Additive solutions differentially influence the effects of hypoxic storage on red blood cell in vitro quality

**Background:** Hypoxic storage of red blood cells (RBC), achieved by depletion of oxygen (O₂) and carbon dioxide (CO₂), is reported to influence RBC metabolism and maintain 2,3-DPG. Differences in blood processing methods and RBC additive solutions may also impact RBC metabolism during storage. This study assessed the impact of hypoxic and conventional normoxic storage conditions using three RBC additive solutions, SAG-M, AS-3 or PAGGSM.

**Study Design/Methods:** RBC components were prepared from whole blood held overnight (<18 hours) using top-and-bottom separation and resuspended in three different additive solutions; SAG-M, AS-3 or PAGGSM. Pairs of RBC in each additive solution were then pooled and split; one of each pair was processed with the Hemanext® RBC Processing System and the other stored in conventional storage bags. RBC in each study arm (n=8 replicates) were tested on day 2, 7, 14, 21, 28, 35 and 42 for biochemical characteristics and biomodulatory capacity. Data were analyzed by two-way repeated measures ANOVA adjusted for multiple comparisons; p<0.05 was considered significant.

**Results:** Hypoxic storage reduced the percent oxygen saturation (SO₂) of the hemoglobin in the RBC to <20%, with partial pressure of oxygen (pO₂) at 37°C maintained at <20 mmHg throughout storage in all additives. RBC in PAGGSM consumed significantly more glucose (p=0.0032) and RBC in all additives produced more lactate (p<0.0001). Only hypoxic red cells stored in AS-3 had significantly higher ATP (p=0.0132). RBC stored under hypoxic conditions in all additive solutions maintained significantly higher 2,3-DPG concentrations than conventionally stored RBC (p<0.0001 for all additives), and 2,3-DPG was over 10-fold higher on day 7, 14 and 21 in hypoxic RBC stored in SAG-M and PAGGSM. Hemolysis was slightly higher in RBC stored hypoxically in AS-3, PAGGSM and SAG-M, although not statistically significant (p=0.2822, 0.5977 and 0.1555 respectively) and still well below the Council of Europe and AABB limits of <0.8% and <1.0% respectively. Hypoxic storage of RBC in SAG-M also led to significantly higher potassium release (p< 0.0001), as well as increased binding of anti-CD47 2D3 antibody, suggesting a conformational change associated with increased red cell clearance. Supernatants from RBC stored hypoxically or conventionally did not activate human umbilical vein endothelial cells, with no significant differences in secretion of IL-8, IL-6, RANTES or sCD62P, or expression of endothelial cell surface activation markers E-selectin and V-CAM.

**Conclusion:** Hypoxic storage of RBC better maintains 2,3-DPG compared to conventional storage. Based on the present in vitro data on RBC quality, hypoxic storage is suitable for use with AS3 and PAGGSM and not with SAG-M.