Toxicological background from a transfusion medicine perspective and in vitro red blood cell storage studies in PVC-free blood bags.

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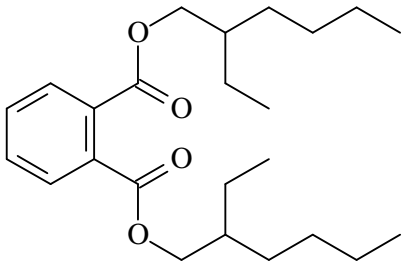

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Transfusion Medicine.

- Transfusion of blood is one of the most important life-saving medical treatments in modern medical service.
- Due to this significant role, blood transfusion is regulated in detail, in Europe based on Commission directives of the European Union.
- Blood is always split into blood components before being used for transfusion.
- Systems of sterile blood containers are used for collection of whole blood and preparation of blood components. Some of those blood bags contain sterile additive solutions.

Transfusion Medicine.

- Technical requirements for blood containers are comprehensive, i.e.
- The plastic material in the blood container systems must not affect the blood components stored in such containers.
- The plastic material must allow sterilization (generally steam sterilization), be stable and transparent.
- Blood containers must allow handling at temperatures between 40°C down to -70°C and must allow centrifugation at up to 5000xg.
- Plastic tubing must allow welding for sealing as well as sterile connection.
- PVC/DEHP meets all the requirements regarding stability, transparency, durability and sealing for the storage of blood components.

Why don't we use other plastics?

- Already mentioned physical and transfusion-medicine requirements

But ……..

- DEHP migrates into stored blood components since DEHP is lipophilic. Lipids present in plasma are chemically related to DEHP and cause extraction of DEHP from the plastic into the blood.
- DEHP has a stabilizing effect on the erythrocyte membrane.
- DEHP is incorporated into the interior and membrane fractions of erythrocytes and reduces osmotic fragility and hemolysis.
- It is also temperature dependent. Migration will increase at higher temperature.
- Hemolysis by the end of storage must not exceed 0,8% of total hemoglobin content according to EU directives.
- This situation has made it difficult to replace PVC/DEHP by other plastic material.

The background within the European Union (1).

- Based on animal studies, DEHP is classified as category 1B for reproductive toxicity according to the LP-Regulation (EC) No 1272/2008. The testis toxicity of DEHP seems to be age dependent, with immature young animals being more susceptible to testicular toxicity by DEHP than older mature animals.
- Recently, the International Agency for Research on Cancer (IARC) has indicated that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. For this reason, DEHP has been classified as possibly carcinogenic to humans (Group 2B). The LOAEL (Lowest Observed Adverse Effect Level) for carcinogenicity (male rat) is specified as 320 mg/kg bw/day.
- On the other hand, reviews of recent epidemiological studies investigating DEHP exposure associated effects on testosterone production, breast tumor etc. is considered either inconclusive or inconsistent.
- DEHP is only physically dispersed in PVC and can therefore leach, migrate or gas out from PVC articles.
- DEHP may migrate from a medical device to the human body, resulting in a certain degree of patient exposure.
- **DEHP from blood bags are integrated in red cell membranes, thereby reducing haemolysis during storage.**

The background within the European Union (2).

- DEHP can be present in air, dust, water, soils, sediments, and food and has become a ubiquitous environmental contaminant.
- The range of DEHP exposure in the general population from all sources excluding medical and occupational exposure has been estimated to be 1 to 30 µg/kg BW/day (median ~ 2-5 µg).
  Children are assumed to have higher exposures to DEHP than adults (median ~5-20 µg).
- A Tolerable Daily Intake (TDI) value of DEHP has been established and published in the EU Risk Assessment Report (RAR 2006). The TDI for DEHP is 48 µg per kg body weight per day.
- However, the use of TDI is not considered appropriate in medical procedures.

Transfusion of blood components.
DEHP exposure of adults .

Blood component	ug/kg BW/day	Reference
RBC, 1 unit, fresh	38	
RBC, 1 unit, stored for 35 days	114	Sampson, de Korte 2011.
PLT i plasma, 1 unit, fresh	64	
PLT i plasma, 1 unit, stored 7 d.	130	
Plasma, 1 unit, thawed aph. fresh	71	
Plasma, 1 unit, thawed apheresis, stored for 7 days	311	
Trauma patient (2.5 L of WB)	1300-2600	Sjöberg 1985
During ECMO (21-46 combined blood products)	3000-10000	Butch 1996

Transfusion of blood components.
DEHP exposure of neonates (4 kg BW).

