PBMCs processed from whole blood with Laminar Wash-based methods yield fewer apoptotic cells than centrifugation

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CURIOX
BIOLOGY AT ITS BEST

Donor leukocytes are in different states of cell health and/or have different tolerance to centrifugation

HYPOTHESIS: Gentle Laminar Wash handling of leukocytes enables more reproducible flow cytometric measurements

EXPERIMENT: Compare Laminar Wash vs Centrifuge processing for leukocytes from individual donors (n=3) and pooled blood

The amount of cells often varies from sample to sample—inconsistent starting number of cells to process

HYPOTHESIS: Uniform retention of cells with LW ensures consistent cell population frequency, regardless of starting cell counts

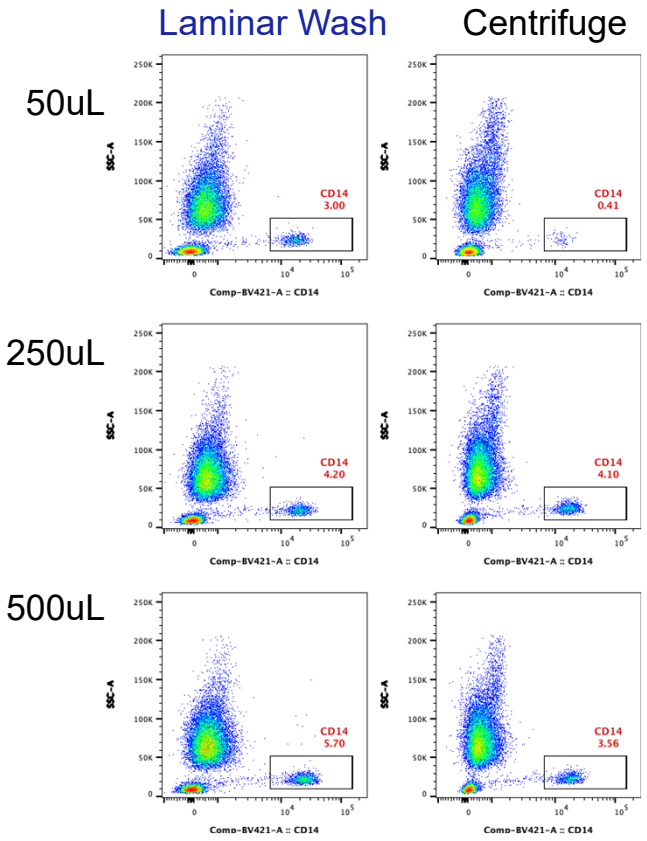
EXPERIMENT: Compare Laminar Wash vs Centrifuge processing for leukocytes from different starting cell counts (n=3)

3 blood donors, and one group of pooled blood
3 starting cell numbers (lysed blood, 50uL, 250uL, 500uL equivalent)
3 technical replicates
2 methods – LW and centrifuge
Quantifiers:

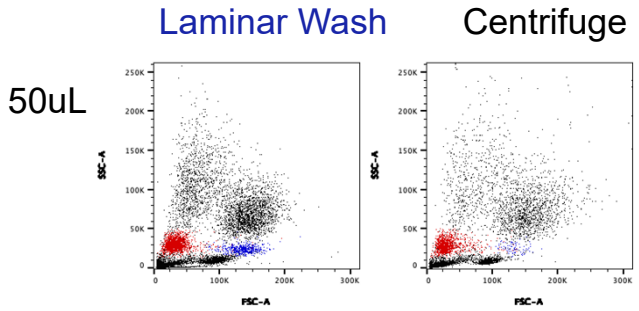
1. Frequencies of leukocytes (CD45), granulocytes (CD66b), monocytes (CD14) and T cells (CD3)
2. Absolute retention of cells

