FLOW CYTOMETRIC DISCRIMINATION OF DIFFERENT ABO PHENOTYPES

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Background

Serologic variant ABO subgroups are defined by weakened red blood cell (RBC) agglutination with anti-A, anti-B and anti-A,B. Furthermore, Aweak subtypes can be suspected if Anti-A isoagglutines are unexpectedly missing. A_2 and A_2B are usually distinguished from other weak ABO subtypes by positive reactions with anti-A_{hel}. Still, distinction of A and B subgroups is challenging using serologic and molecular methods. The aim of this study was to optimize flowcytometry to differentiate phenotypes with weakened A antigen expression.

Methods

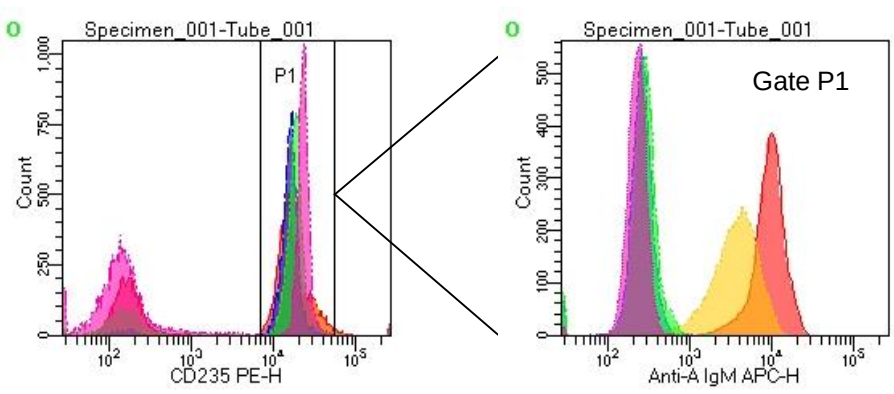
Analysis was performed on a flowcytometer (FACS Canto II, Becton Dickinson, Allschwil, CH) applying identical instrument settings for all samples analysed. BD FACSDiva software was used for graphical presentation (histogram). RBCs were incubated with anti-A (BIRMA-1, Merck, Darmstadt, D). Next, antigen-antibody complexes were stabilized with 1.5% glutaraldehyde ($C_5H_8O_2$) before adding secondary antibody (Alexa Fluor® 647 AffiniPure Goat Anti-Mouse IgG, Jackson ImmunoResearch Europe Ltd, UK) followed by another round of glutaraldehyde. Finally, RBCs were stained with anti-Glycophorin A (GPA, CD235a APC, Becton Dickinson AG, Allschwil, CH) and only GPA positive events were gated.

Results

As shown in Figure 1, Anti-A staining of RBCs without applying incubation with glutaraldehyde reveales broadly overlapping staining pattern of A+ RBCs and more important, Aweak RBCs become indistinguishable from A- control RBCs. Upon use of glutaraldehyde differential expression of A antigens by A variants becomes detectable by FACS (Figure 2).

37 samples typed by serology were assessed by FACS using glutaraldehyde fixation: 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 two samples of Aweak RBCs (Figure 3).

Figure 1: Staining pattern of A+ RBCs with Anti-A IgM (BIRMA-1) without applying glutaraldehyde fixation



Sample color:	AB0 blood group	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

Variant expression of A-antigen is shown for A_1, A_2, A_{weak} and non-A RBCs. Note: Overlapping staining of Aweak and non–A RBCs. (MFI = mean fluorescence intensity)

