Flow Cytometric Evaluation of Red Blood Cells Transformed with Variable Amounts of Synthetic A and B Glycolipids

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Background

According to national guidelines or directives, monoclonal ABO reagents may be required to detect A_x and B_{weak} subgroup red blood cells (RBCs). Many routine laboratories do not have access to naturally-occurring ABO subgroups that can be used as weak controls for these reagents. Group O RBCs modified with synthetic analogs of blood group A and/or B glycolipids (KODE™ technology) to mimic weak ABO subgroups could be used for quality control purposes.

Aim of the Study

Extensive serological testing of KODE™ RBCs has previously been carried out. An extended evaluation of KODE™ RBCs using flow cytometry was performed to explore the correlation between the concentrations of synthetic glycolipids and A/B antigen site density of the resulting RBCs. The aim of this study was to examine if KODE™ RBCs mimic the distinct flow cytometric patterns of naturally-occurring ABO subgroups and to identify the optimal concentration of glycolipid required.

Materials & Methods

Samples: KODE™ RBCs were prepared according to a previously described procedure.[1] RBCs were modified with 15 different concentrations of synthetic glycolipids, ranging from 1000 µg/mL to 0.06 µg/mL for KODE™-A and 5000 µg/mL to 0.3 µg/mL for KODE™-B. The concentration was decreased by doubling dilution steps. For both KODE™-A and KODE™-B RBCs, repeat samples were produced for four selected concentrations as a consistency measurement and all KODE™ batches were tested in triplicate.

Flow Cytometry: Sensitive and specific flow cytometry was used to characterize and semiquantify the synthetic A and B antigen levels on group O RBCs. Relevant control RBCs (A_1, A_2, A_x, B, B_{weak} and O) were included in each run.
Primary antibodies: Anti-A (ES-15, Serologicals Limited, West Lothian, UK) Anti-B (9621A8, Diagast, France). Secondary antibody: PE-labelled rat-anti-mouse Ig kappa light chain (Becton Dickinson, CA, USA).

Figures

KODE™ -A RBCs tested with anti-A

Figure 1.

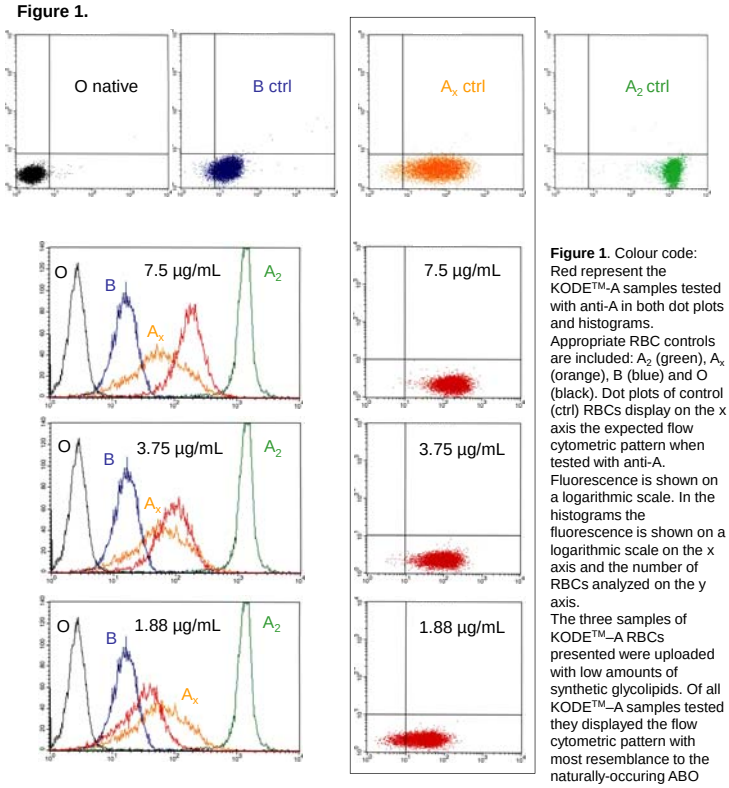

Figure 1. Colour code: Red represent the KODE™-A samples tested with anti-A in both dot plots and histograms. Appropriate RBC controls are included: A_2 (green), A_x (orange), B (blue) and O (black). Dot plots of control (ctrl) RBCs display on the x axis the expected flow cytometric pattern when tested with anti-A. Fluorescence is shown on a logarithmic scale. In the histograms the fluorescence is shown on a logarithmic scale on the x axis and the number of RBCs analyzed on the y axis.
The three samples of KODE™–A RBCs presented were uploaded with low amounts of synthetic glycolipids. Of all KODE™–A samples tested they displayed the flow cytometric pattern with most resemblance to the naturally-occuring ABO subgroup A_x.

