Improved separation of Foxp3^{+} and Foxp3^{-} cells in a whole blood flow cytometry assay

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Introduction

The anti-FOXP3 **clone 3G3** belongs among the group of very few and unique monoclonal antibodies that are reactive with both human as well as mouse FOXP3 protein. Its use is limited due to the reports of low yields of FoxP3+ cells. Most human Treg studies were done with **clones 259D and PCH101**.

Objective

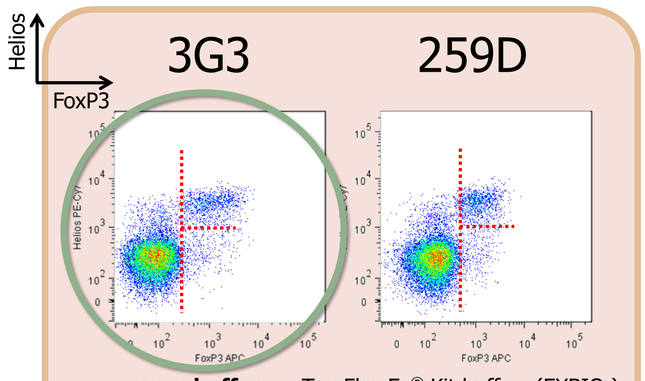
To develop new buffers that will enhance 3G3 staining to allow reliable quantitation of FoxP3^{+} cells in whole blood.

Results I

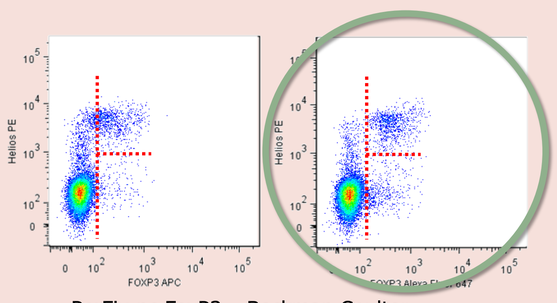
- **new fixation/permeabilization procedure**
  - hypotonic solution with 1% formaldehyde (10 min fixation and simultaneous erythrocyte lysis)
  - sodium dodecyl sulphate/saponin mixture (added directly to the „lysate", 10 min incubation)
  - pre-blocking with BSA/human IgG, 10 min
  - intracellular staining 30 min in the blocking buffer

Results II

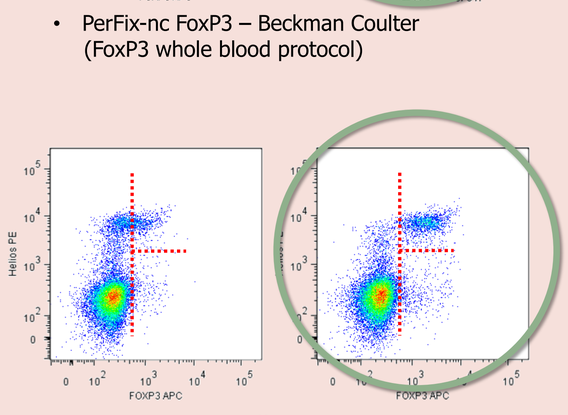
- **Unlike other buffer sets the new method provides better staining with 3G3 than with 259D**



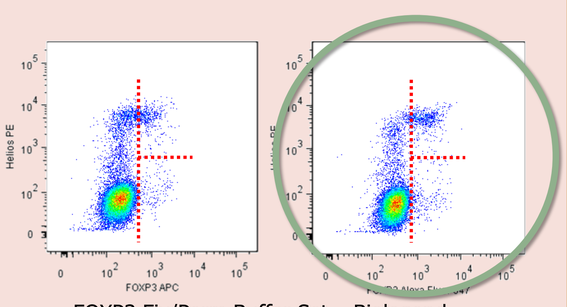
- **new buffers** – TregFlowEx® Kit buffers (EXBIO) (whole blood protocol)



- PerFix-nc FoxP3 – Beckman Coulter (FoxP3 whole blood protocol)



- FoxP3 Staining Buffer Set - Miltenyi Biotec (PBMCs)