Blood component	ug/kg BW/day	Reference
Rec. Blood for exch. Transf. fresh	1660	
Rec. Blood for exch. Transf. 24 h	2660	Sampson, de Korte 2011.
Single dose RBC (20 mL) fresh	40	
Single dose RBC (20 mL) 35 d.	125	
PLT in plasma, day 0 (20 mL)	75	
PLT in plasma stored 7 d (20 mL)	121	
Exchange transfusion	840-4200	Sjöberg 1985
During ECMO treatment	>10000	Schneider 1989, Karle 1997

Background of the present study.

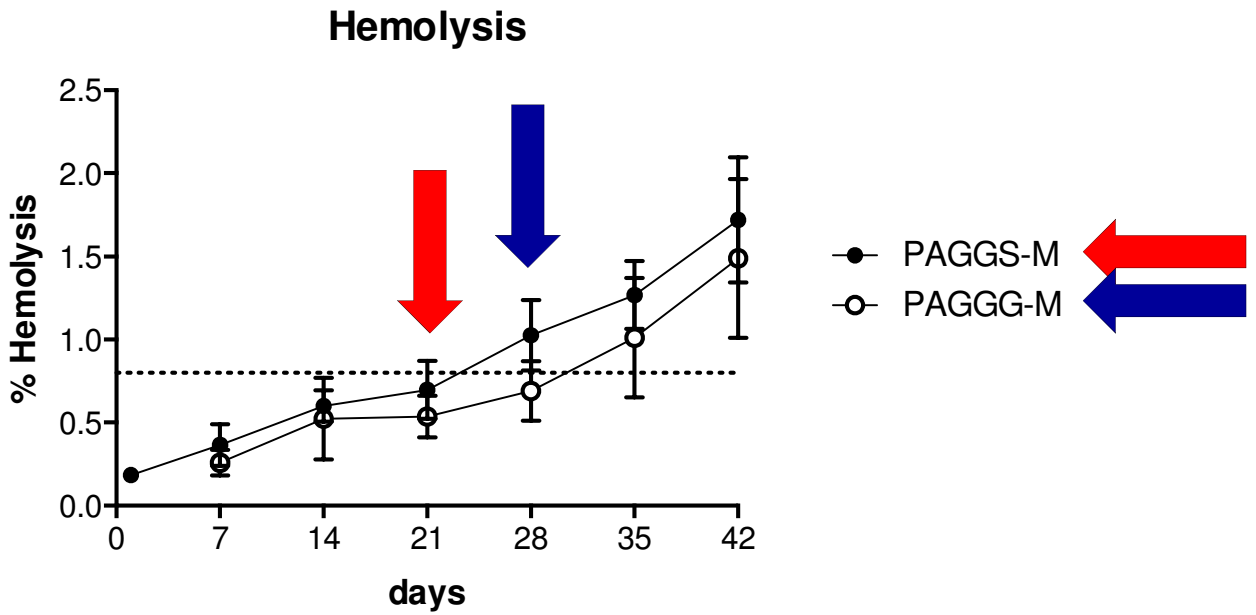
- Whole blood from blood donors are generally separated into 3 different blood components, viz.
- Red Blood Cells (RBCs).
- Plasma.
- White Blood Cells and Platelets. The preparation of Platelets is associated with additional preparation steps.
- The study focused on red cell storage in a first step, since hemolysis problems associated with the absence of the DEHP red cell membrane stabilizing effect was expected, based on previous studies in different countries. RBCs must not exceed the European maximum limit of 0.8% hemolysis (percentage of the whole hemoglobin content in RBC units).

Composition of RBC additive solutions (in mmol/L) used in the study.

	SAG-M	PAGGS-M	PAGGG-M
NaCl	150	72	-
Na_2HPO_4		16	8
NaH_2PO_4		8	8
Adenine	1.25	1.4	1.4
Guanosine		1.4	1.4
Glucose	45	47	47
Na-gluconate		-	40
Mannitol	30	55	55
pH	5.7	5.7	8.2
Osmolarity (mOsm/L)	376	345	275

