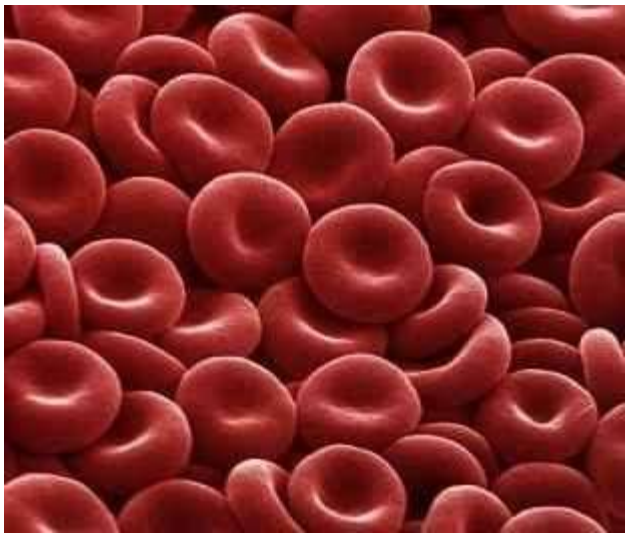
Red Cell Hemolysis during Storage and Transportation



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Introduction

- Blood and blood components are labile biological products which needs to be kept under right conditions to ensure that they remain viable, safe and clinically effective

- Blood and blood components needs to transported under right conditions to ensure viability and safety

Factors affecting the quality of RBCs

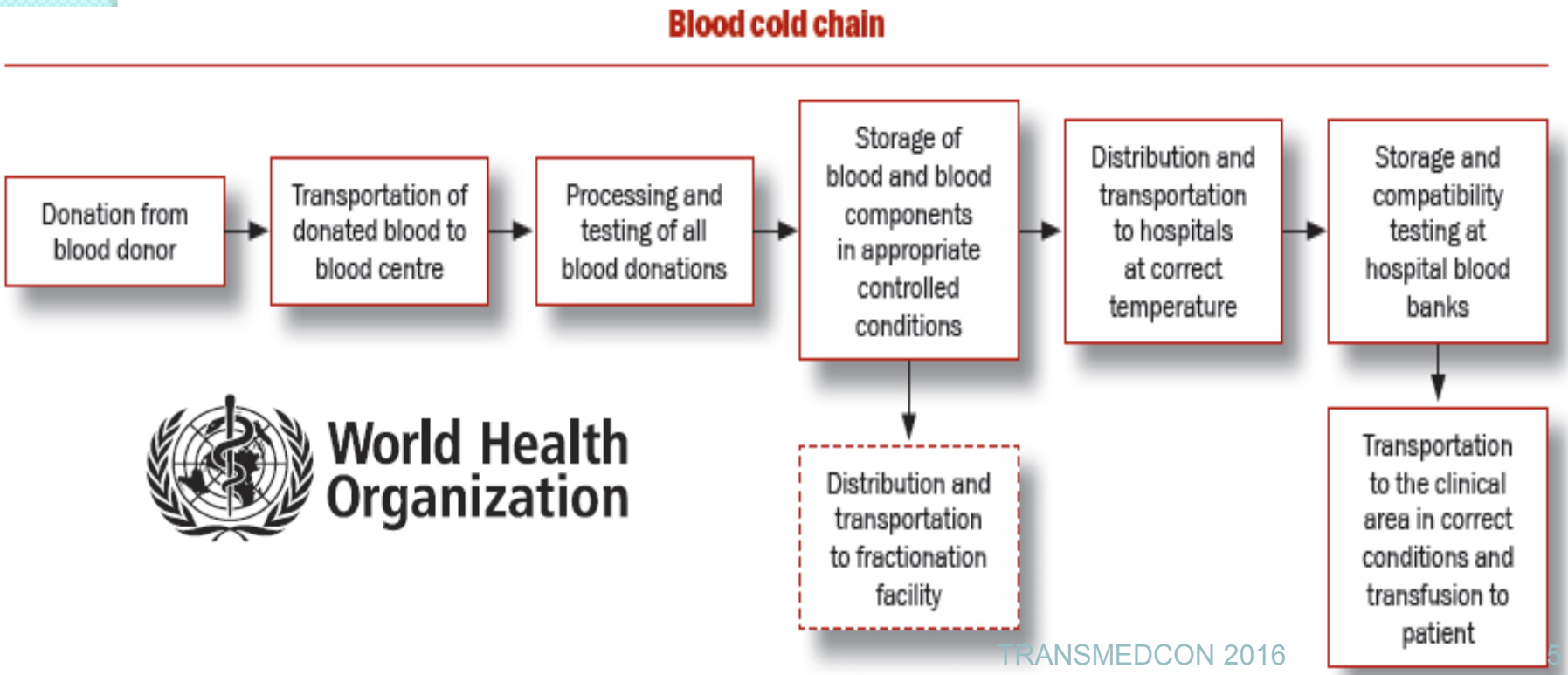
- During Storage
  - Temperature
  - Time lapses during collections and component preparation
  - Storage container
  - Leucocyte reduction
  - Irradiation

