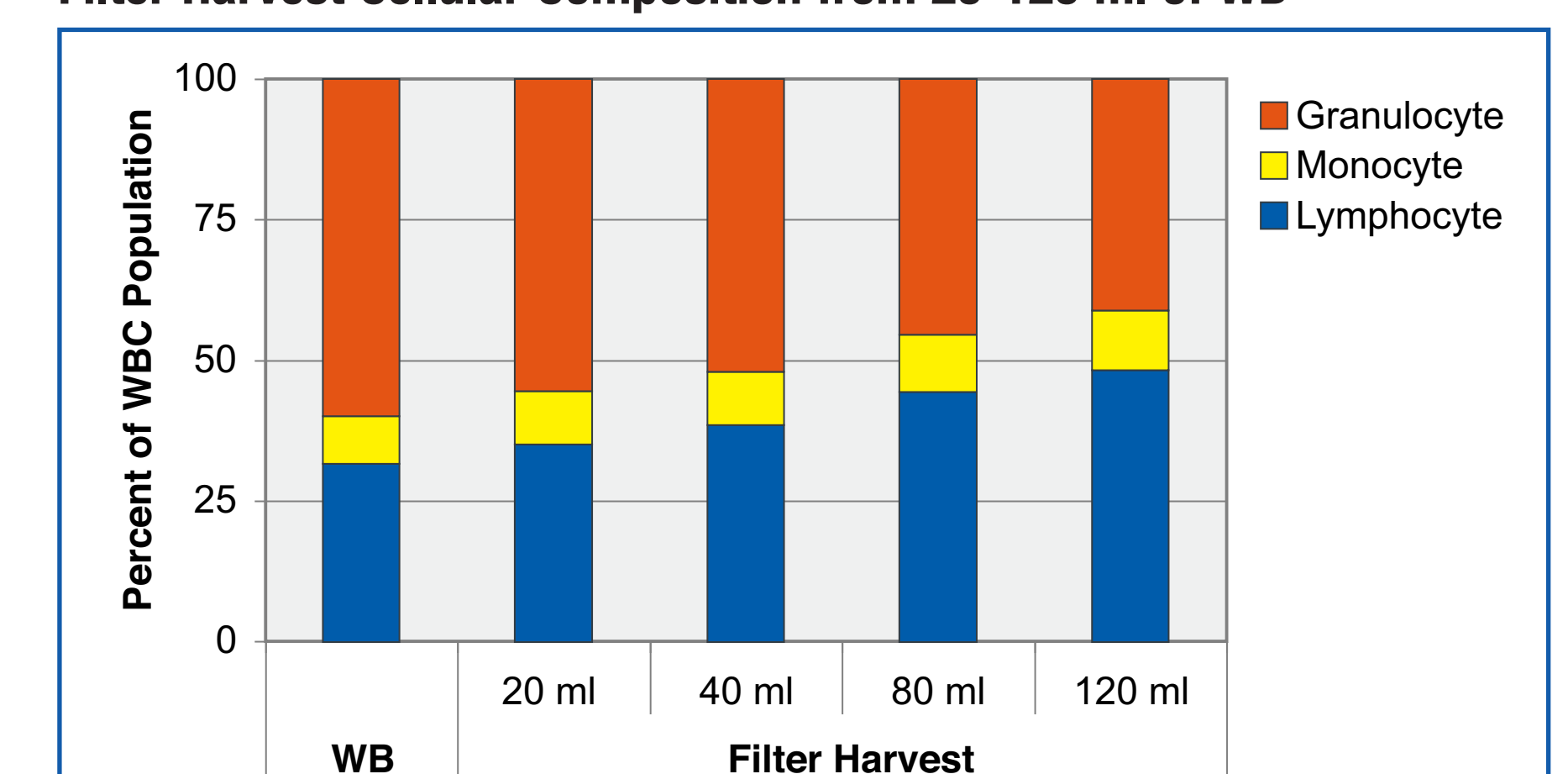
- The bar graph shows total cell number for MNC and granulocytes (left and right, respectively).
- As expected, the number of MNC and granulocytes post-filter harvest (FH) increases with increasing volume of WB. There is a slight decrease in the percent recovery of MNC as the WB volume goes up (number above bar).
- There is a dramatic decrease in granulocyte recovery as the WB volume increases. This results in a relatively greater enrichment of MNC as compared to granulocytes with increasing volume.
- When the volume is increased to 150 ml, there is a significant reduction in percent MNC recovery (data not shown). Thus, 120 ml is the upper limit recommended for this filter harvest set design.

