

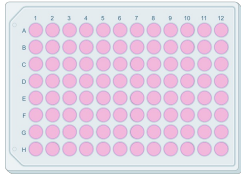
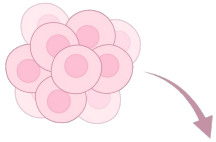
Automated PBMC isolations – gain in time, number of cells and quality of samples

Ewa Cendrowicz

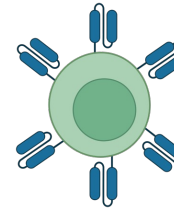
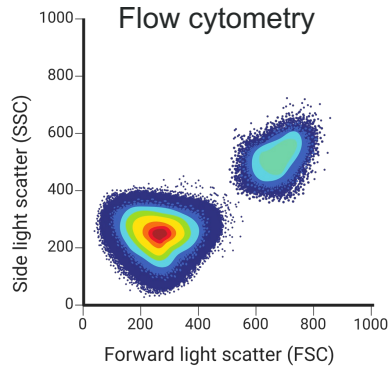
11-May-2023

Why do we need to isolate PBMCs?

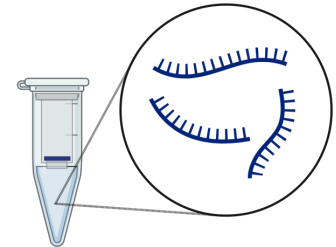
One of the most standard procedure in clinical laboratories



Cell-based assays
(including functional assays)



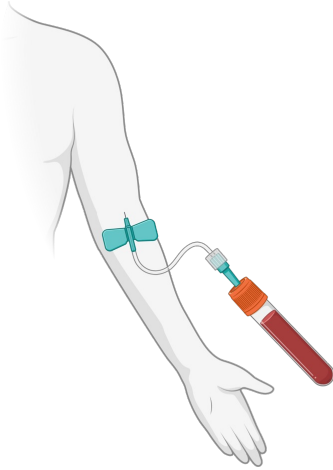
CAR T cells



DNA/ RNA extraction for further analysis

Why do we need to isolate PBMCs?

The best possible representation of the sample at the moment of blood collection

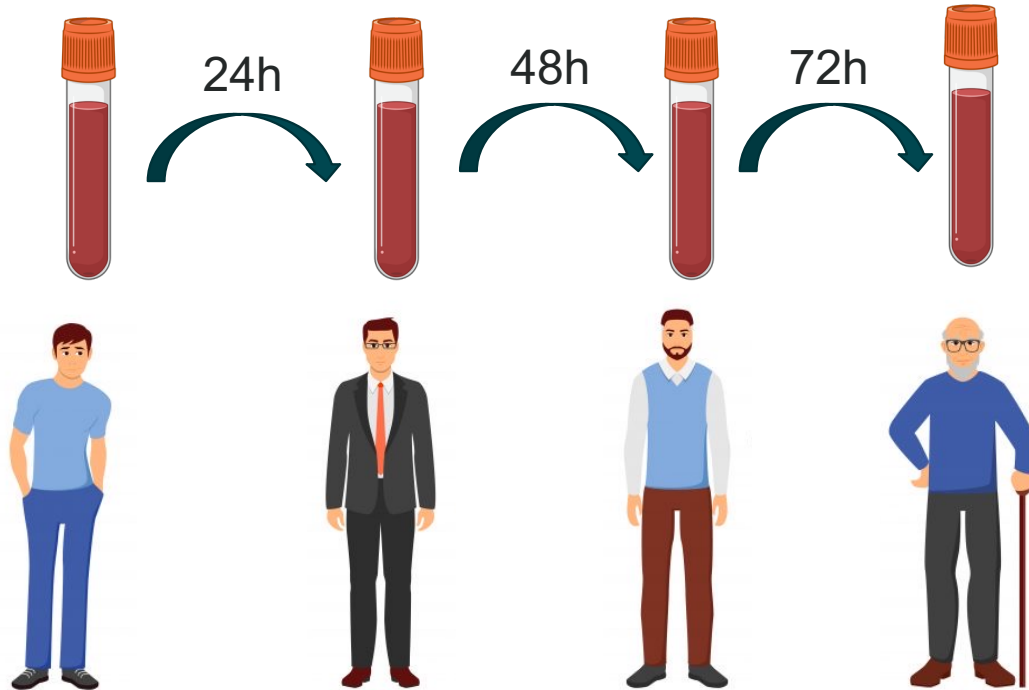


Immediate analysis?



Why do we need to isolate PBMCs?

