INTRODUCTION

FoxP3 is a nuclear antigen expressed specifically in regulatory T cells (Tregs). Tregs play a critical role in the maintenance of the dominant self-tolerance and have been subject of extensive research efforts for the last two decades. Today the detection of FoxP3 staining is laborious, time consuming (3-4 hours) and poorly reproducible.

A new commercially available method, named PerFix-nc, allows simultaneous intra- and extra-cellular staining without any wash step. This new permeabilization and fixation procedure allows a reduction of the workflow to 45 min. In these conditions, most common intracellular antigens are easily and efficiently stained.

To this day we aimed at the optimization of the PerFix-nc protocol for the detection of FoxP3, an antigen that is difficult to obtain by currently available techniques. We show that a small modification of PerFix-nc staining procedure allows a clean, efficient and reproducible detection of FoxP3 in whole blood samples.

RESULTS

A new commercially available method, PerFix-nc, allows simultaneous intra- and extra-cellular staining without any wash step. This new permeabilization and fixation procedure allows a reduction of the workflow to 45 min. In these conditions, most common intracellular antigens are easily and efficiently stained.

The discrimination improvement with PerFix-nc allows to gate an additional 1% FoxP3+CD4+ cells with even lower variability. More strikingly, the SN variability has been improved over 4-fold.

Figure 9. External evaluation: In the hands of a clinical research laboratory, multiple blood samples have been analyzed. Preliminary results are illustrated below:

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| Results are significantly improved by the addition of human serum (not provided with eBioscience kit), and clone 206D displays an enhanced S/N ratio (around 7) in this procedure. Figure 2. eBioscience procedure: Comparison of two anti-FoxP3 clones (206D and 259D), with addition of normal human serum to the tube and resuspension of the cells in the same R3 buffer.

• Signal to noise ratio was at least 40% better than the one obtained for the same clone in the PerFix-nc procedure. The discrimination improvement with PerFix-nc allows to gate an additional 1% FoxP3+CD4+ cells with even lower variability. More strikingly, the SN variability has been improved over 4-fold.

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| RESULTS

| RNF6Relative Mean Fluorescence Intensity (RMF) ratio | Staining 60 min - 10µg FoxP3 300µL Perm Buffer - Wash x 2 | Staining 60 min - 10µg FoxP3 300µL Perm Buffer - Wash x 2 | eBioscience | PerFix-nc | RMF6 Relative | 1.00 | 0.99 | 1.00 | 0.99 |
|------------------|--------------------|--------------------|------------------|------------------|------------------|------------------|--------------------|
| CD4 | CD8 \* | CD25 | Viral infection | CD4 | CD8 \* | CD25 | Viral infection | CD4 | CD8 \* | CD25 | Viral infection |
| FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) | FoxP3 (259D-Alexa647) |
| 8% | 8% | 8% | 8% | 8% | 8% | 8% | 8% | 8% | 8% | 8% |

*Galileo PerFix-nc and Kaluza are for research use only. Not for use in diagnostic procedures. Galileo and Kaluza are trademarks of Beckman Coulter, Inc. The deconvolved peaks were registered in the CFP12, Alexa Fluor is a registered trademark of Molecular Probes, Inc.

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It is clear from this experiment that the first wash with PBS plays a critical role in the quality of the staining for both clones.

Nevertheless, and in contrast to the eBioscience procedure (see Figure 2), the clone 206D is more efficient for FoxP3 staining in the optimized PerFix-nc procedure. Figure 4. Emission spectrum detection with 488-nm and 661-nm excitation.

Figure 7. Questionable role of CD25 in the gating of Tregs.

PerFix-nc procedure allows an easy separation of FoxP3+ cells from CD4- FoxP3- cells demonstrating then no benefit in using a CD25 marker for Treg gating. It thus offers the possibility to use more markers for FoxP3 cells characterization.

Figure 8. Reproducibility studies:

Eight replications were performed on the same blood sample using either PerFix-nc or eBioscience procedures.

In order to analyze the variability of the results mean % of positive cells and SN ratios, and the corresponding standard deviations are shown: The discrimination improvement with PerFix-nc allows to gate an additional 1% FoxP3+CD4+ cells with even lower variability. More strikingly, the SN variability has been improved over 4-fold.

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