- Temperature is most important

- If blood is stored or transported outside of these temperatures for long,
  - Viability
  - Functional efficacy

Blood Cold Chain

To maintain appropriate temperature during storage and transportation of blood and blood components from the point of collection to the point of use.



Transportation of RBCs

- Transportation of blood from collection site to component preparation
  - $+2^0$ c to 10^0 c
  - For platelet separation $+20^0$ c
- Transportation of whole blood and red cell components from one blood bank to another
- $+2^0$ c to $+10^0$ c
- Red cell component : at no given point should the ice be allowed to in direct contact with blood

Table 1. Storage and transport conditions for whole blood and red cells

Condition	Temperature range	Storage Time
Transport of pre-processed blood	+20 °C to +24 °C	Less than 6 hours
Storage of pre-processed or processed blood	+2 °C to +6 °C	Approx. 35 days
Transport of processed blood	+2 °C to +10 °C	Less than 24 hours

*Manual on the management, maintenance and use of blood cold chain Equipment : World Health Organization

RBC storage

- For RBCs storage at $+2^0$ c $\pm$ 4^0 c
  - Slows RBC metabolism and facilitates extended storage
- However, at this temperature significant chemical changes take place during storage
- These changes collectively known as storage lesion

Problems with stored Red Blood Cells

Storage lesions of RBCs

A series of physical and biochemical changes in the red cell occurring during preservation that reduces subsequent survival and function after reinfusion

Storage effects on RBCs

- Metabolic effects
- Biomechanical or membrane effects
- Oxidative effects
- RBC integrity is negatively influenced by storage lesion
- Metabolic modulation, shape changes, altered rheologic properties and oxidative injury collectively contributes to progressive RBC degradation
- Resulting into RBC lysis

Red cell hemolysis

Morphological alteration can cause cell injury

May result into red cell lysis

Cause release of hemoglobin

which get accumulated in suspending fluid

Can reflect storage and transport condition

Plasma Hb : considered as marker of storage lesion of red cells

Quality requirement: Hemolysis

- FDA has recommended a maximum of 1% hemolysis for deglycerolized RBCs and has approved and licensed additive solutions for long term storage of packed RBC units with less than 1% hemolysis at the end of storage period.
- In European guidelines: 0.8% hemolysis

***Does the storage duration of blood products affect outcomes in critically ill patients Spinella et al, Transfusion August 2011**

Hemolysis in stored RBCs

- Hemolysis of stored red cells is a normal process
- Some degree of hemolysis is acceptable
- Increases with storage time
- Plasma Hb: serve as a quality indicator

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Asian J Transfus Sci. 2011 Jan; 5(1): 15–17.

Evaluation of the red cell hemolysis in packed red cells during processing and storage

R. N. Makroo, Vimarsh Raina, Aakanksha Bhatia, Richa Gupta, Abdul Majid, Uday Kumar Thakur, and N. L. Rosamma

Introduction:
Storage of red cells causes a progressive increase in hemolysis. In spite of the use of additive solutions for storage and filters for leucoreduction, some amount of hemolysis is still inevitable. The extent of hemolysis, however, should not exceed the permissible threshold for hemolysis even on the 42nd day of storage.

Study Design and Methods:
Eighty units of packed red cells, 40 stored in SAGM post leucoreduction and 40 in ADSOL without leucoreduction filters, were evaluated for plasma hemoglobin by HemoCue Plasma Hemoglobin analyzer on the day of collection and on the 7th, 14th, 21st, 28th, 35th and 42nd days thereafter. The hemoglobin and hematocrit were also noted for all these units by the Beckman and Coulter analyzer. Percentage hemolysis was then calculated.