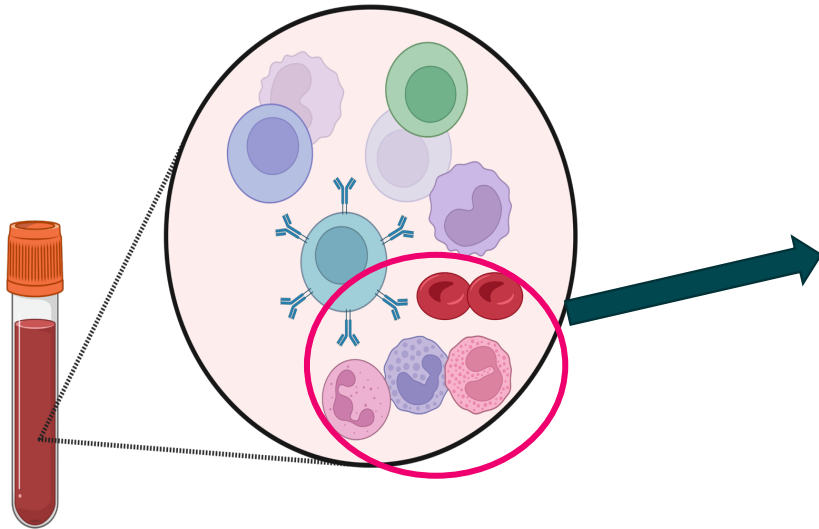
Blood is a biological material



- Ongoing biological processes
- Changes in the expression of markers
- Cell death
- Release of cytokines
- Many other....

Why do we need to isolate PBMCs?

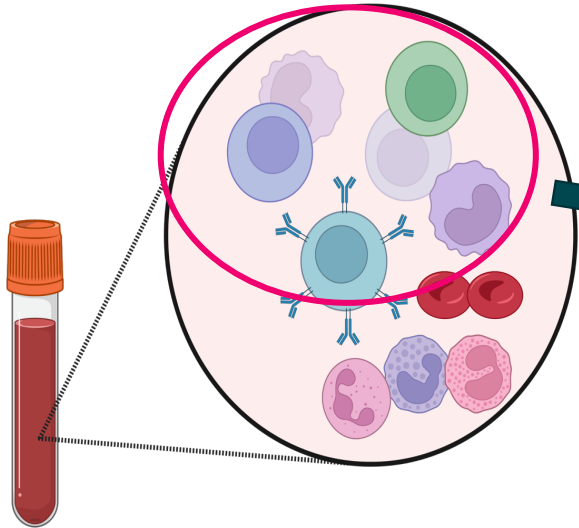
Preservation of immune cells



RBCs and PMNs are sensitive to freezing, thawing and culturing

Why do we need to isolate PBMCs?

Increase the number of cells of interest



RBC:WBC = 600:1
PMNs – 60-70% of WBCs
MCs – cells of interest

Why do we need to isolate PBMCs?



Batched analysis

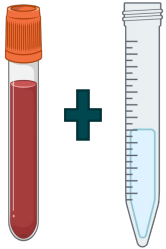
Direct comparison of various time points

Flow cytometry applications:

- The same compensations for all samples
- One preparation of the machine
- No day-to-day variation of the machine
- One-time cocktail preparation

Procedure PBMC isolation

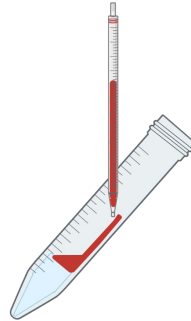
Dilute blood
with PBS



Prepare a
tube with
ficoll



Carefully
apply blood
to ficoll

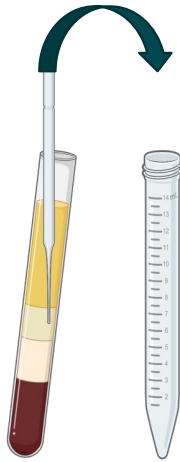


Spin down
without brake
(~30 min)

