

Labile Blood Components

Collected, Processed and Stored in DEHP-free Containers



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INTRODUCTION

Use of diethylexyl phthalate (DEHP) as a polyvinyl chloride (PVC) plasticizer for medical devices is now discouraged since this plasticizer has been found to be toxic in animal experiments. During blood storage, DEHP leaches slowly from PVC bags into blood components intended to be transfused to patients. Toxicological studies show that DEHP is a reproductive toxicant potentially leading to a lower potential of sexual reproduction. The European Directive 2007/47/EC advises to use alternatives to DEHP-PVC as far as possible. The plasticizer DINCH is an alternative to DEHP in the manufacture of blood bags. DINCH has a very low migration rate and an excellent toxicological profile:

- no testicular toxicity, nor impairment of fertility, nor teratogenicity;
- no carcinogenic potential.

Although alternative PVC plasticizers are known for decades, demonstration has to be made whether blood component storage is not adversely influenced, especially because DEHP is known to be a red blood cell membrane stabilizer. The present study focuses on the in vitro effects on leukocyte-reduced red blood cells (RBC) in SAG- M additive solution prepared and compares these effects in DINCH-PVC container and in DEHP-PVC container during 42 days.

MATERIALS & METHODS

. CONTAINERS:

Quadruple bag systems with whole blood filter (MACOPHARMA) made of DINCH-PVC and DEHP-PVC respectively. DINCH-PVC has been used to produce 30 units of whole blood in line filtration systems. 30 standard units in DEHP-PVC have been used in order to compare characteristics of blood components stored in both plastic materials.

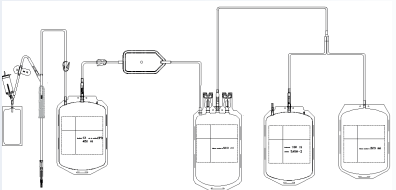
. TESTS, METHODS:

The red blood cell counts, residual leukocytes, residual platelets, hemoglobin, hematocrit, blood gases and pH were analyzed on the suspension of red blood cells. After centrifugation of samples, the supernatant was used for the determination of potassium, phosphorus, glucose, plasma hemoglobin, and lactate dehydrogenase (LDH). 1 ml of RBC suspension was deproteinized by trichloroacetic acid (ATP) or perchloric acid (2-3 DPG), placed on ice for 5 min. After centrifugation, the supernatant was assayed for 2-3 DPG immediately and stored at -80 °C for determination of ATP. Assays of G6PD and pyruvate kinase were performed after lysis of red blood cells previously washed. The statistical comparison and biological criteria descriptives between the two types of PVC was carried using SPSS PSAW.

. BLOOD COMPONENTS:

450 – 480 ml of blood were collected from 60 normal voluntary blood donors and drawn in quadruple-bag blood container systems with whole blood filter for the preparation of RBC and plasma and 63 ml of citrate phosphate dextrose (CPD) solution. 100 ml of SAG-M solution was used as an additive for RBC. 30 whole blood units were stored at 2 - 6°C during 8 hours and 30 units overnight during 18-24 hours.

This device is completely DEHP-free plasticizer because of 100% PVC components are plasticized with the DINCH (bags, tubings, plugs, connectors...).



The table below describes the nature and timing of the analysis

Analysis performed	Total Blood before deleucocytation	D0 or D1	Day 14 or Day 15	Day 35 or Day 36	Day 42	Analysis performed	Total Blood before deleucocytation	D0 or D1	Day 14 or Day 15	Day 35 or Day 36	Day 42
Weight (g)	X	X	X	X	X	Plasma Hemoglobin (mg/l): Spectro-photometric method in second derivative of Evolution 600 (Thermo)	X	X	X	X	X
Volume (ml)	X	X	X	X	X	Hemolysis (%)	X	X	X	X	X
Determination of red blood cells Count on MAXM AL (Beckman Coulter)	X	X	X	X	X	Determination potassium (K+) and sodium (Na+) (mEq/l) Measurement by ion selective electrode on ADVA 1650 (Siemens)	X	X	X	X	X
Hemoglobin (g/l) Hematocrit (Ht%) Measurement MAXM AL (Beckman Coulter)	X	X	X	X	X	LDH assay (U/l)	X	X	X	X	X
Residual Leukocytes (106/l) Count on EPICS XL (Beckman Coulter)	X	X	X	X	X	Enzymatic determination of Uv 340 nm on Evolution 600 (Thermo)	X	X	X	X	X
Residual Platelets (109/l) Count on MAXM AL (Beckman Coulter)	X	X	X	X	X	Determination of ATP (μmol/g Hb) Photometric endpoint of Evolution 600 (Thermo)	X	X	X	X	X
pH, pO2 and pCO2 Measured by selective electrode ABL 5 (Radiometer)	X	X	X	X	X	Dosage of 2-3 DPG (μmol/g Hb) Photometric endpoint of Evolution 600 (Thermo)	X	X	X	NA	NA
Determination of Glucose (g/l) Colorimetric method with glucose oxidase on ADVA 1650 (Siemens)	X	X	X	X	X	Lactate (mg/l) Enzymatic determination with lactate oxidase on ADVA 1650 (Siemens)	X	X	X	X	X
						Determination of Phosphorus (g/l) Phosphomolybdate colorimetric method on ADVA 1650 (Siemens)	X	X	X	X	X

