

# 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<sub>x</sub> and B<sub>weak</sub> 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:** Extensive serological testing of KODE™ RBCs has previously been performed. An extended evaluation of KODE™ RBCs using flow cytometry was performed to explore the correlation between the concentrations of synthetic glycolipids and A/B 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.

**Material and Methods:** KODE™ RBCs were prepared according to a previously described procedure [Frame *et al.*, *Transfusion* 2007;47:876-82]. RBCs were modified with 15 different concentrations of synthetic glycolipids, ranging from 1 mg/mL to 60 ng/mL for KODE™-A and 5 mg/mL to 0,3 µg/mL for KODE™-B. The concentration was decreased by doubling dilution steps. Sensitive and specific flow cytometry [Hult & Olsson, *Transfusion* 2006;9S:32A] was used to characterize and semiquantify the synthetic A and B antigen levels on RBCs. Relevant control RBCs (A<sub>1</sub>,A<sub>2</sub>,A<sub>x</sub>,B,B<sub>weak</sub> and O) were included in each run. For both KODE™-A and KODE™-B RBCs, repeat samples were produced for four selected concentrations and all KODE™ batches were tested in triplicate.

**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<sub>x</sub> and most B subgroups indicating a more variable antigen site density on the cells in the population. The concentrations of synthetic glycolipids that 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.

**Discussion and Conclusion:** Using very low amounts of synthetic glycolipids, KODE™-A and KODE™-B RBCs can be made to mimic A<sub>x</sub> and B<sub>weak</sub> subgroup control RBCs, respectively, according to this flow cytometry method. 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.