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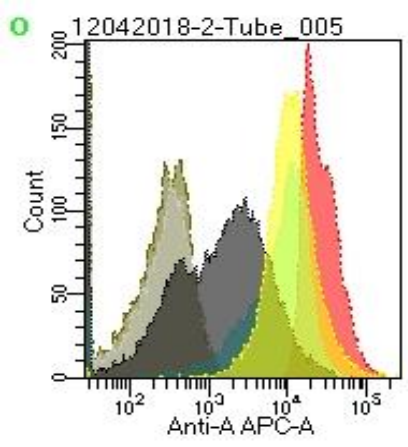
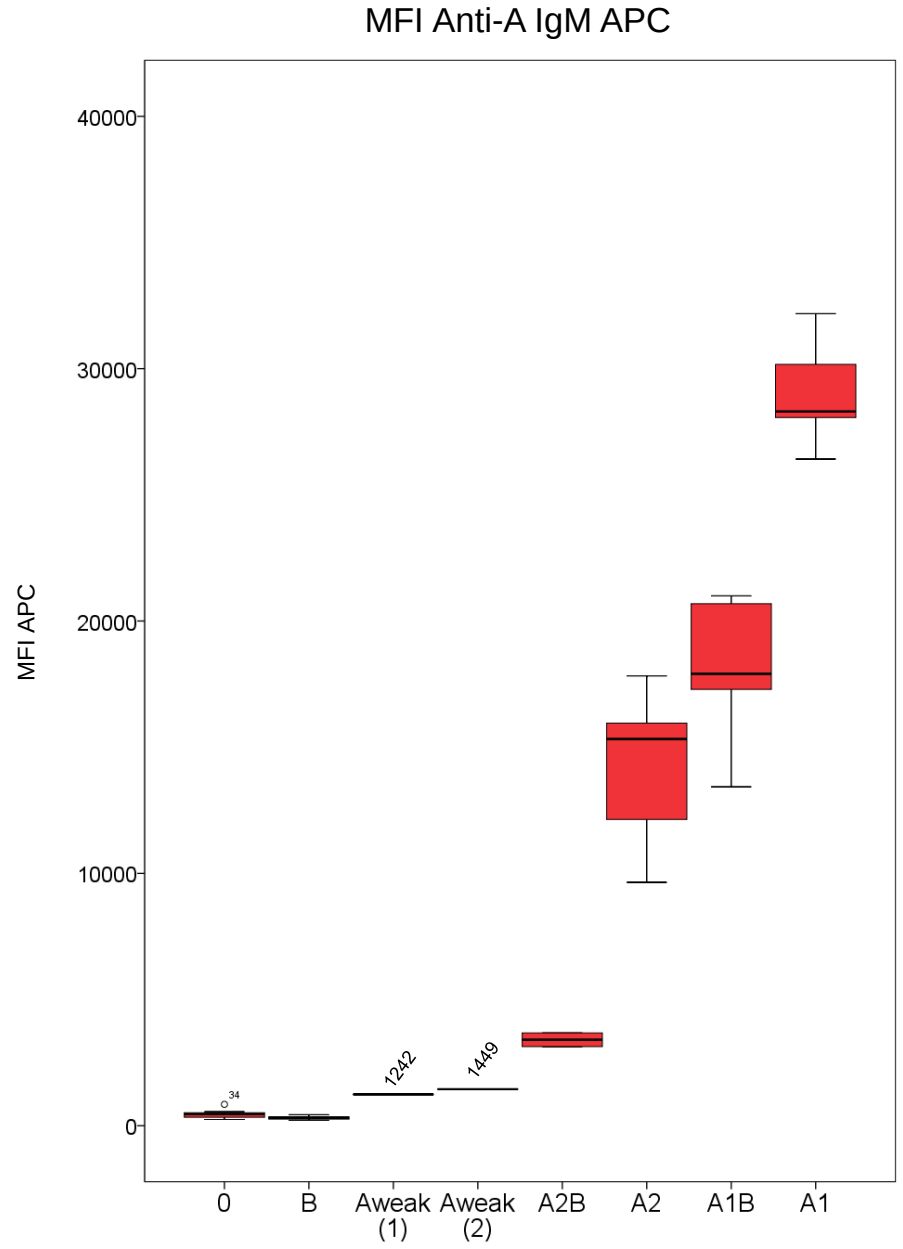
Figure 2: Differential staining pattern of A epitopes using anti-A IgM (BIRMA-1) with glutaraldehyde fixation of antigen/antibody complexes



Sample color:	AB0 blood group	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 shows relative quantification (MFI, by flowcytometry) of A antigen expression on RBCs' surface which correlates with ABO phenotypes. A_{weak} samples express significantly lower amount of A antigens (MFI: 1'449 and 1'242) as compared to regular A phenotypes and remain distinguishable from negative control RBCs.

Figure 3: Anti-A MFI APC of different AB0 blood types using glutaraldehyde to stabilize antibody staining



Conclusion

By evaluating different staining approaches of RBCs the most solid results were obtained if the staining protocol was completed by incubation with glutaraldehyde. With this recipe we observed reproducible MFIs corresponding to ABO phenotypes. Furthermore, we could clearly discriminate A_{weak} samples from common ABO and non-A phenotypes, supporting the specificity of flowcytometry. Further studies of $A_{variants}$ are needed to validate our approach.

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