Comparison of Hemolysis in Circulating Cell-free DNA Stabilization Tubes

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Introduction

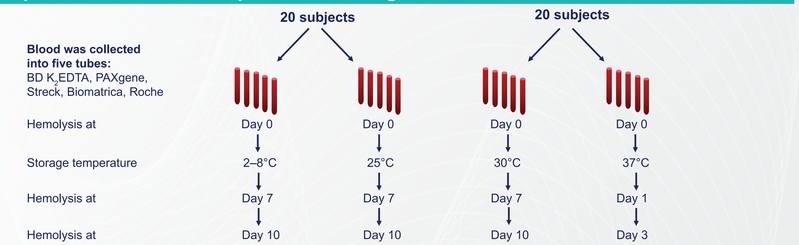
Tubes with additives to stabilize circulating, cell-free DNA (ccfDNA) are used to collect and store whole blood for purification of ccfDNA from plasma. The whole blood ccfDNA stabilization chemistry of additives varies based on the tube manufacturer and may impact downstream ccfDNA research applications and/or important sample quality parameters such as hemolysis. Hemoglobin released into plasma of ccfDNA stabilizing tubes from hemolysis of red blood cells may interfere with downstream PCR assays, although, its impact on ccfDNA-based assays is not clear. Hemolysis can make the boundary between the plasma and cellular fractions of the specimen difficult to see during plasma transfer leading to incomplete plasma transfer or contamination of the plasma with cellular fraction. Hemolysis is also a general indicator of poor sample integrity.

The PAXgene® Blood ccfDNA Tube (RUO)*, is a plastic, evacuated tube containing an additive that anticoagulates blood and stabilizes ccfDNA in whole blood. The tube contains a non-crosslinking stabilization solution that prevents release of intracellular DNA into plasma and stabilizes ccfDNA levels. The tube is also designed to minimize hemolysis during blood storage. This study evaluated hemolysis levels in plasma of PAXgene Blood ccfDNA Tube (PreAnalytiX®) in comparison with an EDTA tube and three other brands of ccfDNA stabilization tubes from Streck®, Biomatrica®, and Roche®.

Methods

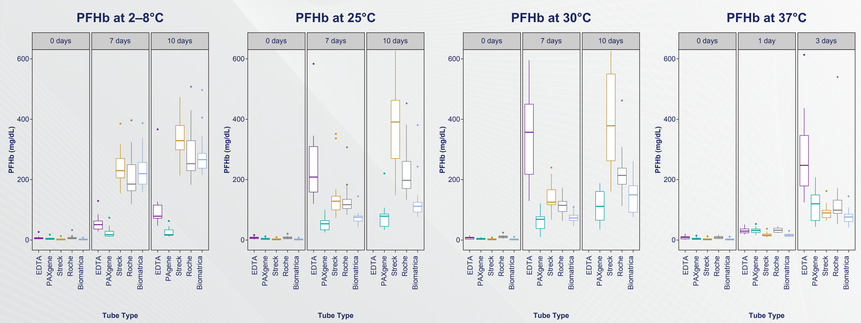
Blood was collected from 40 apparently healthy subjects into BD K_2EDTA tubes, and into stabilization tubes from PAXgene Blood ccfDNA Tubes, Streck Cell-free DNA BCT®, Roche Cell-Free DNA Collection Tubes, and Biomatrica LBgard® Blood Tubes. Tubes with blood were stored at 2–8°C, 25°C and 30°C for 10 days and at 37°C for 3 days. Twenty (20) subjects were used for 2–8°C and 25°C and the second 20 for 30°C and 37°C storage temperatures. Blood was collected and processed per the recommended handling conditions for each tube type. At each test time point, a 1 mL aliquot of whole blood was sampled from each tube into a secondary container. Aliquots were centrifuged, and the plasma visually assessed for hemolysis and then tested for plasma-free hemoglobin (Beckman Coulter® UniCel® DxC 680i) and Hemolysis Index (Roche cobas® 6000). Mean biases for plasma-free hemoglobin (PFHb) and Hemolysis Index (HI) between PAXgene and other tubes were estimated at two storage time points for each storage temperature.