Observations:
Hemolysis progressively increased with the storage period in all the stored red cell units (SAGM as well ADSOL). However, on day 42nd of storage, free hemoglobin in all the red cell units was within the permissible level (which is 0.8% according to the Council of Europe guidelines and 1% as per the US FDA guidelines). The mean percentage hemolysis was slightly higher in the SAGM-containing bags with an integral leucoreduction filter as compared to the bags containing ADSOL. However this difference was marginal and not statistically significant.

Conclusion:
Hemolysis of the red cells increases with storage. However, maximum hemolysis does not exceed the permissible limits at any time thereby indicating the effect of optimum processing and storage conditions on red cell hemolysis

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Asian J Transfus Sci. 2007 Jul-Dec; 1(2): 47–51.

Red cell hemolysis during processing and storage

R. B. Sawant, S. K. Jathar,* S. B. Rajadhyaksha,* and P. T. Kadam*

Introduction:
Apart from the visual assessment, measurement of plasma hemoglobin in the supernatant from red cell units provides an objective measure of the extent of hemolysis during storage.

Study Design and Methods:
Packed red cells (N=50), 25 units each in triple (CPD-A1 and SAGM) and quadruple (CPD-A1 and ADSOL) blood bags were evaluated for plasma hemoglobin by the tetramethylbenzidiene (TMB) method on day 1, 7, 14, 21 and 28 of collection. The hemoglobin, hematocrit, MCV, LDH and potassium levels were also noted. Whole blood units (N=25) were used as controls.

Results:
Hemolysis increased in all the stored red cell units. Plasma hemoglobin increased significantly in the first week of storage. The hemolysis, LDH and potassium levels were found to be significantly higher in the red cell units harvested from the triple blood bags. However, on day 28 of storage, free hemoglobin in all the red cell units was much below the 0.8% hemolysis.

Conclusion:
Hemolysis of the red cells increases due to processing and during storage and is maximum during the first week. Adequate process control and proper storage facilities should be ensured to minimize the hemolysis of red cells during processing and storage.

Prestorage leukoreduction does not increase hemolysis of stored red cell concentrates

Manish J. Gandhi *, Eugene Shapiro, Lynn Emmert, D. Michael Strong, Thomas H. Price

TRANSFUSION AND APHERESIS SCIENCE

ierhealth.com/journals/tras

Transfusion and Apheresis Science 36 (2007) 17–22

Results: In the first-phase LR-RCs exhibited an average 0.06% hemolysis vs. 0.02% for non-LR units. In the second-phase the average % hemolysis before and after filteration on day-2 (LR: 0.04% & non-LR: 0.04%) was similar. While on days:15 (LR: 0.09%, non-LR: 0.05%) and 30 (LR: 0.16%, non-LR: 0.13%) % hemolysis was slightly more in LR as compared to non-LR. It was the opposite for day 40 (LR: 0.19%, non-LR: 0.31%). However, none of these differences were statistically significant.

Conclusions: The % hemolysis increased as the age of the unit increased. There was no significant statistical difference between LR-RC and non-LR-RCs. This data did not confirm our hospitals' concerns regarding increased hemolysis following LR.

Assessment of changes in plasma hemoglobin and potassium levels in red cell units during processing and storage

Nishant Saini *, Sabita Basu, Ravneet Kaur, Jasbinder Kaur

Red cell units undergo changes during storage and processing. The study was planned to assess plasma potassium, plasma hemoglobin, percentage hemolysis during storage and to determine the effects of outdoor blood collection and processing on those parameters. Blood collection in three types of blood storage bags was done – single CPDA bag (40 outdoor and 40 in-house collection), triple CPD + SAGM bag (40 in-house collection) and quadruple CPD + SAGM bag with integral leukoreduction filter (40 in-house collection). All bags were sampled on day 0 (day of collection), day 1 (after processing), day 7, day 14 and day 28 for measurement of percentage hemolysis and potassium levels in the plasma of bag contents. There was significant increase in percentage hemolysis, plasma hemoglobin and plasma potassium level in all the groups during storage ($p < 0.001$). No significant difference was found between any parameter analyzed for outdoor and in-house collected single CPDA red cell units. There was significant lower percentage hemolysis ($p < 0.001$) and potassium (day 7 to day 14 – $p < 0.05$ and day 14 to day 28 – $p < 0.001$) in red cell units from day 7 onward until day 28 of storage in the leukoreduced quadruple bag as compared to the triple bag. The in-house single CPDA red cell units showed significantly more hemolysis ($p < 0.001$) as compared to the triple bags with SAGM additive solution after 28 days of storage. There is gradual increase in plasma hemoglobin and plasma potassium levels during the storage of red blood cells. Blood collection can be safely undertaken in outdoor blood donation camps even in hot summer months in monitored blood transport boxes. SAGM additive solution decreases the red cell hemolysis and allows extended storage of red cells. Prestorage leukoreduction decreases the red cell hemolysis and improves the quality of blood.