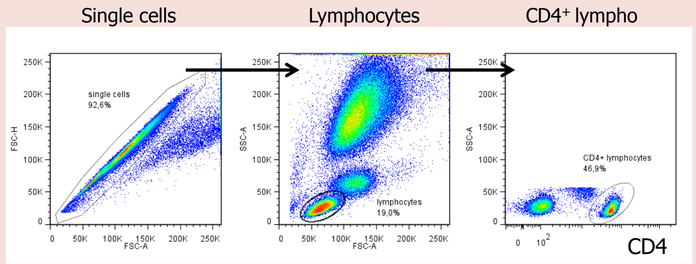


- FOXP3 Fix/Perm Buffer Set – Biolegend (PBMCs)

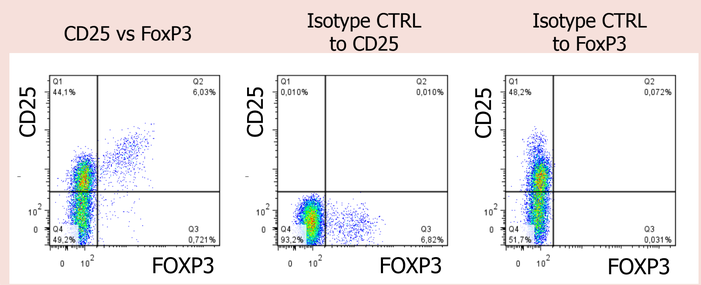
Results III

- **Buffers preserve light scatters, facilitate lymphocytes gating**

CD4^{+} T-cells gating



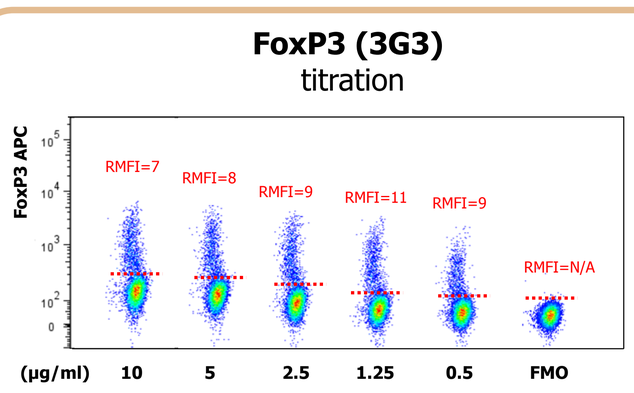
„Treg" gating according to the isotype controls



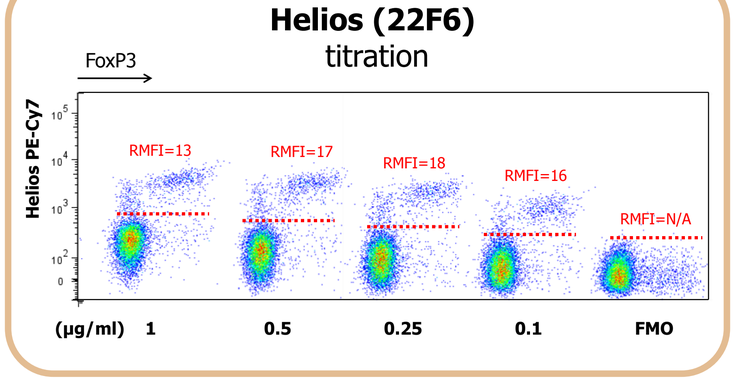
Results IV

- **Intracellular staining provides high RMFI**

FoxP3 (3G3)
titration

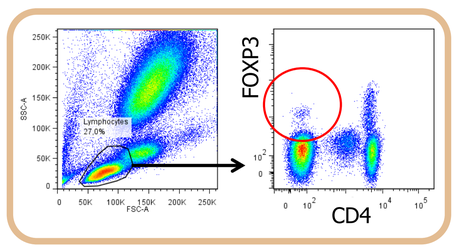


Helios (22F6)
titration

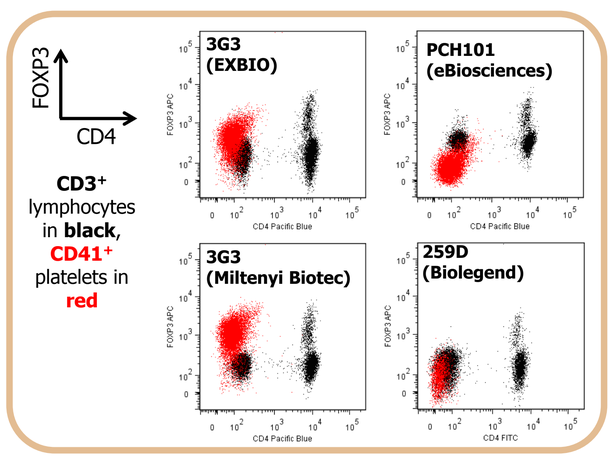


Results V

- **Platelets interference**
  - FOXP3^{+} platelets were found in lymphocyte gate



