VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2017) **112**, 33–39

DOI: 10.1111/vox.12472

ORIGINAL PAPER

Storage of red blood cells in a novel polyolefin blood container: a pilot *in vitro* study

H. Gulliksson,[1,2] S. Meinke,[1,2] A. Ravizza,[3] L. Larsson[2] & P. Höglund[1,2]

[1]*Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden*
[2]*Karolinska Institutet, Stockholm, Sweden*
[3]*Hematronic SpA, Mirandola, Italy*

Vox Sanguinis

Background and Objectives The present general plasticizer di-2-ethylhexyl-phthalate in polyvinylchloride (PVC) blood bags is only physically dispersed in PVC and will therefore leach into blood components. The objective of this study was to perform a first preliminary red blood cell (RBC) storage evaluation in a new blood bag manufactured of polyolefin without any inclusion of potentially migrating substances.

Study Design and Methods This is a RBC storage study for 42 days. Blood collection was performed in a polyolefin-based PVC-free blood bag. RBCs were prepared within 8 h. Two different RBC additive solutions were used, either PAGGS-M or PAGGG-M. We weekly measured pH, K^+, glucose, lactate, haemolysis, red cell ATP and 2,3-DPG.

Results RBC storage in PAGGS-M resulted in high haemolysis levels already after 21 days, exceeding the European maximum limit of 0·8%, and low ATP levels by the end of the storage period. With PAGGG-M, haemolysis exceeded 0·8% after 28 days of storage. For additional parameters, the results were comparable to those of previous studies in conventional blood bags.

Conclusion This is a first preliminary study of RBC storage in a new type of blood bags. PAGGG-M gave encouraging results except for its inability to prevent increased haemolysis. There will be room for further development of RBC additive solutions to address the haemolysis problems. Plasma should also be tested regarding the stability of coagulation and activation pathway variables. There may also be a potential for future use of the bag for preparation of pooled buffy-coat-derived platelets.

Key words: blood component production, blood components, blood safety, haemoglobin measurement, red cell components.

Received: 17 June 2016,
revised 15 September 2016,
accepted 12 October 2016,
published online 21 December 2016

Introduction

From the time of introduction of plastic blood containers in the 1950s until today, essentially all conventional blood bags for blood collection and preparation of blood components are produced from polyvinylchloride (PVC) polymer and are plasticized with di-2-ethylhexyl-phthalate (DEHP) [1, 2]. Not until in the 1970s, the migration of DEHP from the plastic into blood components was fully recognized [3–5]. Ten years later, plasticizer effects on erythrocyte membranes were reported [6–10]. The situation may briefly be summarized in the following way, (1) DEHP migrates into stored blood components due to its lipophilic characteristics, implicating that the presence and levels of plasma lipids will increase migration; (2) for this reason, the quantity of DEHP extracted may vary between blood from different donors; (3) DEHP has a stabilizing effect on the erythrocyte membrane as DEHP is incorporated into the interior and membrane fractions of erythrocytes thus reducing osmotic fragility and thereby haemolysis; (4) the migration of DEHP is temperature

Correspondence: Hans Gulliksson, Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Huddinge F79, Stockholm SE-141 86, Sweden
E-mail: hans.gulliksson@karolinska.se

dependent, increasing at higher temperature and (5) at a fixed temperature, the migration of DEHP is essentially linear over time.

PVC plasticized with butyryl-n-trihexyl-citrate (BTHC) has primarily been used for the storage of platelets, but can also be used for storage of red blood cells (RBC) [11, 12]. Recently, a new plasticizer has received significant interest, viz. di-iso-nonyl 1,2-cyclohexanedicarboxylat (DINCH). In a publication from Sanquin (the Netherlands), encouraging *in vitro* red blood cell (RBC) storage results were presented using blood bags manufactured of PVC-DINCH [13]. Four different red cell additive solutions were tested, viz. SAG-M, PAGGS-M, PAGGG-M, and AS-3. Acceptable *in vitro* results were found at day 42. The only exception was haemolysis in SAG-M environment. For the other three additive solutions, haemolysis values at day 42 were similar to values reported for storage in SAG-M in PVC-DEHP blood bags.