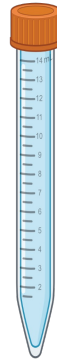


Procedure PBMC isolation

Carefully
remove the
PBMCs from
the layer



Fill up with
PBS to wash



Spin down
5 min



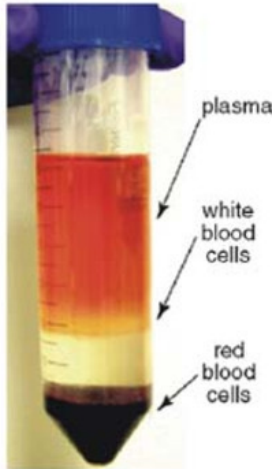
Fill up with
PBS to wash



Spin down
5 min

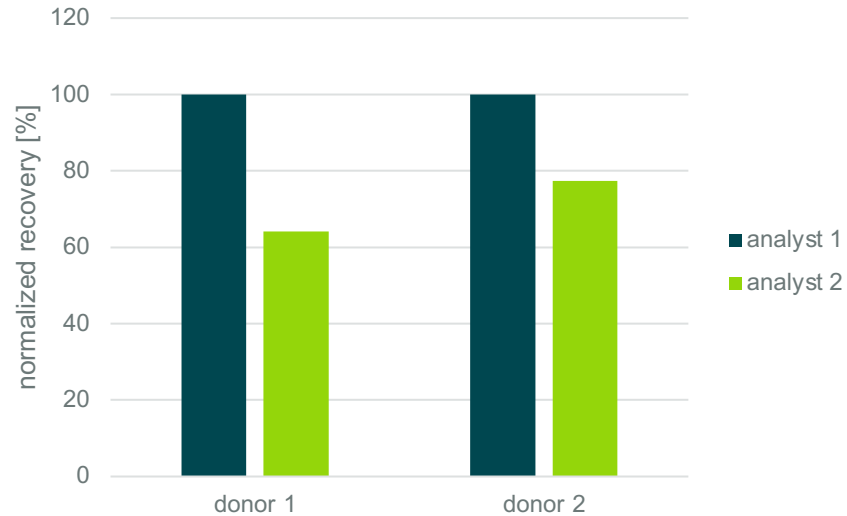


Remove the PBMC ring



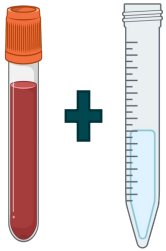
Comparison of Ficoll isolations by 2 analysts

- The same donors
- The same volume
- The same time
- The same centrifuge
- 2 analysts

