FLOW CYTOMETRIC SEMI-QUANTIFICATION OF H SUBSTANCE ON RED BLOOD CELLS

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Background

The H blood group contains one antigen, the H antigen, which is present on virtually all red blood cells (RBC) and is the acceptor substrate of both the *A* and *B* gene encoded glycosyltransferases. In blood group O the H antigen remains unmodified and therefore O RBCs show the highest amount of H antigens. The reverse applies to AB RBCs which possess least amount of H antigens. Individuals with the so called Bombay phenotype carry homozygous *Hnull* alleles (*h* | *h*) and do not produce any H antigens. The Para-Bombay phenotype retains some H antigens on RBCs either induced by a weakly active (*H+w* | *H+w*) or silenced *FUT1* gene (*h* | *h*), associated with an active *FUT2* gene.

Aim

In this study, we aimed to develop an adapted flowcytometry protocol allowing to quantify the relative amount of H substance present on RBCs for classification of ABO phenotypes.

Methods

The analyses were performed on a flowcytometer (FACS Canto II, BD Biosciences, CH) and measured with identical instrument settings. List mode data were evaluated and visualized using BD FACSDiva software. RBCs were incubated with increasing concentrations of monoclonal anti-H antibodies (BRIC231-PE and with a 1:1 mixture of BRIC231-PE/BRIC231, IBGRL, UK) to define saturating concentration of anti-H. After rinsing the cells with PBS, micro-aggregates were mechanically dissolved. RBCs from 29 blood donors with different ABO phenotypes (O (5), A1 (5), A2 (5), B (5), A1B (5), A2B (5)) and 3 samples with confirmed Bombay (2) and Para-Bombay (1) phenotypes were assessed.

Results

Type O RBCs were incubated with a 1:1 mixture of conjugated/non-conjugated BRIC231 (see methods) to reach saturating staining of H antigen binding sites. Non-conjugated BRIC231 antibodies shielded approx. half of H binding sites allowing to titrate BRIC231-PE for complete coverage of the rest of H binding sites. In contrast, non-O type RBCs reached saturation of H-binding sites just by using BRIC231-PE. At saturating concentration of BRIC231-PE, RBCs revealed a distinct staining pattern according to A phenotype. Figure 1 depicts the MFI (mean fluorescence intensity) upon staining with BRIC231-PE as a proxi for H antigen sites of various A phenotypes. Figures 2 – 5 reveal prototypic staining pattern of ABO phenotypes with BRIC231-PE including Bombay and Parabombay blood type.

Figure 1: RBCs incubated with increasing concentrations of monoclonal anti-H (BRIC231) antibodies. For O-RBCs a 1:1 mixture of anti-H was used.

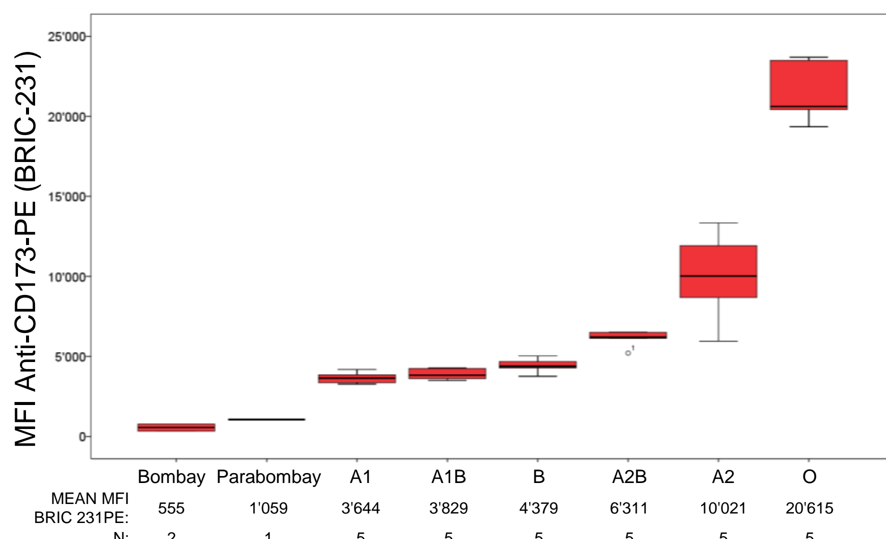


Figure 2: A_1 Erythrocytes

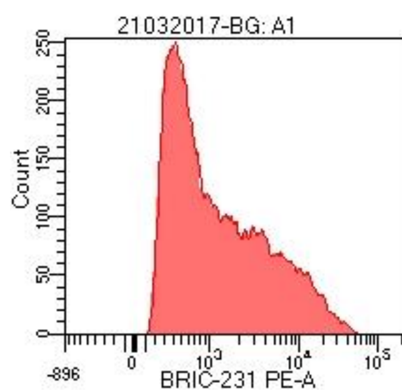


Figure 3: O Erythrocytes

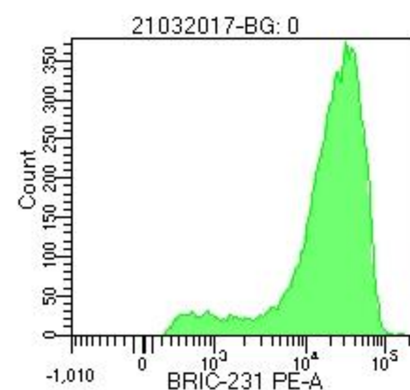


Figure 4: Bombay RBCs

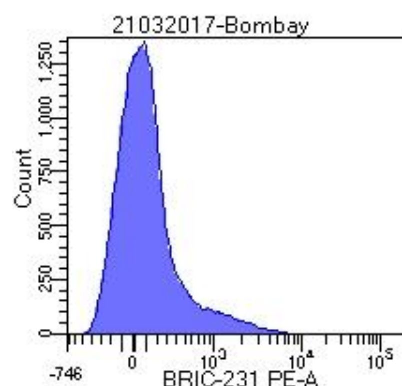
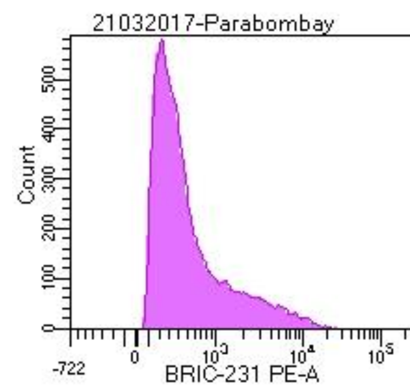


Figure 5: Parabombay RBCs



The histograms display distinct BRIC231-PE staining pattern (H-expression) depending on A/O phenotypes.

Conclusion

Modified flowcytometry protocol is able to detect variant expression of H antigens on RBCs. Thus, our flowcytometry method may complement serologic and genetic ABO typing and more intriguing, it may be used to confirm Bombay and Para-Bombay phenotypes. It will be of interest to further evaluate the protocol by typing of A-/B- variants for variant H expression.

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