

Automated Detection of Low-Level A-Antigen on Red Blood Cells (SP334)

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Introduction: RBC phenotyping is used to identify compatible components for alloimmunized transfusion recipients. Additionally, efforts to develop “universal” donor RBCs by enzymatic conversion of group A and B cells may require very sensitive methodologies for high-throughput confirmation of antigen removal. We investigated the utility of a completely automated fluorescence cytometric immunohematology platform (3Ti Aegis) for highthroughput phenotyping of red cells with varying levels of A-antigen expression.

Materials and Methods: All samples were tested in a completely automated fashion using the 3Ti Aegis workstation. Data acquisition utilized an integrated, commercially-available capillary cytometer. Off-line data analysis required approximately 30 seconds per sample.

Results: We first titrated a commerciallyavailable blend of murine monoclonal anti-A antibodies (Ortho) and the ES-15 monoclonal anti-A antibody for optimal detection of Aantigen on A1, A2, Ax, Ael, and Am RBCs. Consistent with our previous data, both the blended typing reagent and ES-15 could readily detect A-antigen on A1 and A2 cells at high-dilutions (> 1:100). However, the assay was initially ineffective at reliably detecting low levels of A-antigen on some A-subgroup cells, even when using undiluted antibodies. Sensitivity was markedly improved through enzyme (ficin) pretreatment of cells prior to testing and by slightly extending (from 1 to 5 mins) the antibody- RBC incubation period. Multiple examples of A1 (n=10) and A2 (n=10) cells were then tested using antibodies at a 1:100 dilution, while Ax (n=3), B (n=103) and O (n=10) red cells were tested using undiluted antibodies. Each RBC-antibody combination was tested in triplicate. Using this approach, each example of A1, A2, and Ax RBCs was clearly and reliably phenotyped, while group O cells were reproducibly negative under these conditions. Interestingly, all (n=54) ficin-treated B RBCs samples were weakly, but reproducibly, reactive with ES-15 (but not the monoclonal blend), consistent with reports of low-levels of A-antigen expression on B RBCs. We also performed automated crossmatches between group O, group B, and group A or AB plasma samples (n=40 each) with examples of group A1, A2, B, and O (n=5 each) cells. The expected reactivity was seen in each case. Reactivity between O plasma and Ax (n=3) cells was occasionally seen.

Conclusions: These results demonstrate that the completely automated Aegis platform is sufficiently sensitive to perform high-throughput RBC A antigen phenotyping and crossmatch for routine blood bank use, and may also be applicable as a quality control approach to validate enzymatic antigen removal from red cells prior to transfusion.