

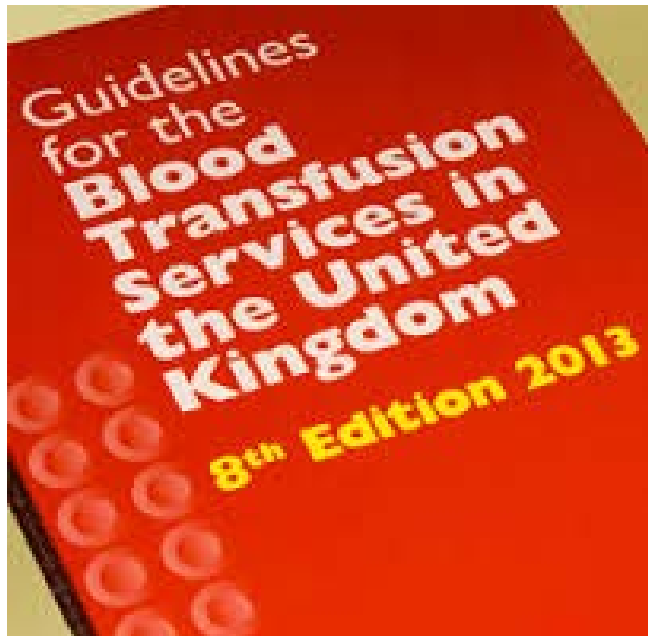
Delta Hb:

A Potential Novel Parameter For Quality Monitoring Of Red Cell Concentrates

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Guidelines for Blood Transfusion Services in the United Kingdom



- Each primary process should be validated to give haemolysis of $<0.8\%$ of the red cell mass at the end of component shelf life at day 35
- Within the UK this is a regulatory requirement, is part of regular QC and is used as a measurement of component quality
- In order to define haemolysis 3 parameters are measured: the haematocrit and a measurement of free and total haemoglobin in the sample

Outline of NHSBT Routine Testing Process:



Red Cell Concentrate

Weighed
Sampled



Measure on
haematology
analyser

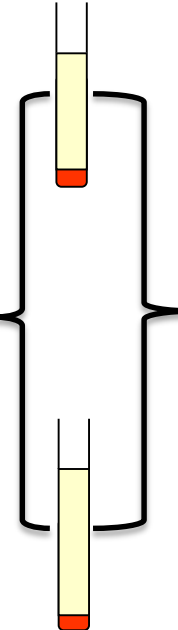


Centrifuge sample
10 mins at 4000 rpm
(1 spin or 2 spins)



1 spin

2 spin



HemoCue
Plasma low-Hb
photometer



Harboe method
Measures
spectrophotometric
plasma haemoglobin

$$\% \text{Haemolysis} = \left(\frac{\text{Spt Hb}}{\text{Tot Hb}} \right) \times (100 - \text{Hct})$$