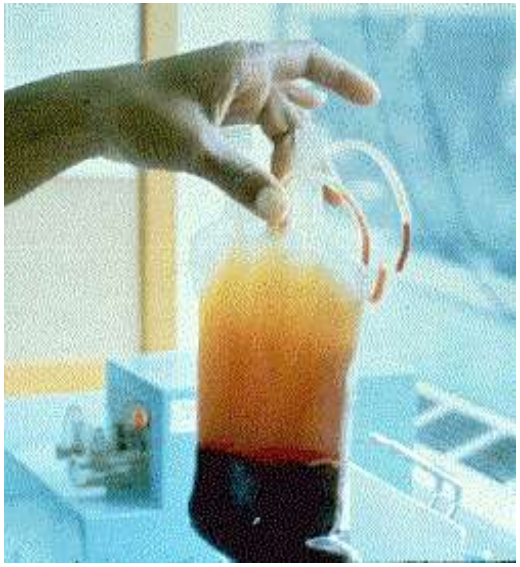
Transfusion and Apheresis Science 52 (2015) 319–325

Hemolysis

- Hemolysis is very important parameter to assess the quality of stored RBCs

Plasma Hb- Determination

Visual assessment



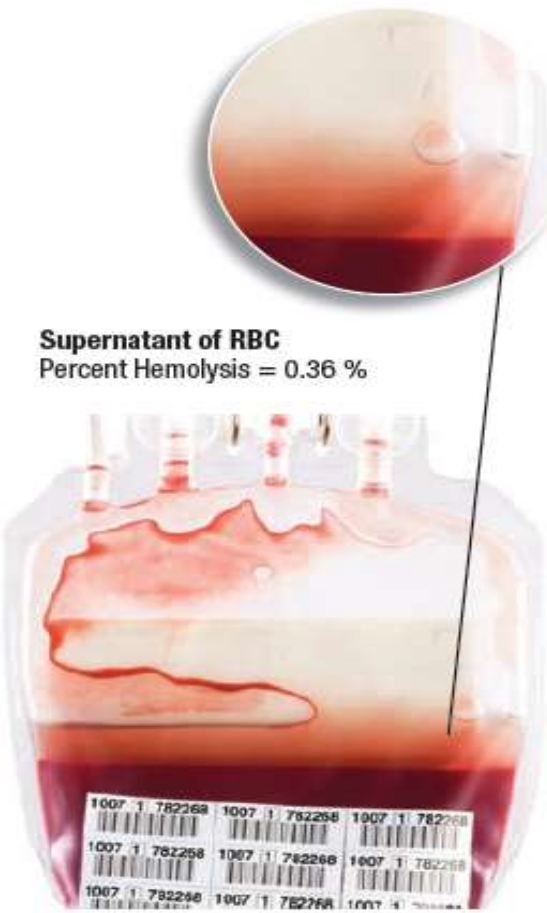
Quantitative methods:

- spectrophotometric assays,
- photometric method
- microplate technique

Visual Assessment

Visual Assessment Guide

Supernatant of RBC
Percent Hemolysis = 0.11 %



Supernatant of RBC
Percent Hemolysis = 0.36 %

Supernatant of RBC showing higher levels of hemolysis
Percent Hemolysis = 1.14 %

Photographs were taken after allowing the RBC to settle for 4 days to permit an evident visualization of the supernatant. This assessment may be performed earlier or later.

The CSA standard will define acceptable levels of hemolysis as < 0.8% at expiry.