KODE™ -B RBCs tested with anti-B

Figure 2.

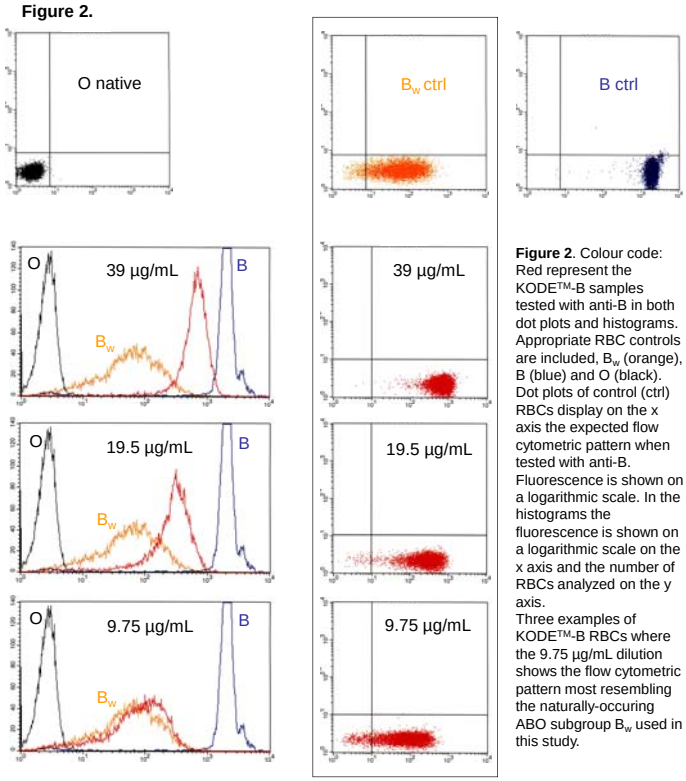

Figure 2. Colour code: Red represent the KODE™-B samples tested with anti-B in both dot plots and histograms. Appropriate RBC controls are included, B_w (orange), B (blue) and O (black). Dot plots of control (ctrl) RBCs display on the x axis the expected flow cytometric pattern when tested with anti-B. Fluorescence is shown on a logarithmic scale. In the histograms the fluorescence is shown on a logarithmic scale on the x axis and the number of RBCs analyzed on the y axis.
Three examples of KODE™-B RBCs where the 9.75 µg/mL dilution shows the flow cytometric pattern most resembling the naturally-occuring ABO subgroup B_w used in this study.

Results

Flow cytometric testing of KODE™ RBCs modified with high concentrations of synthetic glycolipids revealed a uniform and even distribution of antigens in the cell population as shown by a single narrow peak in the FACS histograms. When lower concentrations were used, peaks tended to broaden to a pattern found in A_x and most B subgroups indicating a more variable antigen site density on the cells in the population. The concentrations of synthetic glycolipids which produced KODE™ cells that resembled the naturally-occurring subgroup control RBCs used in this study are ~2-4 µg/mL for KODE™-A and ~10 µg/mL for KODE™-B. Repeat testing demonstrated good correlation between flow cytometric runs and KODE™ batches.

Conclusion

Using very low amounts of synthetic glycolipids, KODE™-A and KODE™-B RBCs can be made to mimic A_x and B_{weak} subgroup control RBCs, respectively, according to this flow cytometric study. With higher concentrations of synthetic glycolipids, the KODE™ RBCs demonstrated a more uniform and even distribution of antigens among the cells. This is in contrast to naturally-occurring subgroups in which some cells express almost no A or B antigen whilst others have close to normal levels. The reason for this is unknown. KODE™ RBCs obviously lack A/B-carrying glycoproteins but it is not fully understood to what extent glycolipid versus glycoprotein A/B epitopes contribute to the phenotype of weak subgroups. This study indicates that KODE™ RBCs with weak expression of A and/or B antigen have characteristics compatible with use as quality controls for monoclonal ABO reagents and could be a valuable addition in the serological laboratory.

References

1. Frame *et al.*, Synthetic glycolipid modification of red blood cell membranes. Transfusion 2007;47:876-82
2. Hult A & Olsson ML. Genetically defined ABO subgroups exhibit distinct flow cytometric patterns. Transfusion 2006;46:32A.