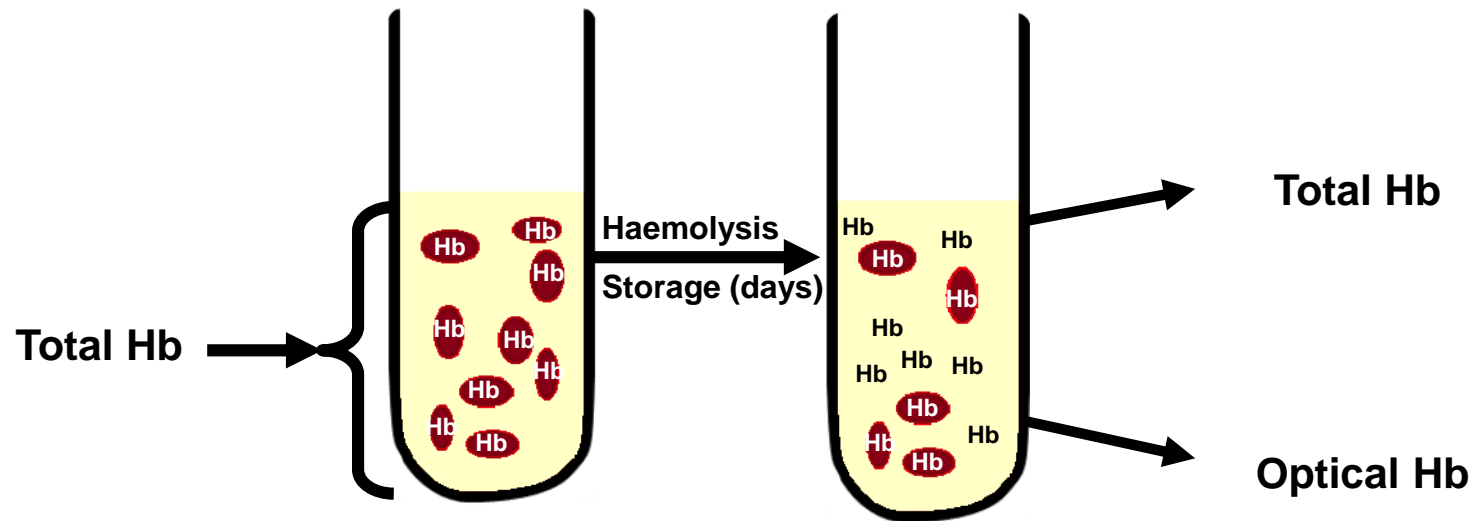
Purpose of study:



- Can we use a single platform method to measure haemolysis in red cell concentrates
- Sysmex XN-1000 is able to measure haematocrit, soluble and total Haemoglobin
- Eliminates pre-analytical variables including:
 - User error
 - QC and calibration of different equipment
 - Time taken for various processes (centrifugation, Harboe, HemoCue)
- Can we use an alternative measurement to infer the level of haemolysis?

Alternative Testing Process: Our Hypothesis

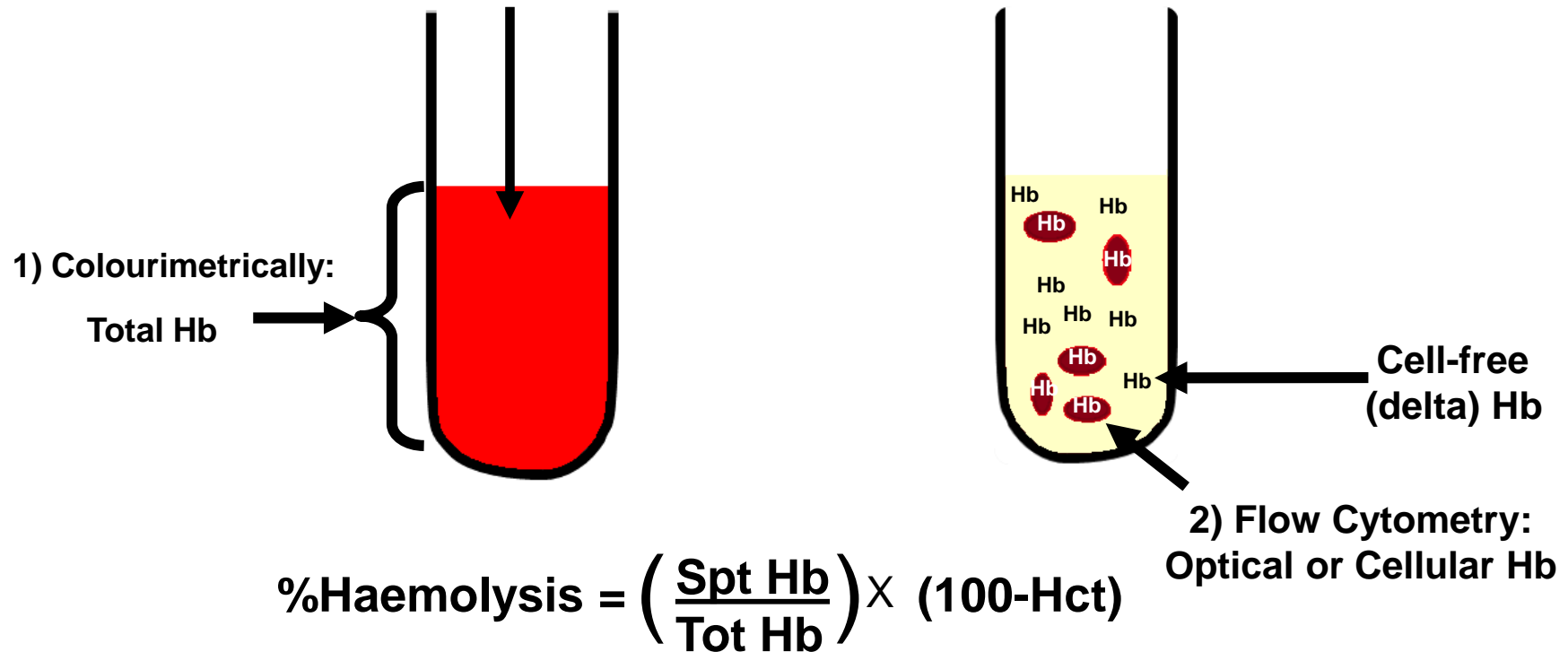
What happens to red blood cells as they age?