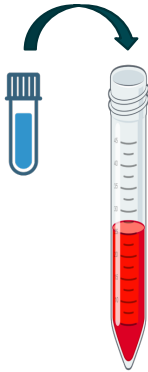


Procedure AutoMACS

Mix blood with
buffer



Add
microbeads



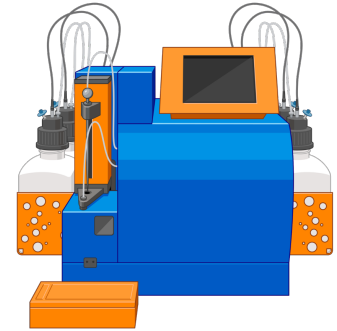
Incubate
3 min



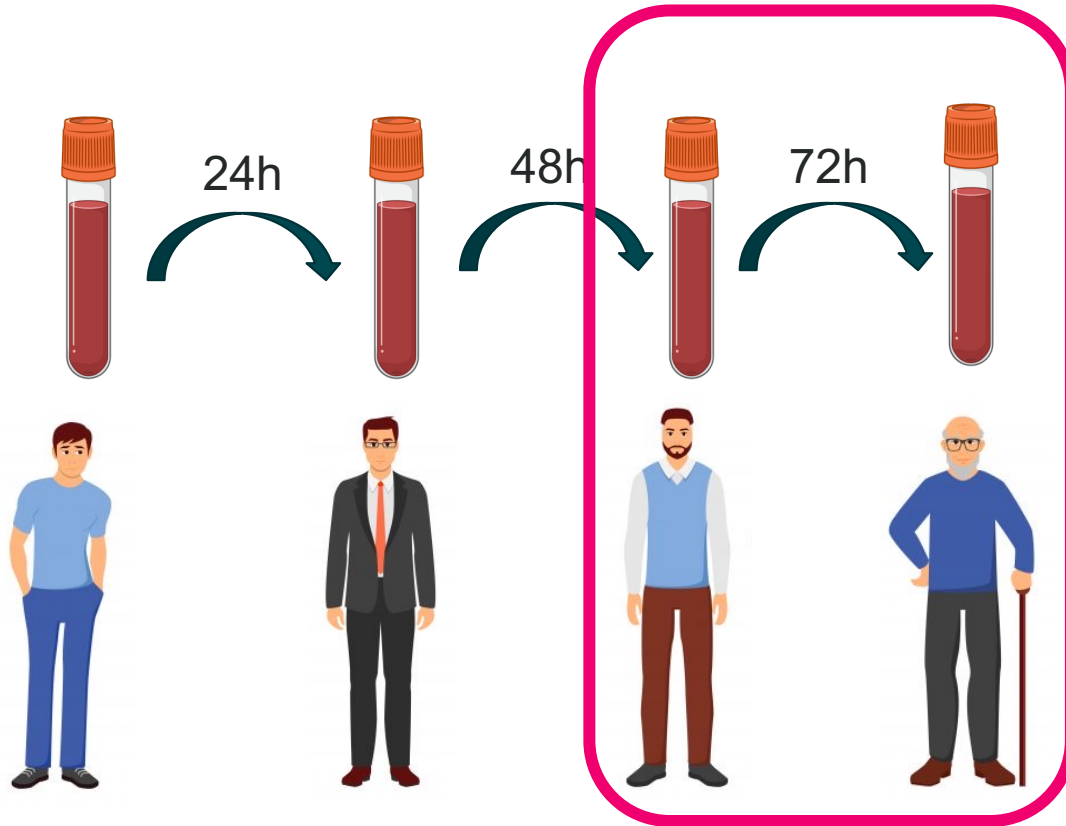
Spin down
3 min



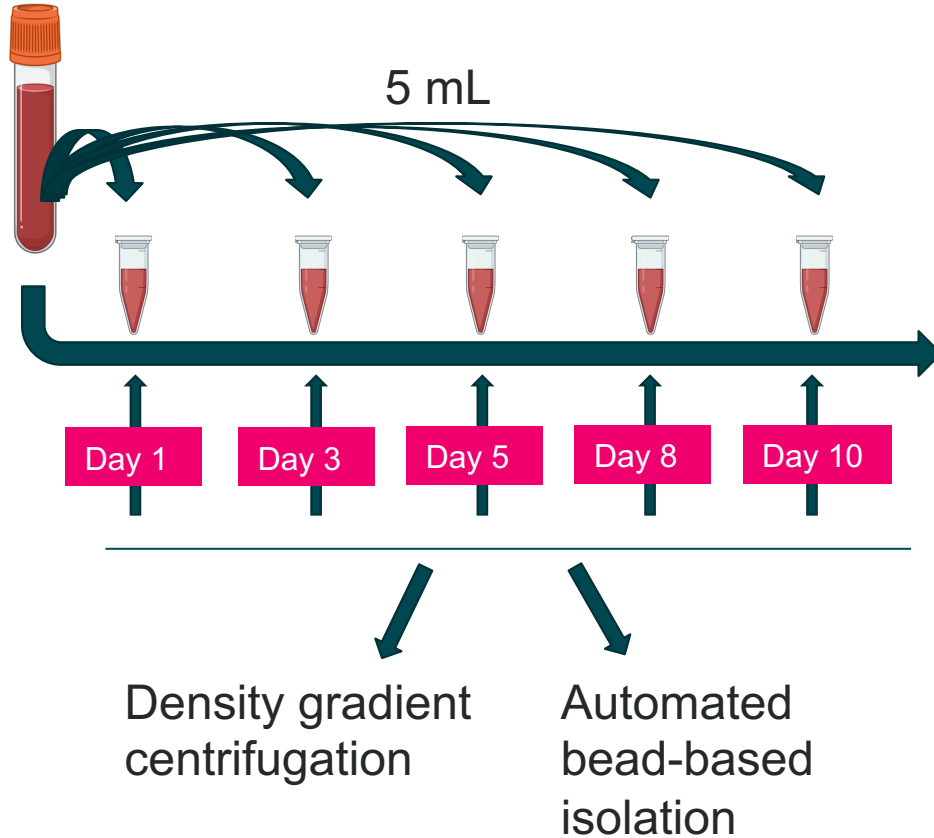
AutoMACS
isolation 10 min



Sample arrival to the BioA laboratory

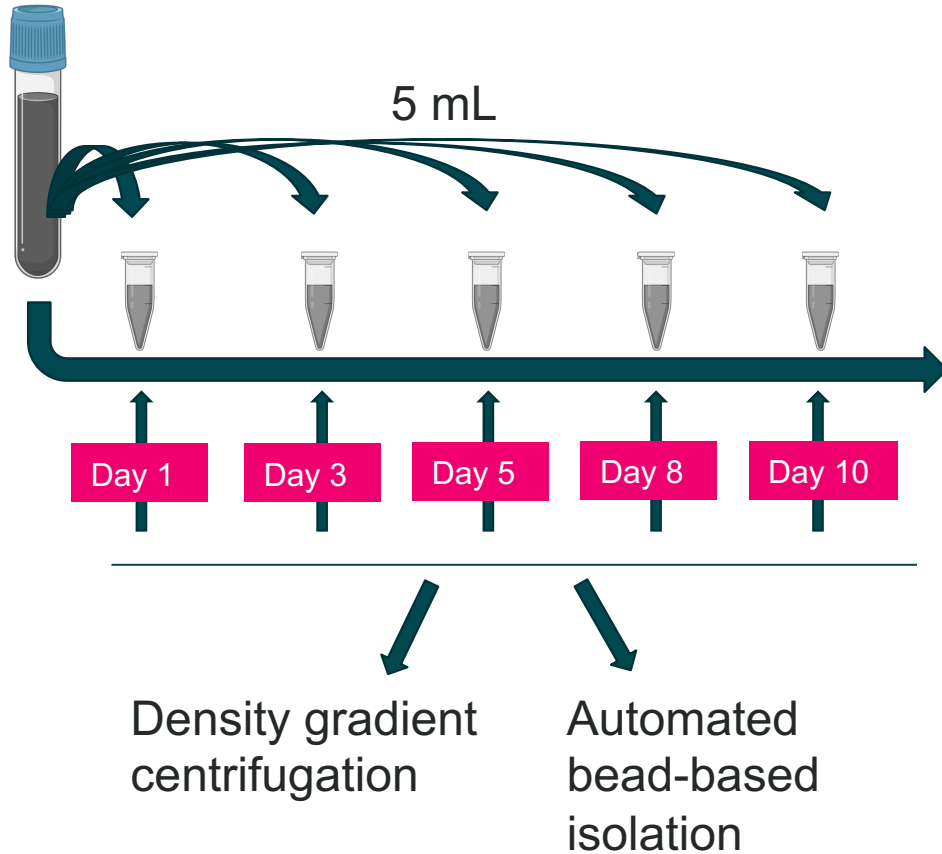


Study setup



Whole blood (K3EDTA)