Figure 2  
Filter Harvest Cell Yields from 20-120 ml of WB



Total number of Mononuclear Cells and Granulocytes in WB or harvested (FH) from indicated volume of WB (20, 40, 80, 120 ml). Filtrations were done in duplicate. 20 ml volume from 3 donors, all others from 6 separate donors. Numbers above the bars indicate percent recovery of MNC or granulocytes from specified WB volume. Recovery is calculated based on cell concentration determination as measured on the Cell-Dyn 1800 hematology instrument. Error bars show standard deviation across donors. FH = filter harvest sample.

Figure 3  
Filter Harvest Cellular Composition from 20-120 ml of WB



Percent of Lymphocytes, Monocytes, and Granulocytes in WB and Filter Harvest samples. WB volume used for the filtration is indicated on the X-axis. Cellular composition determination as measured on the Cell-Dyn 1800 hematology instrument.

### WB Volume Testing – Percent Composition of the Major Cell Populations Before and After Filter Harvest Using 20-120 ml of WB (Fig. 3, Cell-Dyn data):

- The stacked bar graph shows the percentage of lymphocytes, monocytes, and granulocytes in the total nucleated cell population in WB or FH samples from 20, 40, 80 or 120 ml WB filtration.
- The increase in enrichment of MNC relative to granulocytes with increasing WB volume is clearly apparent.

Table 1  
Fresh vs. 24-Hour Whole Blood—Percent Recovery and Viability of MNC and Total WBC Population

Sample Type	MNC Recovery	WBC Recovery	WBC % Viable
Fresh WB			85 +/- 5 %
Fresh WB FH Sample	80.1 +/- 9.9 %	67.4 +/- 4.3%	89 +/- 4 %
~24 hr WB			81 +/- 7 %
~24 hr WB FH Sample	76.4 +/- 5.6 %	68.8 +/- 5.7%	87 +/- 7 %