Laminar Wash retains CD14⁺ monocytes with low starting sample count

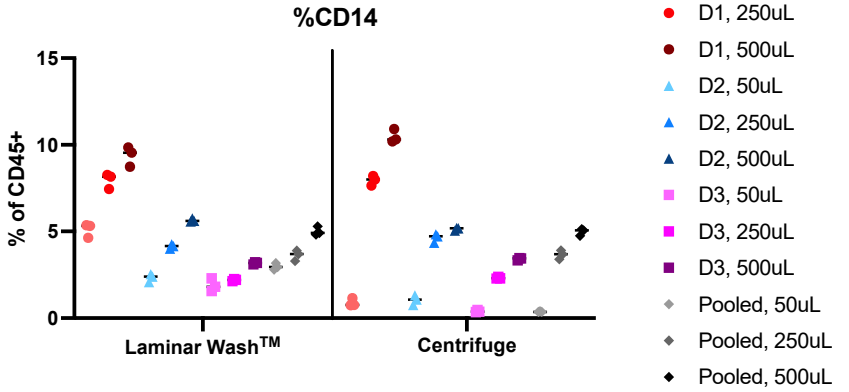
(a)



(b)



(c)



Key observations:

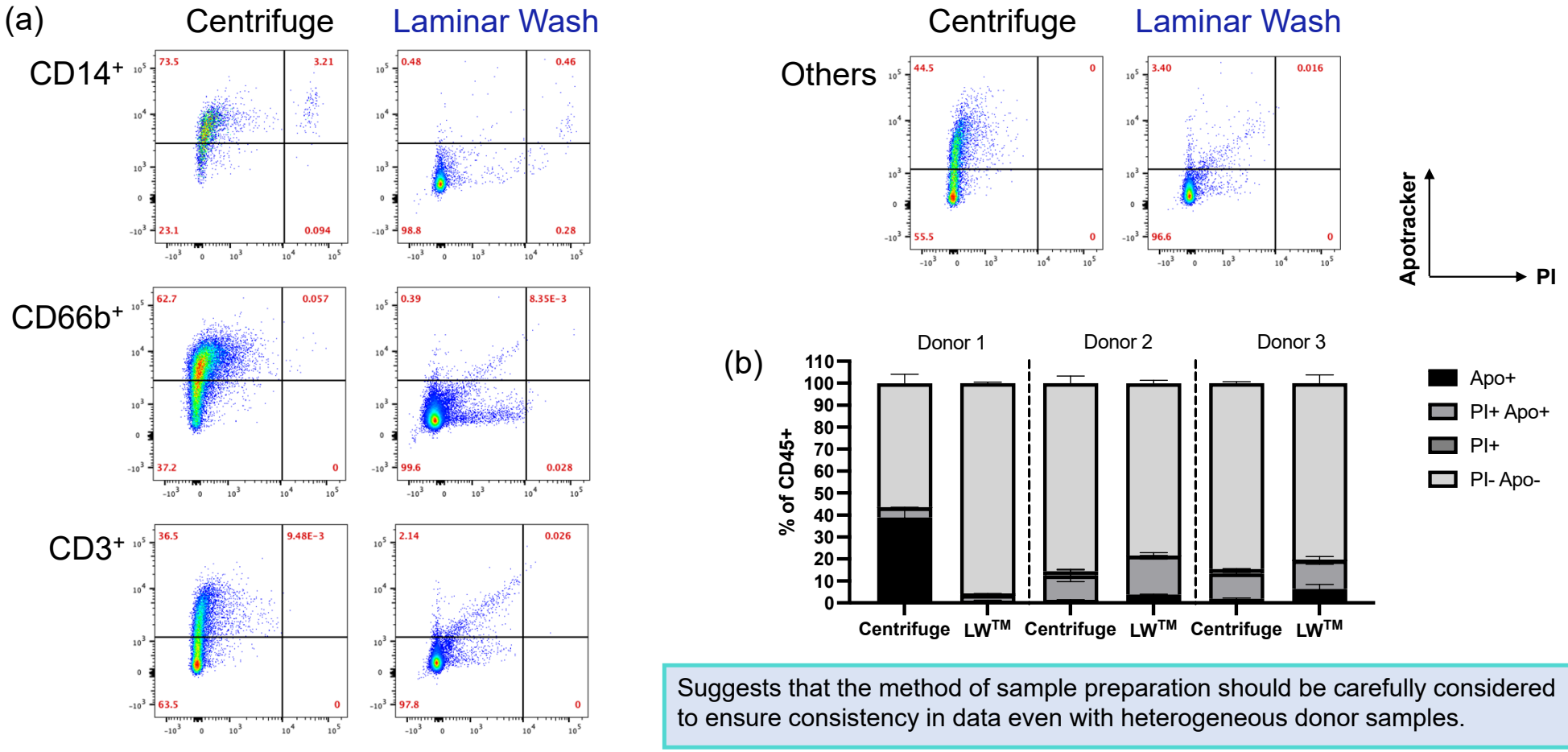
- Monocytes at low starting cell numbers are most 'vulnerable'
- Laminar Wash shows least fanning of frequencies across all starting cell numbers
- Laminar Wash shows least variation of frequencies from pooled sample