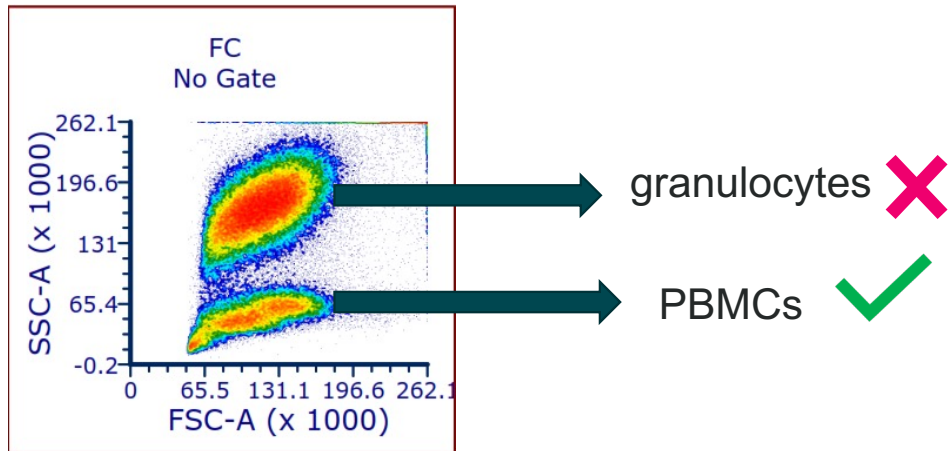
Study setup



Fixed whole blood (Cytochex)

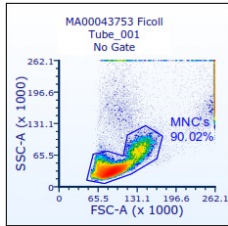
Purity of PBMCs – WB – example of flow cytometry plot

White Blood Cells

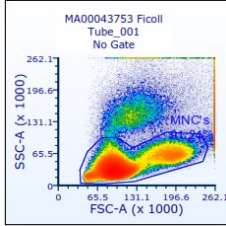


K3EDTA - Ficoll

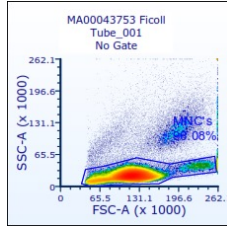
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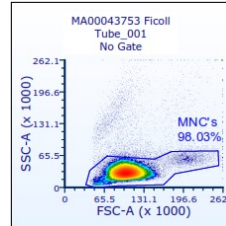
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5



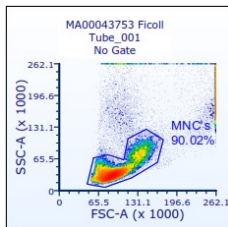
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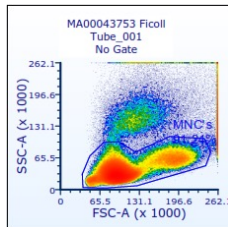
CD45 staining

K3EDTA - Ficoll

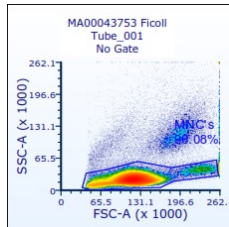
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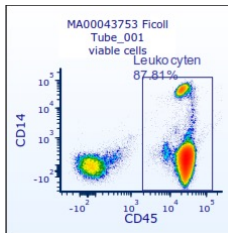
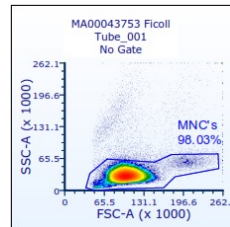
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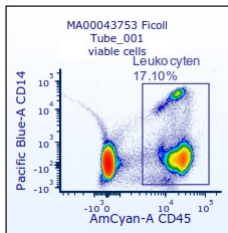
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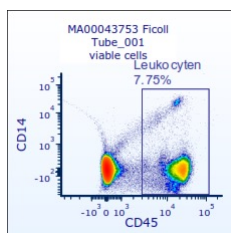
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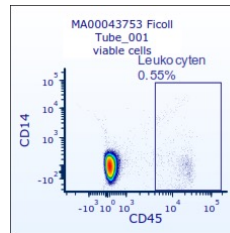
88%