Alternative Testing Process: Our Hypothesis

Haematology analysers can measure Hb in two ways:

Lysercell reagents lyse RBC
within a sample



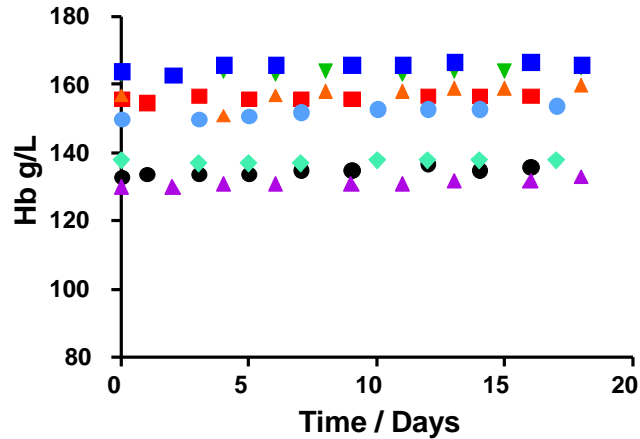
Delta Hb or Spt HB = Total Hb – Optical Haemoglobin

Experiments to test whether Delta-Hb can be used as a marker for haemolysis

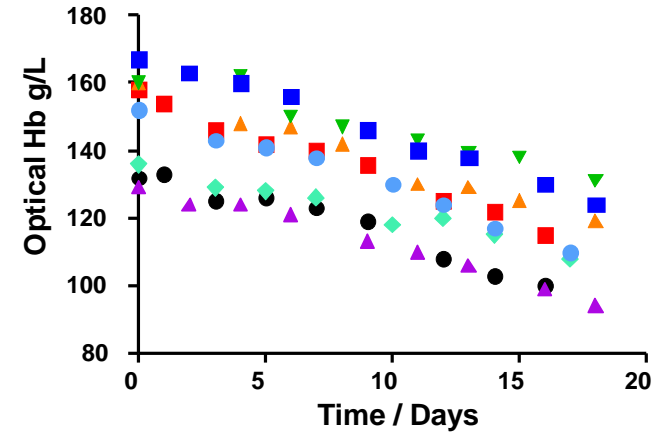
- **Study 1** - Positive Control Experiment: Take whole blood tubes left at room temperature to induce haemolysis and test over 22 days
- **Study 2** - Negative control Experiment: Take fresh whole blood tubes and test within 1 hour of bleeding to show initial level of haemolysis present in red cell concentrates
- Retrospective analysis of previously carried out experiments within CDL
 - **Study 3** - Red cell concentrates up to day 42
 - **Study 4** - Red cell concentrates up to day 35
- **Study 5** - Test red cell concentrates over shelf life maintained at ideal conditions