Visual assessment

- Is non-destructive means to estimate hemolysis in a red cell unit
  - difficult to define a “cut-off” color to decide acceptability of unit
  - Subjective method
  - color of the supernatant varies
- This visual inspection is often deceptive
  - may lead to an overestimation of haemolysis levels

Visual assessment of hemolysis in red blood cell units and segments can be deceptive

K.A. Janatpour, T.G. Paglieroni, V.L. Crocker, D.J. DuBois, and P.V.

TRANSFUSION 2004;44:984-989

BACKGROUND: Blood components that appear hemolyzed are discarded. However, visual inspection is subjective and criteria for excessive hemolysis are poorly defined.

STUDY DESIGN AND METHODS:

Packed RBCs (10 CPDA-1, 10 Adsol) were collected. Half of each unit was leukoreduced. Plasma Hb was measured and compared in segments and units by three methods: 1) a HemoCue Plasma/Low Hb Photometer system; 2) a tetramethylbenzidine (TMB) chemical method, and 3) a free Hb visual comparator.

RESULTS:

Visual assessment tended to overestimate hemolysis. Chemical methods were comparable (r2= 0.894; HemoCue=0.043+[0.770]\TMB; n=400; range, 0.01-0.5 g/dL), although the mean plasma Hb (g/dL) for the HemoCue method was higher than that of the TMB method (0.12 vs. 0.10 g/dL, respectively; p <0.001). No units would have been discarded based on a hemolysis level of at least 0.6 g/dL (approx. 1%) if measured by a chemical method. However, 50 percent of CPDA-1 and 10 percent of Adsol units would have been discarded if only visual criteria were used. Leukoreduction did not increase plasma Hb levels. Discrepancies in plasma Hb levels were noted between units and their corresponding segments.

CONCLUSION:

Visual assessment of hemolysis can result in unnecessary wastage of blood components. HemoCue offers an alternative, objective method to assess plasma Hb in the setting of blood collection and processing facilities for routine quality control and process validation, and may aid in the development of objective criteria for excessive hemolysis in blood components.

Key points

- Red cell hemolysis is known to occur during blood processing, handling, storage and during transport
- Adequate process control should be ensured to minimize the hemolysis of red cells during processing and storage.
- Extent of hemolysis can serve as a quality indicator for stored red cell units

TMH study

A STUDY OF HEMOLYSIS IN RED CELL CONCENTRATES DURING TRANSPORTATION

***Dr Anand Bodade*, Dr S Rajadhyaksha, Dr Priti Desai,**
Dr Shashank Ojha, Dr Preeti Chavan

Aims and Objectives:

- To measure free hemoglobin levels in transported units as a marker of hemolysis
- To check the plasma K^+, Na^+ and LDH levels in RCC before and after transportation

Methods

- Test: 50 RCC (PRBC) units under transportation were studied
- Controls : 50 RCC of corresponding age of test units available on shelf in the blood centre kept under controlled temperature.
- WHO guidelines were followed for transportation

Calculation:

% Hemolysis = $\frac{\text{100-Hct} \times \text{plasma Hb (g/dL)}}{\text{Total Hb of the unit (g/dL)}}$

Results

Parameter	TEST ARM		CONTROL ARM
	Mean		Mean
	PRE	POST	-
Hb (g/dL)	19.45 (15.4-24.1)	18.88 (12.7-22.7)	19.38
Hct (%)	59.63 (50.3-71.7)	58.28 (50.2-70.2)	59.26
pHb (g/dL)	0.10 (0.03-0.33)	0.14 (0.04-0.39)	0.14
%HL	0.20 (0.06-0.70)	0.30 (0.09-0.78)	0.28
Na^+ (mmol/L)	160.48 (121-248)	131.06 (11-155)	131.94
K^+ (mmol/L)	26.52 (4.82-44.55)	31.74 (8-64)	27.10
LDH (U/L)	440.46 (42-1459)	540.90 (59-1727)	411.62
Temp. (˚C)	7.73 (6.4-9.38)	7.43 (6.49-9.38)	4.75
Time (min)	-	110.90 (85-150)	-

Conclusions

- However, in our study though the values were raised none of the units showed hemolysis greater than 0.8% (Europe) or 1% (US) as per available guidelines
- This emphasizes that proper maintenance of cold chain during transportation is very crucial
- Visual assessment of hemolysis leads to inadvertent discard of precious units and therefore routine quantitative analysis for hemolysis must be done
- Maximum extent of hemolysis needs to be set for the permissible threshold considering transfusion to patients, as no guidelines exist in India

Thank you !!