17%



8%



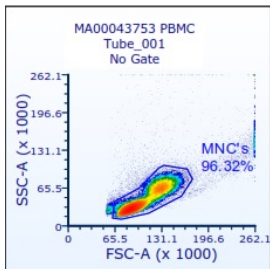
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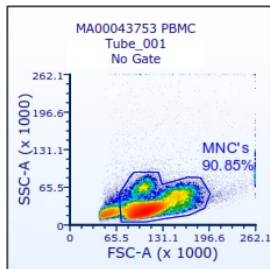
CD45 staining

K3EDTA - AutoMACS

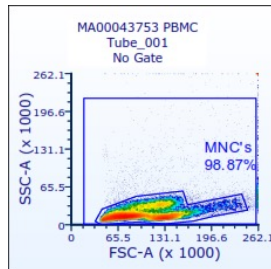
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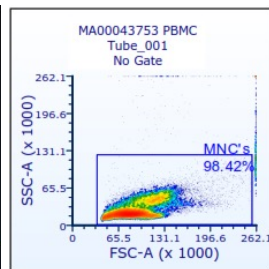
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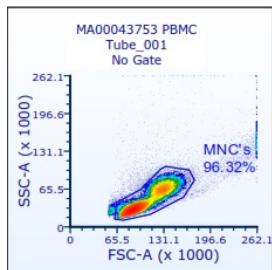
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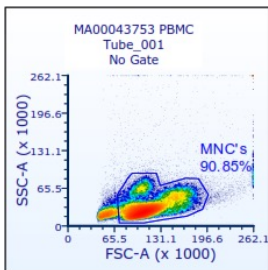
CD45 staining

K3EDTA - AutoMACS

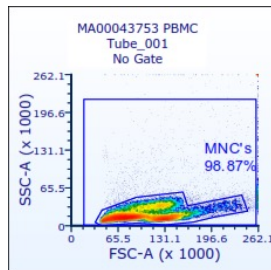
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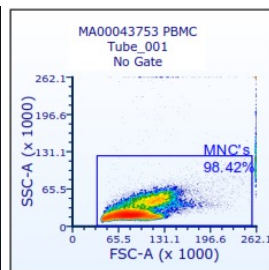
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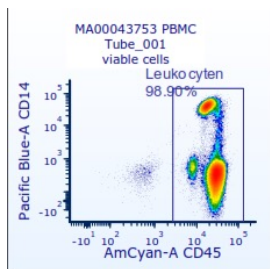
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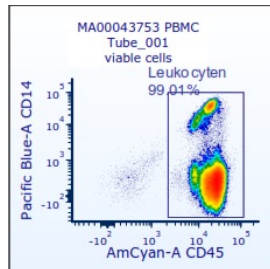
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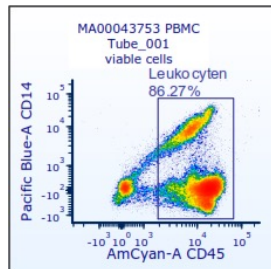
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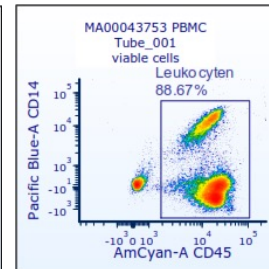
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99%

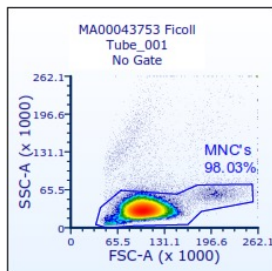


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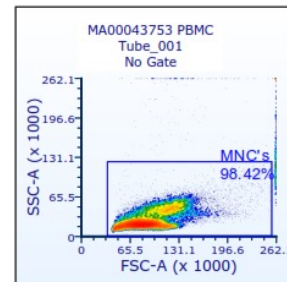
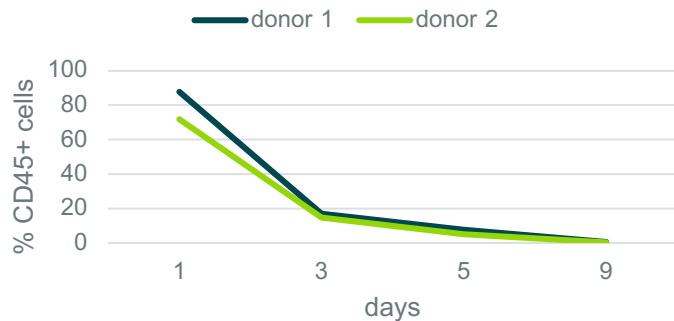