percent +/- Standard deviation, n = 5

filtrations performed on fresh WB and WB ~24 hr old after ON storage at 4°C

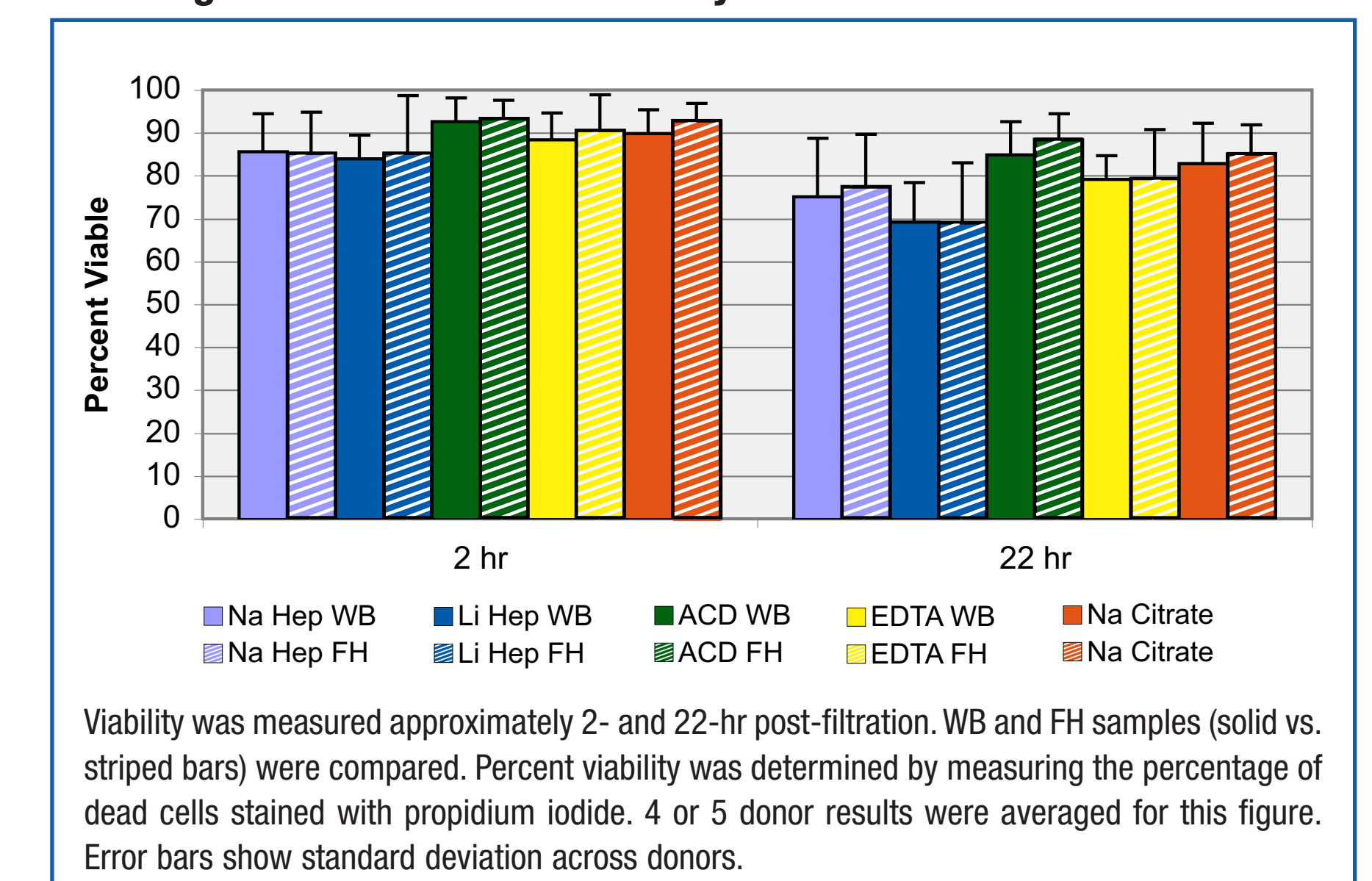
Comparison of MNC and total WBC recovery and viability from matched donors using fresh WB vs. the same WB stored at 4 °C overnight.

### Comparison of Effect of Anticoagulants on Filter Harvest (Fig. 4):

CPDA is the standard anticoagulant used for filter harvest experiments as it is known to be very friendly to blood cells. ACD and CPDA are commonly used anticoagulants in blood banks for blood products. Heparin and EDTA are commonly used in research labs, with NaCitrate also used, but less frequently.

- Viability data from samples, measured at ~2 hrs and ~22 hrs post-filter harvest, suggest that Heparin has a small negative effect on WBC viability. This effect is more apparent with stored WB or filter harvest samples (Fig 4). Although this effect is small, it has been reported by others.

Figure 4  
Anticoagulant Effect on Cell Viability Before and After Filter Harvest



Viability was measured approximately 2- and 22-hr post-filtration. WB and FH samples (solid vs. striped bars) were compared. Percent viability was determined by measuring the percentage of dead cells stained with propidium iodide. 4 or 5 donor results were averaged for this figure. Error bars show standard deviation across donors.

- The recovery data from 5 donors show no statistically significant differences between anticoagulants (data not shown). There may be a small negative impact on recovery of all WBC populations in the presence of EDTA.

## Conclusions

- Pall's easy-to-use MNC filter harvest system results in high MNC recoveries (typically >75% from 50 ml) in <15 minutes.
- Dynamic range of the filter harvest system enables processing of 20-120 ml, with no significant difference observed between fresh and day-old WB samples.
- The filter harvest system is compatible with WB collected in any of the commonly-used anticoagulants.
- Approximately 200 million MNC can be isolated from a single filter harvest using 120 ml WB.