Study 1 - Proof of principle: Positive Control Experiment

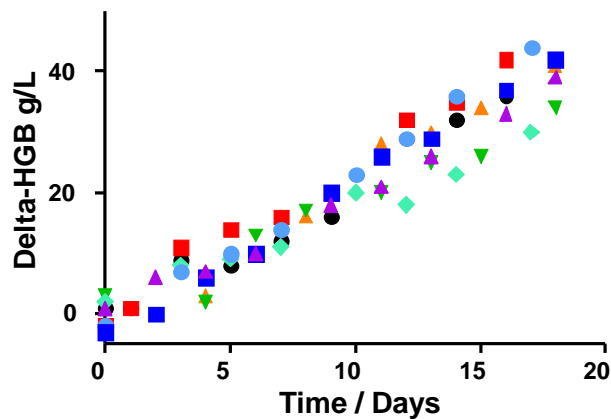
Total Hb



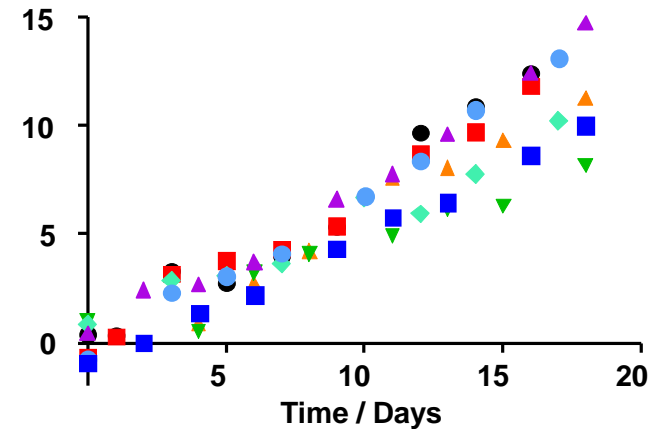
Optical Hb



Difference Between Total and HGB-O



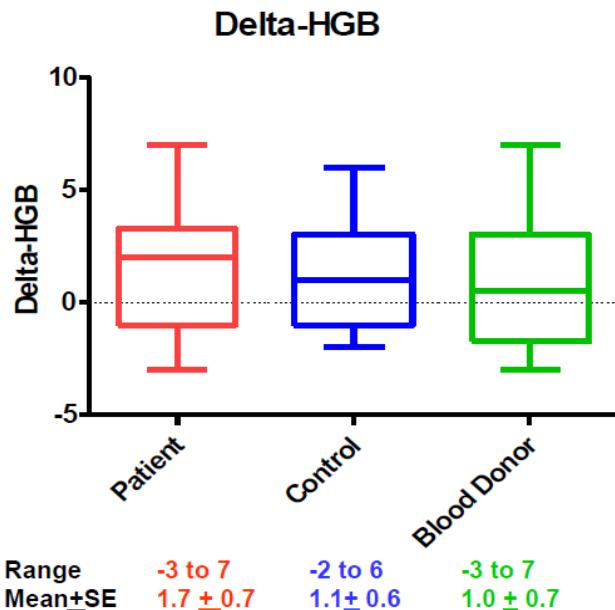
% Haemolysis



Study 2 - Proof of principle: Negative controls (specificity and sensitivity)

- Fresh EDTA samples, tested within 1 hour of sampling should:
 - Give equivalent total Hb and optical Hb values
 - Show minimum amounts of haemolysis

