Quantification of residual red blood cells in platelet concentrates and fresh frozen plasma by flowcytometry

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Introduction: Blood component products are considered pharmaceuticals and must therefore comply with stringent product specifications. Measurement of residual red blood cells (rRBC) in platelet concentrates (PC) and fresh frozen plasma (FFP), requires quantification techniques that perform 2 to 3 log below linearity range of regular cell counters. Flowcytometry seems to be suitable for accurate rRBC counting in blood products.

Method: Based on selective and ample expression of glycophorin-A by the RBC membrane, we designed a staining protocol for RBCs in PC and FFP using a commercially available, phycoerythrin (PE)-labeled, anti-glycophorin-A monoclonal antibody (aGlyA-PE,murine IgG2b, clone GA-R2 (HIR2), Pharmingen). Stained RBCs and reference beads (TruCount tubes, BD) were quantified by flowcytometry applying a specifically designed gating strategy which allows simultaneous counting of aGlyA-PE positive events (rRBCs) and microbeads. The rRBC concentration and total amount of rRBCs per unit were calculated based on known concentration of microbeads. Briefly, aGlyA-PE first was diluted with FACSFlowTM (pH 7.4, Becton Dickinson) containing 0.5% BSA (bovine serum albumin) in order to eliminate agglutinating activity of the antibody but still maintaining adequate surface staining. 500 ul of the stained aliquot was then used to resuspend the microbeads according to manufacturer's recommendation. Flowcytometrically determined relative concentration of RBCs and beads were then used for calculation of number of rRBCs in the unit tested. The protocol was validated by measuring geometrically diluted PCs and FFPs, that have been spiked with known number of blood group identical RBCs.

Results: The optimal dilution factor of aGlyA-PE for use in PC and FFP is 1:50 (v/v) and 1:250 (v/v), respectively. Prediluted aGlyA-PE proved to be stable and fully active for more than 14 days, if stored at +4°C. Applying linear regression analysis of spiking experiments, the quantified rRBCs in PC and FFP correlate strongly with the expected numbers in the respective products (R2PC = 0.9997, R2FFP = 0.9928 resp.).The coefficient of variation for determination of rRBCs in PC and FFP was below 5% (VPC = 3.0%, VFFP = 0.7%) assessing routine products.

Conclusion: 1. Our protocol provides a reliable and accurate method for enumeration of rRBCs in blood products using conventional flowcytometry. 2. Compared with standard methods such as chamber counting, our method minimizes technician related inaccuracy and has the capacity of high throughput of samples. 3. Flowcytometric quantification of residual cell contaminants may become indispensable for evaluation and validation of new manufacturing techniques and continuous quality control of blood products.