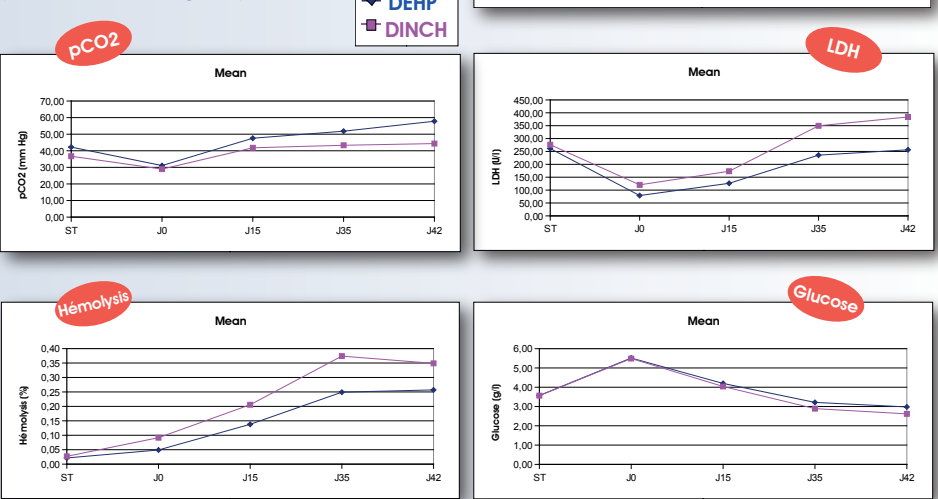
RESULTS

Results RBC after 42-day storage

	Mean	SD	Mini	Maxi
Weight (g) DEHP	259,67	23,59	217	303,3
Weight (g) DINCH	267,18	21,63	231,6	303,1
Volume (ml) DEHP	215,75	22,26	175,5	256,9
Volume (ml) DINCH	222,81	20,40	189,2	256,7
Red Cells (tera / l) DEHP	6,13	0,31	5,56	6,79
Red Cells (tera / l) DINCH	6,14	0,37	5,41	7,05
Hemoglobin (g/l) DEHP	184,56	11,97	161	207
Hemoglobin (g/l) DINCH	187,06	10,02	171	206
Hematocrit (Ht%) DEHP	56,92	3,35	49,9	61,8
Hematocrit (Ht%) DINCH	58,26	3,18	51,3	64,4
pH DEHP	6,51	0,05	6,38	6,6
pH DINCH	6,48	0,04	6,36	6,57
pO2 (mm Hg) DEHP	43,8	32,25	20	142
pO2 (mm Hg) DINCH	76,2	35,16	28	133
pCO2 (mm Hg) DEHP	57,8	13,15	34	102
pCO2 (mm Hg) DINCH	44,33	8,93	25	67
Glucose (g/l) DEHP	2,97	0,45	2,34	3,76
Glucose (g/l) DINCH	2,61	0,46	1,73	3,56
Lactate (mg/l) DEHP	1936,86	51,35	1820	2007
Lactate (mg/l) DINCH	1899,43	65,88	1740	1990
Phosphorus (mg/l) DEHP	182,3	14,17	152	206
Phosphorus (mg/l) DINCH	181,13	15,26	151	224
Plasma Hb (mg/l) DEHP	1093,06	499,09	470	2672
Plasma Hb (mg/l) DINCH	1613,5	839,60	325	3530
Hemolysis (%) DEHP	0,25	0,12	0,11	0,65
Hemolysis (%) DINCH	0,34	0,16	0,09	0,73
K+ (mEq/l) DEHP	50,8	5,12	40,8	59,8
K+ (mEq/l) DINCH	50,09	4,86	40,7	59,2
Na+ (mEq/l) DEHP	114,06	4,54	105	127
Na+ (mEq/l) DINCH	115,86	3,13	109	124
LDH (U/l) DEHP	256,46	97,22	129	520
LDH (U/l) DINCH	383,86	179,05	84	735
ATP (μmol/g Hb) DEHP	17,01	5,40	10,3	34,8
ATP (μmol/g Hb) DINCH	18,55	6,62	6,5	38,8

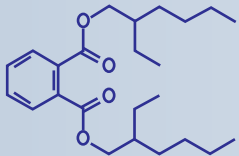
DISCUSSION

For RBCs, only 6 parameters differ lightly but significantly, namely: pO2 (greater for DINCH group) and pCO2 (lower for DINCH group), hemolysis (0.35% for DINCH vs. 0.26% for DEHP groups respectively after a 42-day storage), LDH and plasma hemoglobin (both greater in the DINCH group), : glucose consumption (equivalent in both group).



CONCLUSION

DEHP
(di[2-ethylhexyl] phthalate).



DINCH-PVC is more permeable to gases than DEHP-PVC, leading to a greater cell metabolism. Haemolysis and LDH levels sign a lower cell membrane protective action of DINCH compared to DEHP. However these differences are very low and all RBCs studied meet requirements for labile blood products. We are currently completing the plasma study in both containers after a 6 and 12-month storage period.

DINCH
(1,2-Cyclohexanedicarboxylic acid, diisononylester).