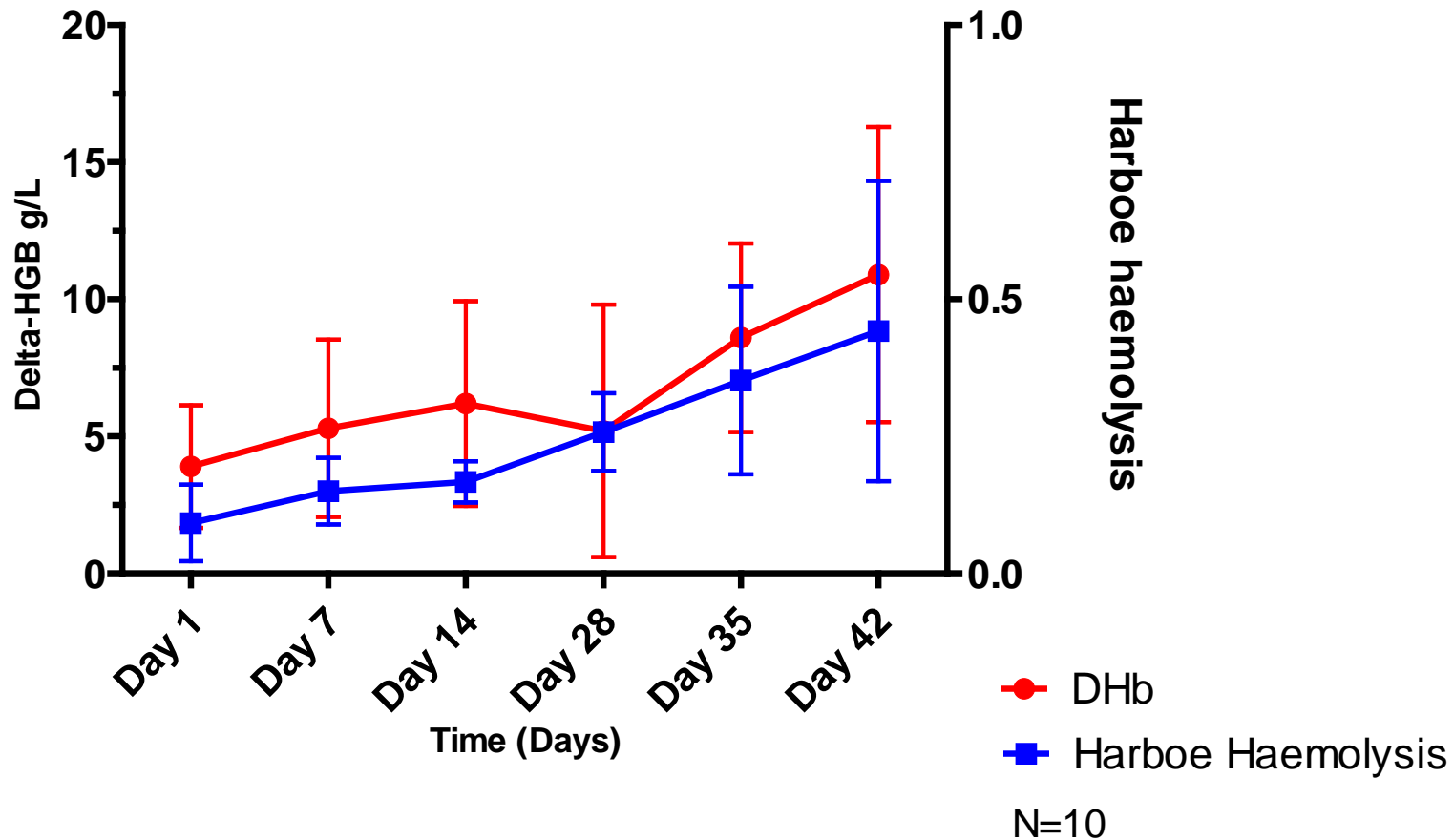
Change in Mean RBC Parameters From Individual Patients (n=18),
Their Controls (n=19) and EDTA Blood Donor (n=20) Whole Bloods



Study 3 - Proof of Principle - Retrospective analysis over time

- Another study was performed on 10 whole bloods and manufactured into red cell concentrates (RCCs) in Cambridge by CDL.
- RCCs were tested at days 1, 7, 14, 28, 35 and 42
- RCCs were sampled and tested using the following methods:
 - Sysmex XN-1000 for total Hb and HGB-O
 - Harboe method for %haemolysis