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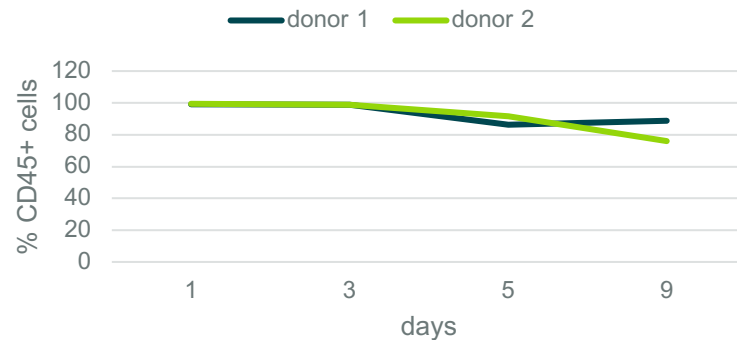
Ficoll vs AutoMACS – K3EDTA



Ficoll K3EDTA

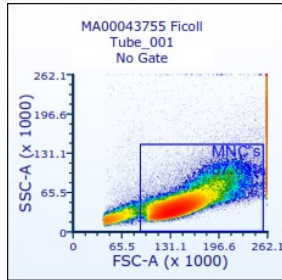


AutoMACS K3EDTA

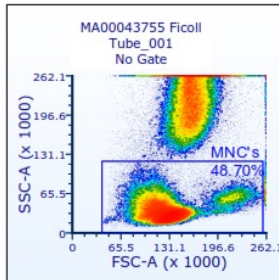


CytoChex blood - Ficoll

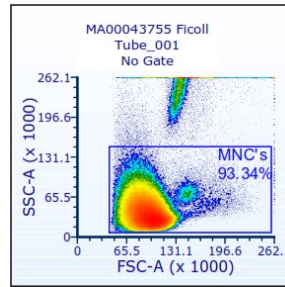
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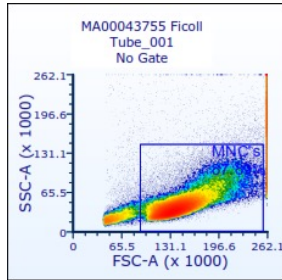
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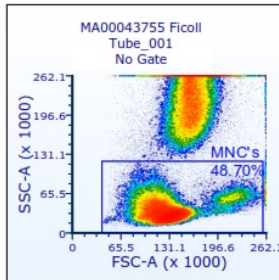
CD45 staining

Cytochex blood - Ficoll

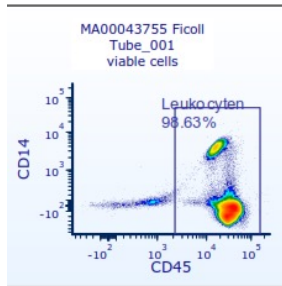
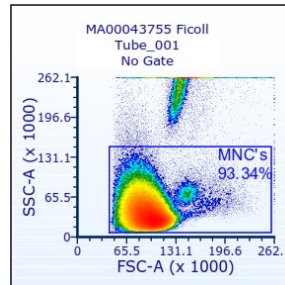
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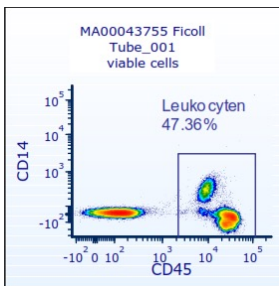
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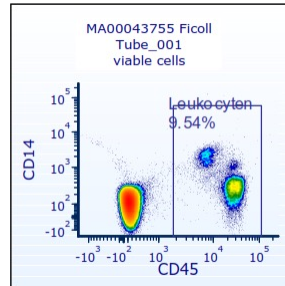
7



99%



47%



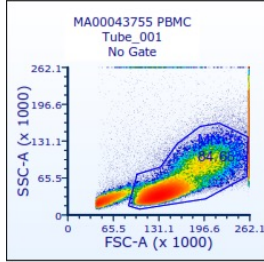
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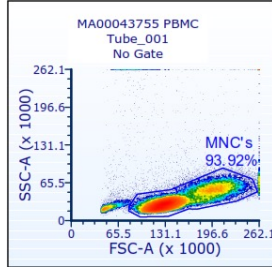
CD45 staining

CytoChex blood - AutoMACS

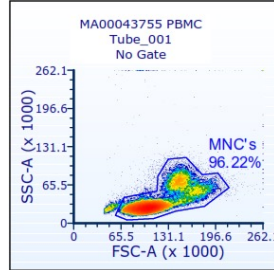
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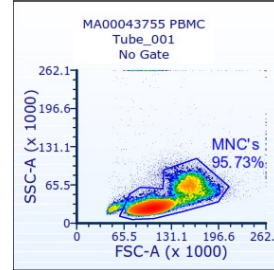
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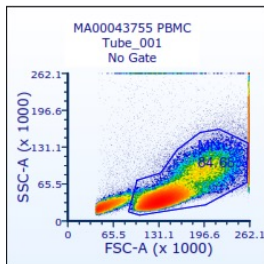
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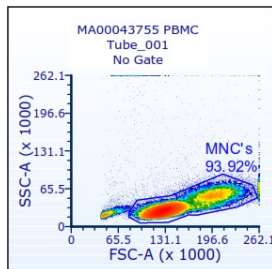
CD45 staining