(a) Looking at live CD45+ single cell events in the Pooled Blood sample, we gate for monocytes by SSC-int and CD14+. There was significant loss of CD14+ population in centrifuged samples at 50uL blood volume, compared to LW or centrifuge and LW at higher blood volumes.

(b) Backgating strategies revealed that the low CD14+ population at 50uL blood volume in centrifuged samples was due to cell death, as monocytes shifted from FSCint to FSClo, with similar FSC intensity as debris.

(c) This loss of CD14+ population at low blood volume was observed consistently among all three individual donor blood samples. The frequency of CD14 population is enumerated across individual donor as well as pooled blood. Within each individual donor, LW consistently showed lower variation in frequency of CD14 across different blood volumes, compared to centrifuge (intra-sample consistency). Inter-sample variation is typically addressed by pooling blood from individual donors to obtain an 'averaged' value in various population frequencies. Comparison of CD14 frequency at corresponding blood volumes between individual donors and pooled blood also revealed greater differences with centrifugation.

Heterogenous sensitivity to centrifugation-induced apoptosis of leukocytes from individual donors



Suggests that the method of sample preparation should be carefully considered to ensure consistency in data even with heterogeneous donor samples.

Whole blood from three donors in independent experiments lysed in bulk with ammonium chloride lysis buffer. Apoptosis and viability after washing by either Laminar Wash or centrifugation were evaluated by Apotracker (BioLegend) and Propidium Iodide (PI), incubated at RT for 30 min. Washing protocols: 9x on Laminar Wash 96-well plates or twice in centrifuge tubes.

(a) Whole blood (50uL) was RBC-lysed and distributed to LW plate or tubes for washing. The cells were then stained with antibodies for T cells, monocytes and granulocytes, along with apoptotic marker Apotracker and viability marker (PI). Donor 1 had high Apotracker frequency in all cell types examined after undergoing centrifugation, but not after Laminar Wash. This was not observed in the other two donors, whose cells were indifferent to pre-staining wash treatment.

(b) Enumeration of frequencies of live (Apo-PI-), apoptotic (Apo+PI-), necrotic (Apo+PI+) or dead (Apo-PI+) cells in CD45+ of each donor by LW or centrifuge. The high apoptotic frequency in response to centrifugation was not uniform across three independent donors.

Conclusions and Perspectives

- Demonstrated differences in quantitative output (cell population frequency, viability) from the same sample, depending on quantity of starting material as well as processing method (LW vs centrifuge)
- Laminar Wash-processed samples showed improved intra- and inter-sample consistency of CD14+ population frequencies across different starting cell counts and individual donors vs pooled samples.
- The high variation in centrifuged samples may be attributed to heterogeneous sensitivity of donor cells to processing methods, as seen by high apoptotic frequencies in centrifuged samples but not Laminar Wash processed samples.
- We have previously demonstrated the non-uniform loss of myeloid populations in independent high-parameter flow and mass cytometry assays in centrifuge-processed samples.
- There is a need to consider the impact of processing methods and resulting cell stress, particularly with rare cell populations (e.g. antigen-specific T cells) or compromised and ‘tricky’ populations (e.g. tumor infiltrating lymphocytes)
- We are currently evaluating other aspects of cell viability and metabolic activity (e.g., caspase 3 and mitochondrial markers) in relation to sample preparation methodology.