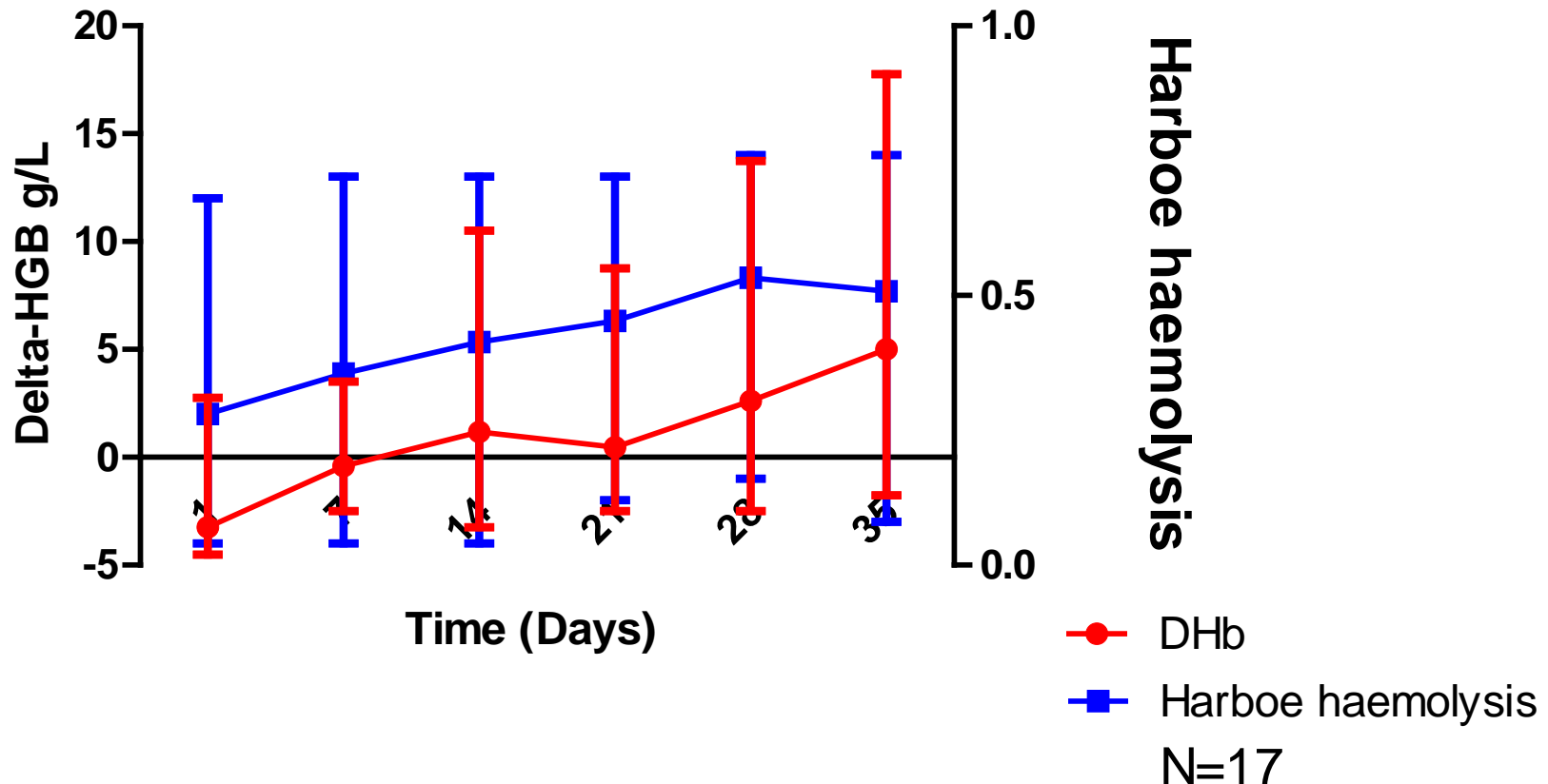
Study 3 - Proof of Principle: How does Sysmex Delta-Hb and Harboe %Haemolysis change with time



Study 4 - Proof of Principle - Retrospective analysis over time

- A fourth study was performed on 17 whole blood units manufactured into RCC top and top (TAT) units in Cambridge by CDL.
- RCCs were tested at days 1, 7, 14, 21, 28, 35
- RCCs were sampled and tested using the following methods:
 - Sysmex XN-1000 for total Hb and HGB-O
 - Harboe method for %haemolysis