Schematic representation of the experimental design

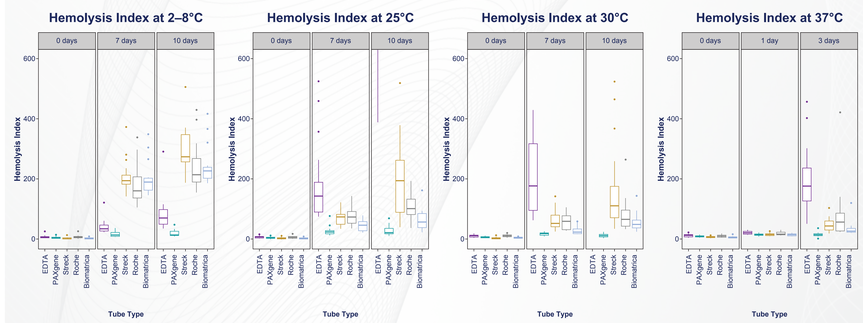


Results

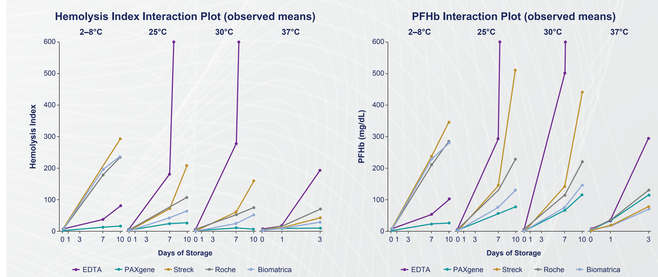
The PAXgene tube exhibits significantly lower levels of plasma-free hemoglobin (PFHb)



The PAXgene tube exhibits significantly lower levels of hemolysis index (HI)

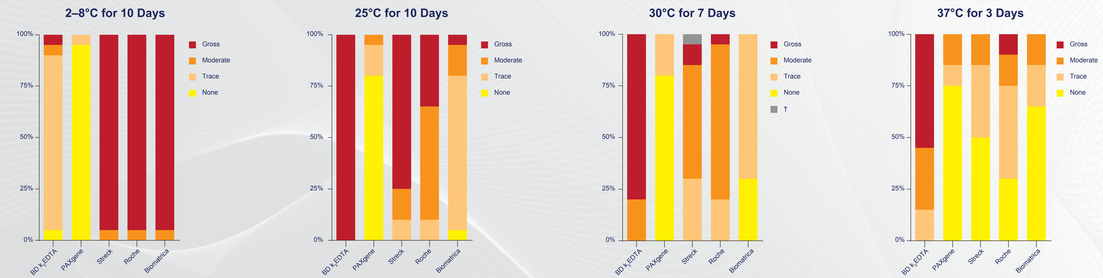


Plasma-free hemoglobin and hemolysis index over time



- The PAXgene Blood ccfDNA Tube maintained lower PFHb and HI levels over the tested time period stored at temperatures between 2–30°C

PAXgene tubes exhibit no "gross hemolysis"



- Visual evaluation of hemolysis in plasma was performed on a scale of 0–3 (0 = none, 1 = trace, 2 = moderate, 3 = gross)
- Each hemolysis category is presented as a percentage of the total number of tubes (n = 20) stored at the indicted duration and temperature

†One specimen was excluded for incomplete fill

The PAXgene tube mitigates hemolysis better than other tubes at most storage temperatures and durations

Analyte	Temp	Time	PAXgene vs. EDTA	PAXgene vs. Streck	PAXgene vs. Roche	PAXgene vs. Biomatrica
PFHb	2–8°C	7 days	✓	✓	✓	✓
PFHb	2–8°C	10 days	✓	✓	✓	✓
PFHb	25°C	7 days	✓	✓	✓	×
PFHb	25°C	10 days	✓	✓	✓	✓
PFHb	30°C	7 days	✓	✓	✓	×
PFHb	30°C	10 days	✓	✓	✓	×
PFHb	37°C	1 day	×	×	×	×
PFHb	37°C	3 days	✓	×	×	×
Hemolysis Index	2–8°C	7 days	✓	✓	✓	✓
Hemolysis Index	2–8°C	10 days	✓	✓	✓	✓
Hemolysis Index	25°C	7 days	✓	✓	✓	✓
Hemolysis Index	25°C	10 days	✓	✓	✓	✓
Hemolysis Index	30°C	7 days	✓	✓	✓	✓
Hemolysis Index	30°C	10 days	✓	✓	✓	✓
Hemolysis Index	37°C	1 day	×	×	×	×
Hemolysis Index	37°C	3 days	✓	✓	✓	✓

- Statistically significant differences were observed between the PAXgene Blood ccfDNA Tube and other collection tubes for hemolysis levels after blood storage
- For HI, the PAXgene Blood ccfDNA Tube was superior to all other tubes when blood was stored up to 10 days at temperatures between 2–30°C
- The PAXgene Blood ccfDNA Tube also exhibited superior performance as measured by PFHb under most storage conditions
- The PAXgene Blood ccfDNA Tube performance appeared similar to other stabilization tubes for up to 3 days storage at 37°C

Conclusions

- The choice of whole blood stabilization for ccfDNA analysis has a significant effect on hemolysis levels after storage
- Whole blood stored in PAXgene Blood ccfDNA Tubes for up to 10 days between 2–30°C exhibited minimal hemolysis, demonstrating better preservation of specimen quality and integrity compared to all other tube brands tested
- The PAXgene Blood ccfDNA Tube did not show any visual "gross hemolysis" up to the maximum claimed whole blood storage duration and temperature

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