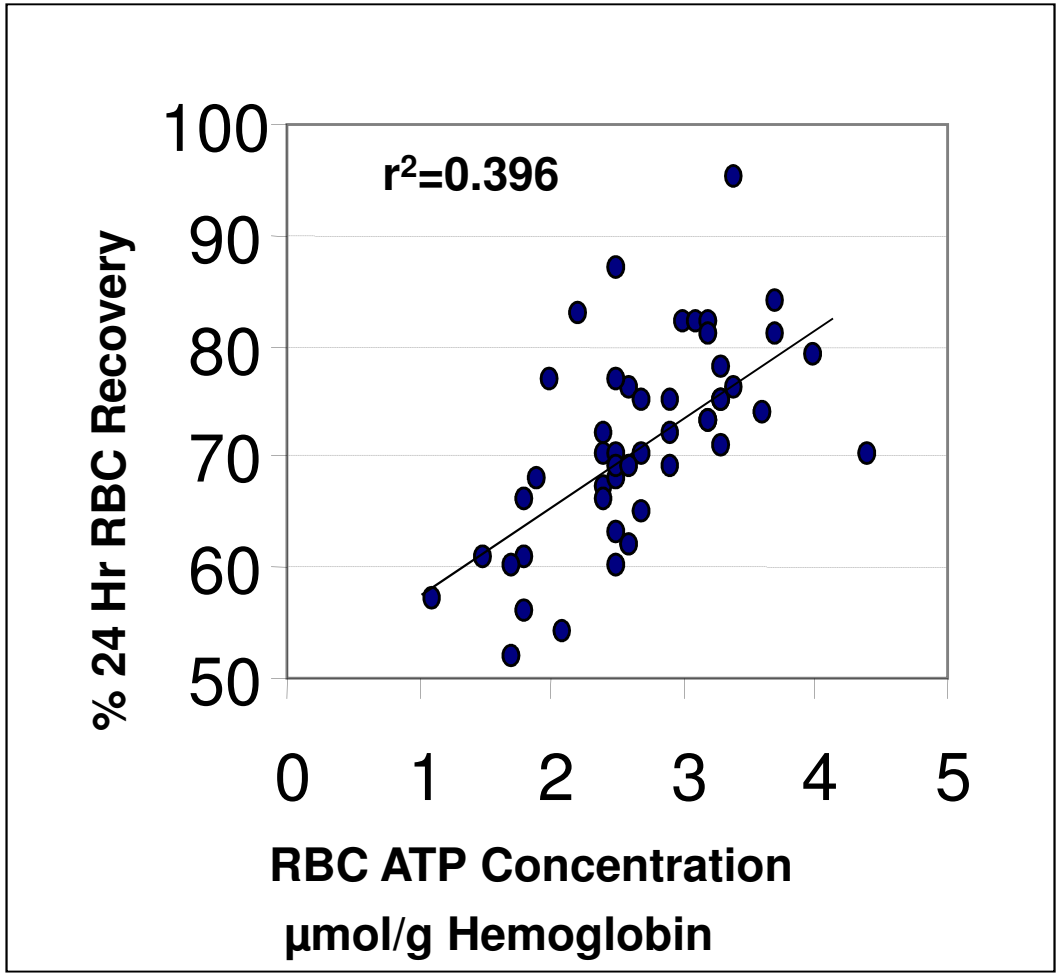
Hemolysis (%)

Data are presented as mean of ten units ± SD.



The dotted line represents the European maximum limit of 0.8%.

Correlation between ATP and viability of erythrocytes.