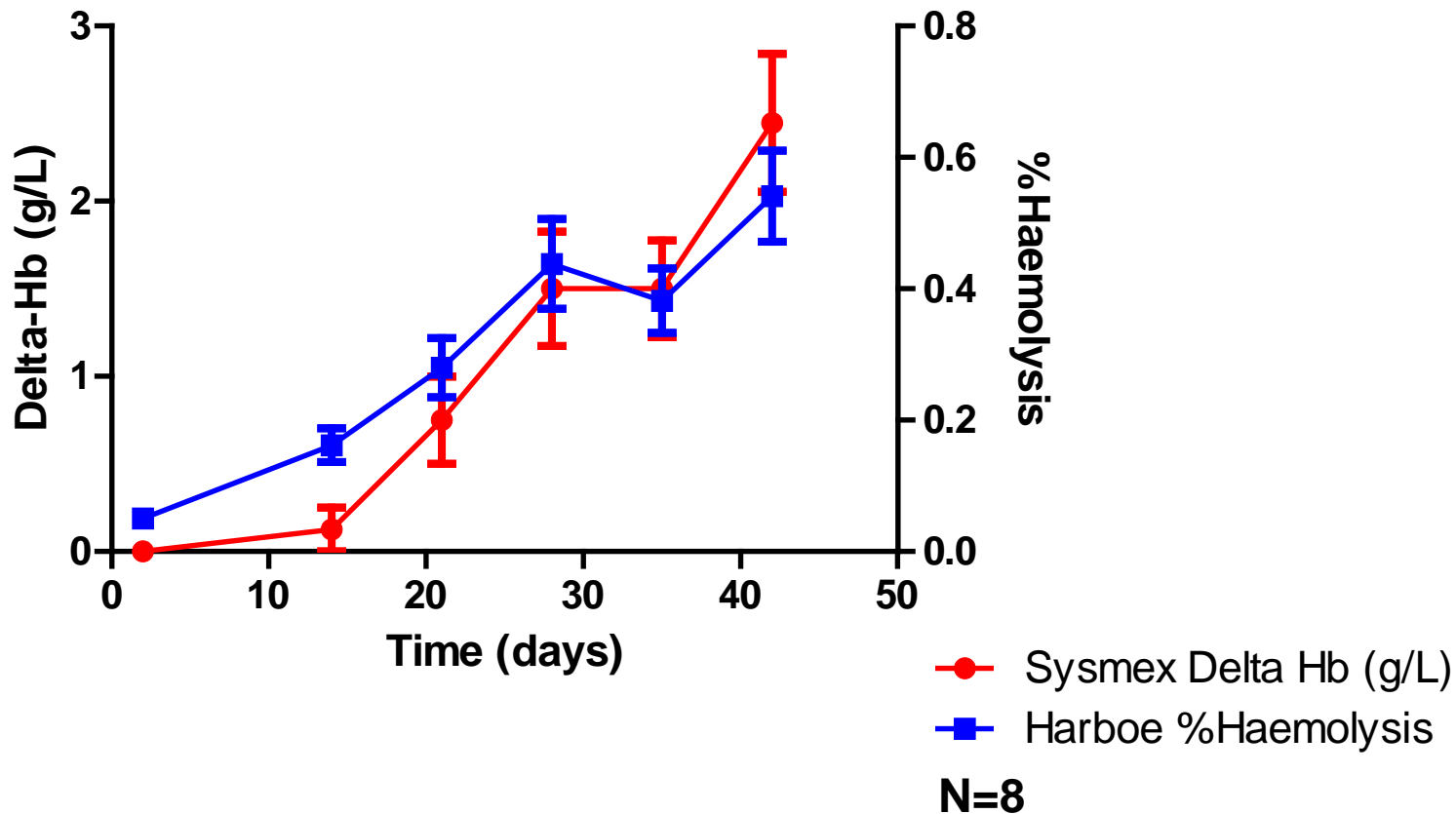
Study 4 - Proof of Principle: How does Sysmex Delta-Hb and Harboe %Haemolysis change with time



