Flow cytometric differentiation of distinct A blood groups

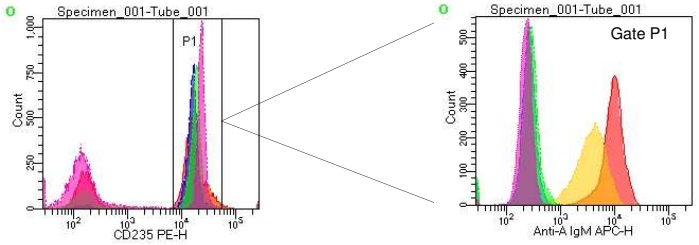
Eduardo Meyer, Stephanie Glaus, Young-Lan Song, Charlotte Engström, Beat M. Frey
Blood Transfusion Service Zurich, SRC, Schlieren, Switzerland

BLUTSPENDE SRK ZÜRICH

Background

Serologically variant ABO subgroups are defined by weakened agglutination with anti-A, anti-B and anti-A,B. Furthermore, a weak subtype can be suspected due to the absence or presence of ABO antibodies in the plasma. A_2 and A_2B are usually distinguished from other weak ABO subtypes by positive reactions with anti-Ahel. Still, detection and distinction of A and B subgroups is challenging for serological and genetic methods. The aim of this study was to optimize flow cytometry for differential detection of weak A antigen expression on erythrocytes.

Figure 1: flow cytometric Anti-A IgM (BIRMA-1) without glutaraldehyde (standard method)



Sample color:	AB0-Bloodgroup	MFI APC (Anti-A IgM)
RED	A_1	9'659
ORANGE	A_2	3'942
BLUE	0	277
GREEN	B	316
PURPLE	A_{weak}	235

Discrimination between the normal blood groups A_1/A_2 and non A as well as between A_1B / A_2B (not shown here) is possible. The serological A_{weak} sample can't be distinguished from O and B. (MFI = mean fluorescence intensity)

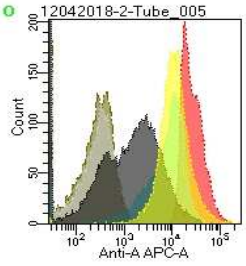
Methods

Analysis was performed on a flow cytometer (FACS Canto II, Becton Dickinson, Allschwil, CH) and measured with identical instrument settings. BD FACSDiva software was used for graphical presentation (histogram). RBCs were incubated with anti-A (BIRMA-1, Merck, Darmstadt, D). Next, antigen-antibody bonding was fixed with 1.5% glutaraldehyde ($C_5H_8O_2$) before a secondary labelled antibody (Alexa Fluor® 647 AffiniPure Goat Anti-Mouse IgG, Jackson ImmunoResearch Europe Ltd, UK) was added. Fixation with glutaraldehyd was repeated. Finally, gating of RBC was ensured by additional staining with anti-Glycophorin A (GPA, CD235a APC, Becton Dickinson AG, Allschwil, CH).

Results

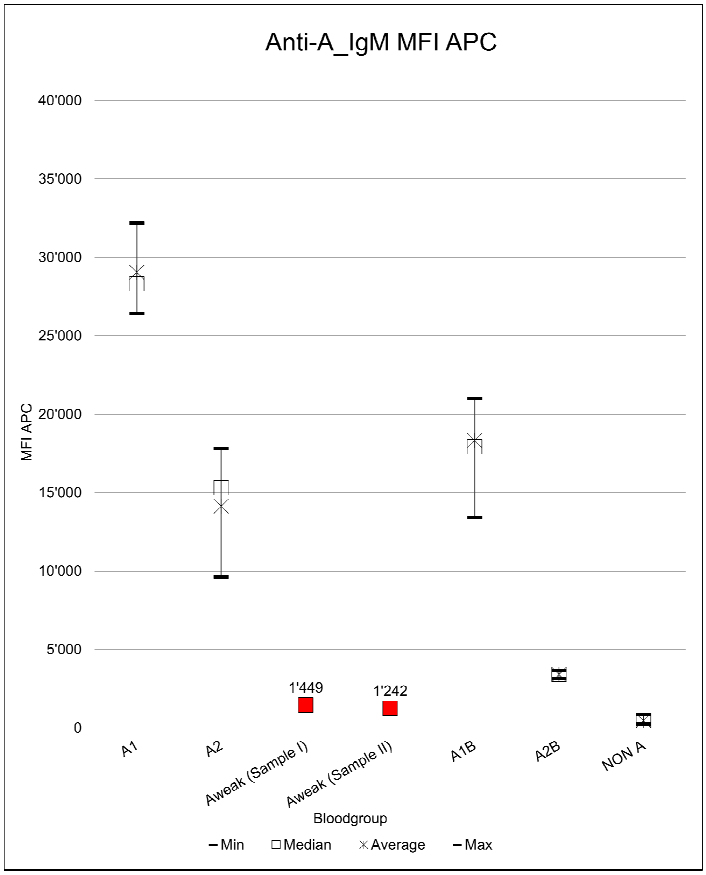
In total 37 serological clearly defined samples (A_1 (7; MFI mean: 29'283), A_2 (7; MFI mean: 15'598), A_1B (7; MFI mean: 19'695), A_2B (2; MFI mean: 13'956), B (7; MFI mean: 404), 0 (7; MFI mean: 468)) and 2 serological weak A subtypes (0-2+ agglutination with different monoclonal anti-A and anti-A,B and no reaction with anti-Ahel) were examined. Distinct flowcytometric patterns based on the number of expressed A antigen-expression of the different ABO phenotypes were displayed. The 2 serologically A_{weak} samples showed a distinguishable intermediate MFI (1'449 and 1'242).

Figure 2: flow cytometric Anti-A IgM (BIRMA-1) with glutaraldehyde



Sample color:	AB0-Bloodgroup	MFI APC (Anti-A IgM)
RED	A_1	29'241
ORANGE	A_2	15'342
GRAY	0	266
BROWN	B	249
BLACK	A_{weak}	1'449

Figure 3: MFI APC comparison between the different AB0 blood groups (under use $C_5H_8O_2$)



Conclusion

By evaluating different staining approaches the most solid results were obtained with dual fixation of antibodies by glutaraldehyde. With this recipe we observed reproducible MFI's corresponding to the expected amount of antigens expressed. Furthermore, we could clearly discriminate the 2 serologically weakened A samples from the common ABO phenotypes, supporting the sensitivity of this flowcytometric method. It must be noted, though, that this method will need to be further proven by investigating additional serologically and genetically defined ABO subgroups.