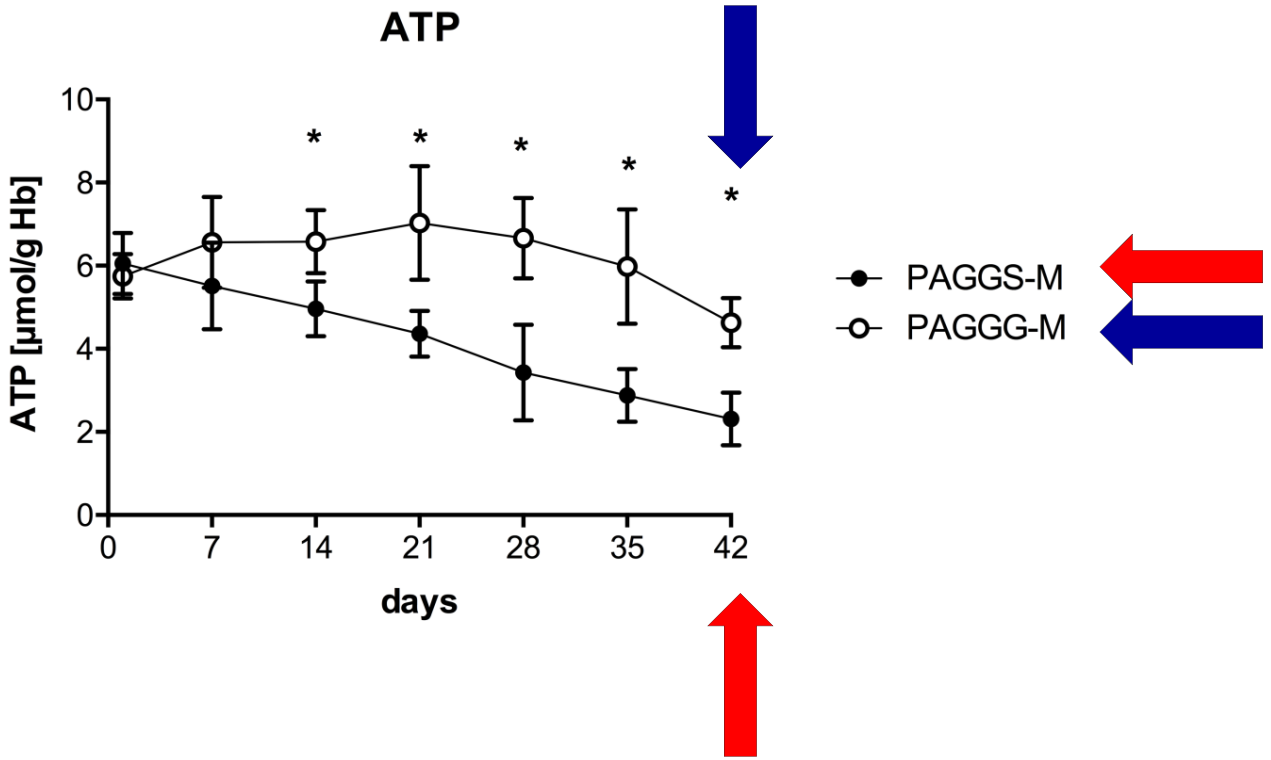
RBC ATP concentration correlates significantly with viability, but the predictive value of the correlation is not good. However, neither measure is precise. Repeat assays of ATP concentration differ by 5%. Repeat measures of RBC recovery also differ with a SD of 5%.

John R. Hess, MD, MPH; University of Maryland, Baltimore, USA:

"Understanding and Improving RBC Storage; BEST Working Party, May 2003 Frankfurt, Germany".

ATP content in RBC units stored in PAGGS-M and PAGGG-M.

Data are presented as mean of ten units ± SD.



Lactate (mmol/L) and extracellular pH.

Lactate [mM]	*	*	*	*	*	*	*
PAGGS-M	4.3 ± 0.6	12.1 ± 1.5	19.1 ± 2.5	23.9 ± 2.7	28.9 ± 3.5	31.6 ± 3.4	34.2 ± 3.6
PAGGG-M	4.9 ± 0.6	15.3 ± 1.9	22.8 ± 3.1	29.7 ±3.6	35.0 ± 3.4	39.0 ± 3.4	40.4 ± 2.9
Extracellular pH							*
PAGGS-M	6.86 ± 0.02	6.72 ± 0.02	6.59 ± 0.03	6.51 ± 0.04	6.42 ±0.04	6.37 ± 0.05	6.32 ± 0.05
PAGGG-M	6.88 ± 0.03	6.74 ± 0.05	6.58 ± 0.08	6.45 ± 0.09	6.35 ± 0.10	6.27 ± 0.10	6.16 ± 0.14

To summarize:

- The physical properties of the new polyolefin plastic are different from those of PVC-DEHP.
- The sealing conditions are a little bit different, implicating that the present sterile connecting devices probably can be used, but new sealing equipment for closing blood bag tubing will be needed.
- Only two different RBC additive solutions were tested. Those studies should be repeated to confirm our results and additionally, other new RBC additive solutions could be of interest for a similar test.
- The RBC additive solution PAGGG-M gave encouraging results except for its inability to prevent excessive hemolysis. It is an alkaline RBC additive solution based on an ion shift mechanism through the red cell membrane resulting in higher intracellular than extracellular pH levels .
- There will be room for further tests of additional available solutions and development of this solution or other RBC additive solutions to address the hemolysis problems.
- In the next step, plasma should also be tested regarding the stability of coagulation and activation pathway variables.
- There also seems to be a potential for future use of the new blood bags for preparation of pooled buffy-coat-derived platelets. However, the use of sterile connecting devices processes in this context needs to be validated.