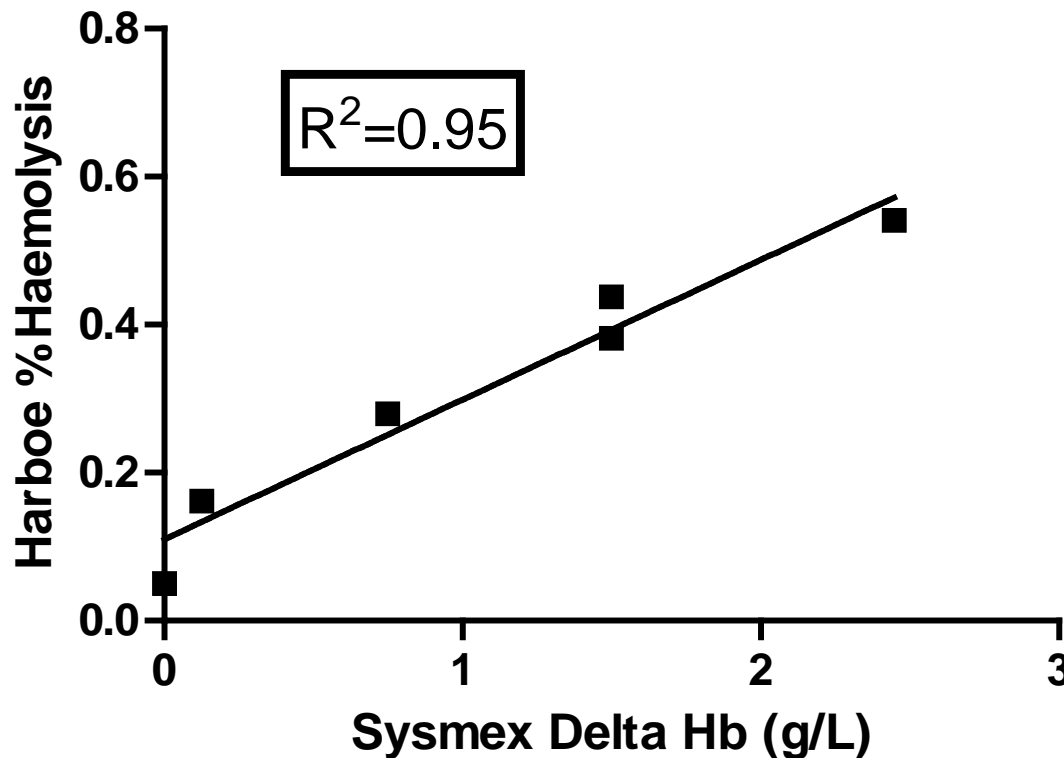
Study 5 – Prospective study - Testing red cell concentrates over time

- A final study was performed on 8 whole blood units collected and manufactured into RCCs in Cambridge by CDL.
- RCCs were tested at days 2, 14, 21, 28, 35, 42
- RCCs were sampled and tested using the following methods:
 - Sysmex XN-1000 for total Hb and HGB-O
 - Harboe method for %haemolysis

Study 5 - How does Sysmex Delta-Hb and Harboe %Haemolysis change over time




How does Delta-Hb measured on the Sysmex correlate with percentage haemolysis measured by the Harboe method ?



Conclusions

- Haematology analysers may offer an alternative single platform approach by measuring Hb in two ways
- All studies showed an increase in delta Hb from day 1 to the end of testing
- Correlation between delta Hb and %haemolysis
- Requirement to test more red cell concentrates from manufacturing facility at end of shelf life
- Compare alternative methods for measuring haemolysis such as the HemoCue
- Compare the effect of alternative centrifuge conditions
- Do red cell fragments and/or microparticles influence the measurement of delta Hb ?
- Why do we get negative values for delta Hb ?

Acknowledgements

- NHSBT hospital services – Colindale/ Cambridge
 - NHSBT research, Prof Willem Ouwehand's group
 - Sysmex UK: Adam Mellick
 - Sysmex Europe: Jarob Saker and Jo Linssen
 - CDL staff
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- A decorative graphic at the bottom of the slide consisting of two overlapping wavy bands in shades of blue.