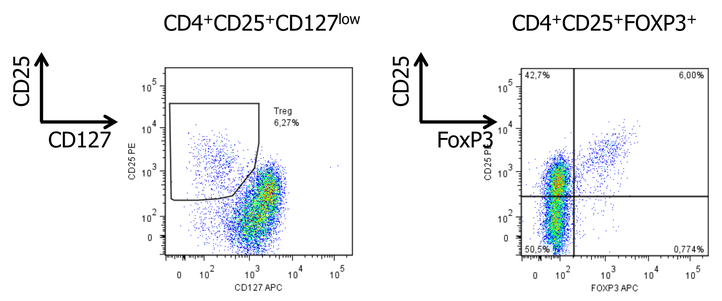
  - Platelets reactivity was clone specific

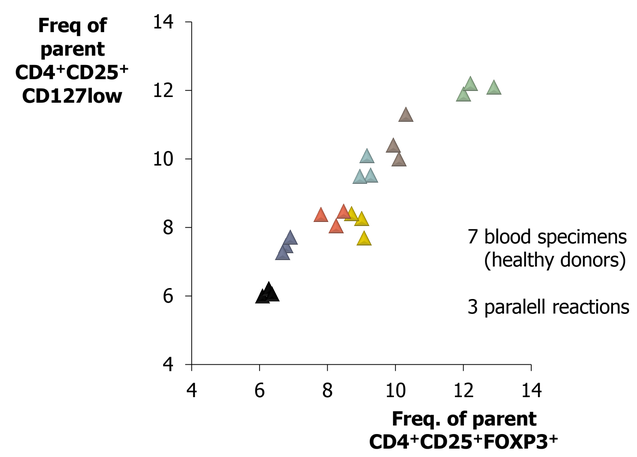


Results VI

- **Treg quantitation with Sm and ICS staining was in a good agreement**

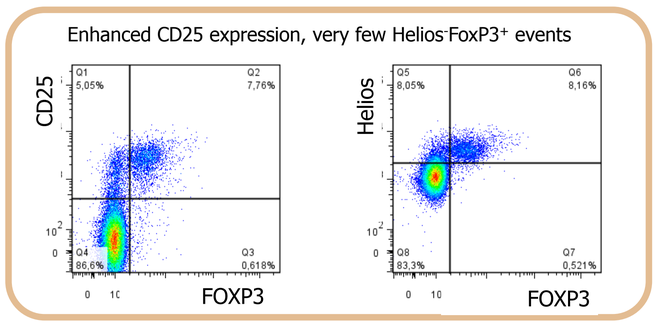
CD4^{+}CD25^{+}CD127^{low} CD4^{+}CD25^{+}FOXP3^{+}



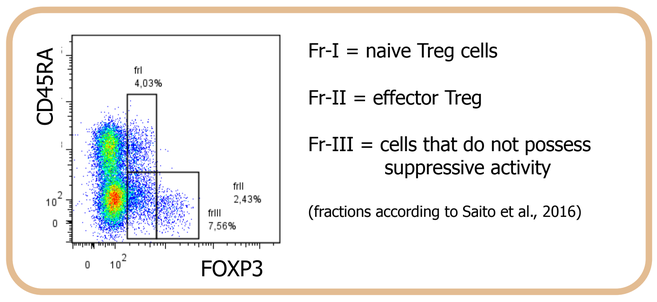


Results VII

- **The method was further tested with: cord blood,**



and CD45RA - Treg fractions I, II and III.



Conclusions

- The developed buffers enhance the 3G3 antibody staining of FOXP3 protein.
- Relative quantitation of Tregs in whole blood from healthy adults showed good agreement with a surface staining procedure.
- Buffers are compatible with staining of other markers, such as Helios and CD45RA.
- Platelets were found to react with 3G3 antibody, however the specificity of the FOXP3 signal is not clear since other FOXP3 antibodies (PCH101 and 259) were unreactive.
- Because there are only a few methods that can process whole blood samples we believe that this development provides a useful tool to investigate blood elements of which function may be regulated by expression of FoxP3.