CytoChex blood - AutoMACS

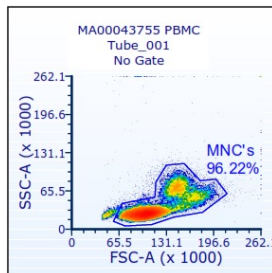
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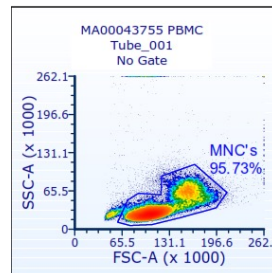
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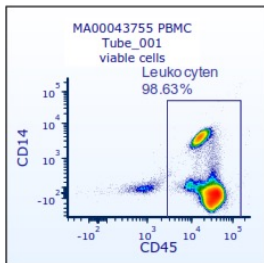
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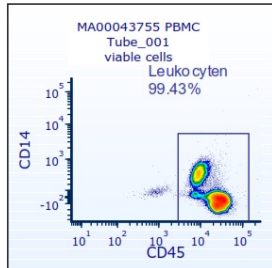
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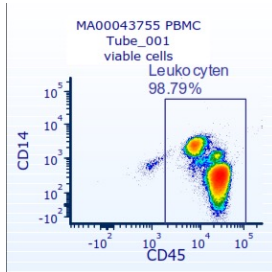
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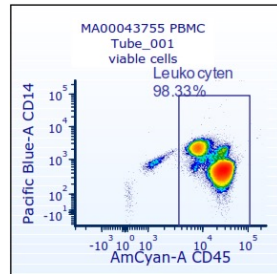
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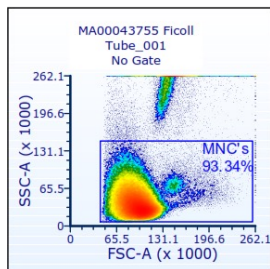


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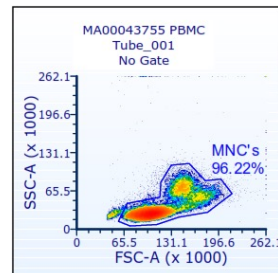
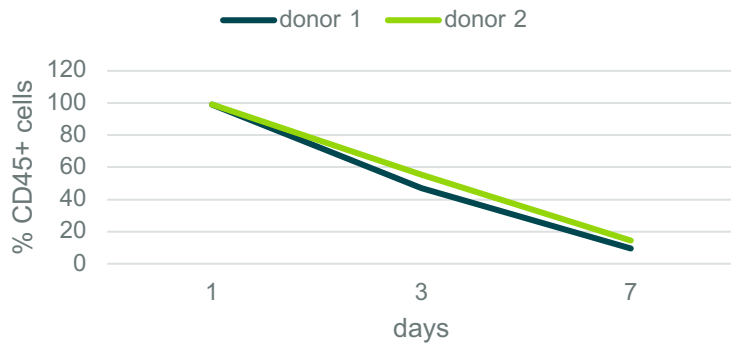


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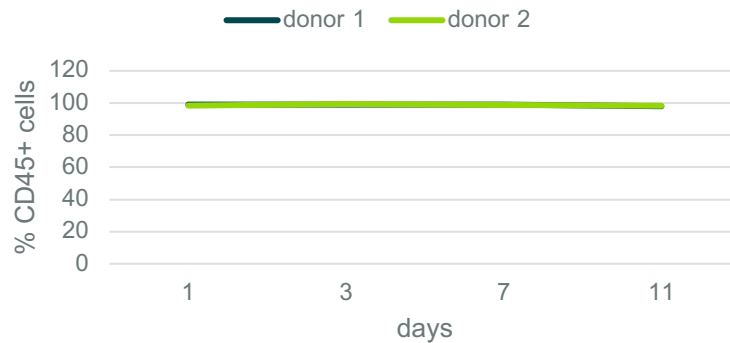
Ficoll vs AutoMACS - CytoChex



Ficoll CytoChex



AutoMACS CytoChex



Conclusions

- We obtained higher recovery of immune cells when using AutoMACS comparing to manual isolation
(67% (auto) vs 0.5% (manual) with K3EDTA for the latest timepoint); (98% (auto) vs 10% (manual) with CytoChex for the latest timepoint)
- Quality of samples is better (quicker acquisition, easier data analysis)
- Automated isolations are 0.5-1 h shorter than the manual isolations (the more samples we isolate, the more time we gain)
- Reproducibility of isolations is better with automated method (less variations of PBMC purity among various timepoints)

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