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 A and B gene-specified glycosyltransferases. In blood group O the H antigen remains unmodified and therefore its RBCs show the highest and the RBCs of blood type AB the least amounts of H antigen. Individuals with the so called Bombay phenotype carry homozygous Hnull alleles (h | h) and do not produce any H antigen. The para-Bombay phenotype retains some H antigen on RBCs either induced by a weakly active (H+w | H+w) or completely silenced FUT1 gene (h | h), mandatory linked with an active FUT2 gene.

Aim

In this study, we aimed to develop an adapted flow cytometric method to quantify the relative amount of H substance present on RBCs in order to distinguish different ABO phenotypes in routine diagnostics as well as to capture rare H-deficient phenotypes.

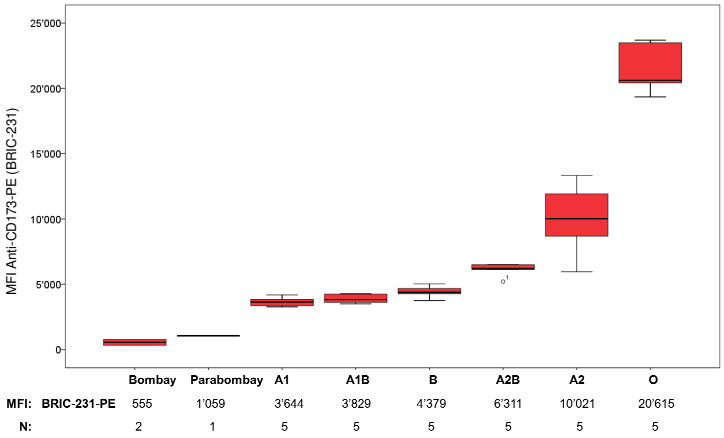
Methods

The analyses were performed on a flow cytometer (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). 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 genetically confirmed Bombay (2) and Para-Bombay (1) phenotype were assessed (Figure 1).

Results

Saturation of H antigen binding sites on type O RBCs was achieved only upon use of a 1:1 antibody mixture (BRIC231-PE/BRIC231) covering approx. half of the H-binding sites by unconjugated BRIC231. In contrast, Non-O type RBCs reached saturation of H-binding sites using pure BRIC231-PE. RBCs coated with BRIC231-PE at saturation revealed a distinct pattern of MFI (mean fluorescence intensity) depending on the ABO phenotype (Figure 1). The amount of H-substance clearly correlates with ABO types (Figure 2 – 5) and confirms Bombay and Parabomby blood type.

Figure 1: RBCs incubated with increasing concentrations of monoclonal anti-H antibodies



This boxplot (Figure 1) gives a good indication of how the MFI (saturation of H-binding) in the ABO-phenotype are spread out.

Figure 2: A_1 Erythrocyte

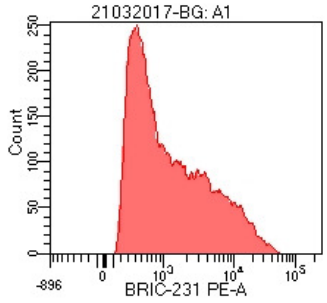


Figure 3: O Erythrocyte

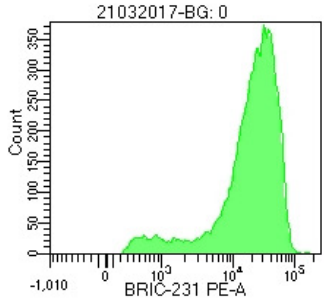


Figure 4: Bombay

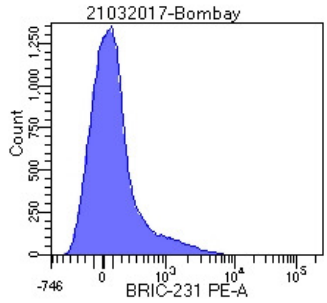
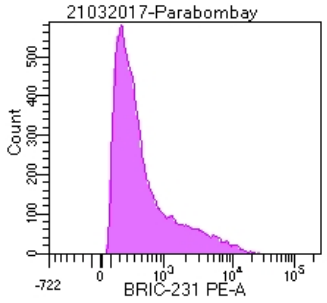


Figure 5: Parabombay



These histograms (Figures 2-5) are an accurate representation of the fluorescence activity of BRIC-231-PE positive RBCs.

Conclusion

Adapted flow cytometry is able to distinguish variant expressions of RBCs H antigen. Thus, our flow cytometric method may complement serologic and genetic analyses in routine ABO typing and more intriguing, when the Bombay or para-Bombay phenotype is suspected. It will be of interest to further evaluate this method for typing of A-/B-variants.

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