However, conventional blood bags manufactured of PVC-DEHP seem to be dominating on the market, associated with their excellent physical properties and a favourable economic situation. On the other hand, the pharmacological profile of DEHP is a concern. The situation is summarized in a recent comprehensive report from an expert group of the European Commission (EC) [14]. DEHP is classified as category 1B for reproductive toxicity according to the CLP-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). However, reviews of recent epidemiological studies investigating DEHP exposure-associated effects on testosterone production, breast tumour, etc. were considered either inconclusive or inconsistent [14].

It may be noted that the general population is already exposed to DEHP from the environment. In addition to this general exposition, patients receiving blood transfusion are consequently exposed to DEHP migrating from the plastics of the blood bags and tubing into whole blood and blood components [15]. There are treatments giving very high doses of DEHP such as ECMO, trauma patients receiving multiple blood components and exchange transfusions in neonates [15]. The effects of DEHP in blood bags were discussed in a recent publication [16].

This study was implemented by the County Councils of Sweden that are responsible for health care in the country in the light of the unfavourable pharmacological profile of DEHP [14]. The objective was to stimulate the development of a non-PVC alternative to existing conventional blood bags based on plastic materials without plasticizers. This study focused on red cell storage in a first step, as haemolysis problems associated with the absence of the DEHP red cell membrane-stabilizing effect were expected, based on previous studies. RBCs must not exceed the European maximum limit of 0·8% haemolysis [17]. International plastic industries were invited to participate, based on their specific knowledge of special plastics for medical use. The project has been performed with financial support from the LIFE+ programme (LIFE10 ENV/SE/037 PVCFreeBloodBag) of EC. The project is also described on the project website www.pvcfreebloodbag.eu.

Materials and methods

Plastic material

The blood bag sets are constructed of multiple parts, viz. bags, tubing, and various connectors. All raw materials are 100% PVC free and contain no intentionally added phthalates. The bags are made of a three-layered, polyolefin-based film. Each layer is made of different types of modified polypropylene (polypropylene belongs to the wide family of polyolefin). Each layer has dedicated characteristics: the outer layer is designed for film durability, the middle one for sealing, the inner layer is specifically designed and tested for blood contact, meeting requirements from ISO 10993-4 (haemolysis), ISO 10993-5 (cytotoxicity) and from USP Class VI (*in vivo* testing for Acute Systemic Toxicity, Intracutaneous Test, Implantation). The tubing is made of a three-layered thermoplastic polyolefin elastomeric compound, a flexible, transparent and durable polymer that is approved for medical applications meeting the requirements of USP class VI and ISO 10993-5. Each layer in the tubing has different functions. The inner layer is in direct contact with blood, the middle layer of the tube ensures the right flexibility of the tubing to avoid kinking and the outer layer provides optimal assembly on the bag. Additional components are made of poly-propylene, poly-ethylene and poly-carbonate, all coloured, hard polymers that are approved for medical applications, including blood contact according to ISO 10993 and USP. Crucial components include: (1) the donor needle, made of steel, poly-carbonate and poly-propylene and (2) the whole blood leukocyte-reduction filter of poly-butylene terephthalate and poly-carbonate. The most important material, in terms of weight, and the one that has the longest contact with blood is primarily the modified polypropylene of the bags but also the thermoplastic polyolefin elastomeric compound of the tubing.

After assembly, the integrated blood bag system consisting of three blood bags (600 ml) has been inserted in

a plastic overwrap made of PET (polyethylene terephthalate) and PP (polypropylene) that preserves the sterile state after sterilization by heat and allows protection of tubing from kinking. No additives such as glues or solvents have been used during the whole process and all assemblies have been reinforced by thermic treatment, thus avoiding use of glues and solvents.

Blood collection, preparation of red blood cells and *in vitro* studies

Totally 20 units of whole blood (WB; 450 ± 45 ml) were collected from normal voluntary donors after obtaining their written informed consent and drawn into test blood bags as described above. Maximum accepted collection time was 15 min. The blood collection bag contained standard CPD solution (63 mL). Cooling plates were applied to reduce the temperature from 37°C to room temperature after blood collection (Butan-1,4-diol, Medicoplast Oy, Helsinki, Finland). All WB units were stored at room temperature (20–24°C) for up to 8 h before the preparation of blood components. After WB filtration for leukocyte reduction, a hard-spin centrifugation programme was used (2988 × g, 10 min, 20–24°C, Sorvall RC 12BP, ThermoFischer Scientific Inc., Waltham, MA, USA). RBCs and plasma were prepared using a manual extractor (Fenwal Inc., Lake Zurich, IL, USA). After processing and addition of either PAGGS-M (100 ml, 10 units; MacoPharma, Lille, France) or PAGGG-M (100 ml, 10 units; Sanquin, Amsterdam, the Netherlands) to RBCs, all RBC units were stored for 42 days at 2–6°C (cf. Table 1 for composition). Those two additive solutions were selected as they were associated with very low levels of haemolysis in a previous study using blood bags manufactured of PVC-DINCH [13]. Haemolysis was expected to be the most severe obstacle in the absence of DEHP in our study. As no automatic sealer suited for this type of plastic tubing was available at this early stage, metal clips were used for sealing of tubing.

Table 1 Composition of RBC additive solutions (in mmol/l) used in the study

	PAGGS-M	PAGGG-M
NaCl	72	–
Na2HPO4	16	8
Na2HPO4	8	8
Adenine	1·4	1·4
Guanosine	1·4	1·4
Glucose	47	47
Na-gluconate	–	40
Na-gluconate	55	55
pH	5·7	8·2

Sampling of the RBC units was performed at days 1, 7, 14, 21, 28, 35 and 42. The sampling also implicated a mixing of the content of the RBC units once a week. Haematocrit and total haemoglobin concentration were measured using Medonic CA 620 Cellguard haematology equipment (Boule Medical, Stockholm, Sweden). White blood cells (WBC) on day 1 were counted using a Cytomics FC500 MPL flow cytometer (Beckman Coulter, Brea, CA, USA). By using routine blood gas equipment (ABL 800 Flex, Radiometer Medical ApS, Brønshøj, Denmark), we measured pH (at 37°C), glucose, lactate and potassium. To determine haemolysis, samples were centrifuged in two steps at 1450 × g for 10 min. The concentration of haemoglobin in the supernatant of the second centrifugation was measured with a HemoCue plasma/low haemoglobin photometer (HemoCue, Radiometer Medical ApS, Brønshøj, Denmark). The percentage of haemolysis was calculated by dividing the supernatant concentration by the total haemoglobin concentration for each sample. ATP concentration was determined using luminometric technique (Orion Microplate Luminometer, Berthold, Pforzheim, Germany) based on methods described previously [18]. 2,3-DPG concentrations were analyzed using spectrophotometry (Jenway 6500 Spectrophotometer, Barloworld Scientific Ltd., Dunmow, Essex, and kit 10148334001, Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. The 2,3-DPG concentrations in mol/l were converted into mol/mol haemoglobin using the total haemoglobin concentration and assuming a molecular mass of 64 000 g/mol.

The differences between the mean values obtained for the two different RBC additive solutions were tested for statistical significance using a *t*-test for each time point and the Holm–Sidak method to correct for multiple comparisons with $\alpha = 5{\cdot}0\%$ and without assuming a consistent SD. GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical evaluation of the results.

Results

The group of voluntary donors comprised 70% men (age 27–70 years) and 30% women donors (age 35–70 years). The RBC units were stored for 42 days and samples were taken once a week to measure *in vitro* storage parameters. Results are presented in Table 2 and Figs 1–3. The units stored in both additive solutions started with similar haemoglobin contents (PAGGS-M: 52·3 ± 6·0 g/unit, mean ± SD, and PAGGG-M: 54·4 ± 6·1 g/unit). However, initially units in PAGGG-M had lower haematocrit (57·2 ± 2·9%; mean ± SD) compared with units in PAGGS-M (63·6 ± 2·2%). During storage, the haematocrit

Table 2 Comparison of *in vitro* parameters of RBC units stored in polyolefin bags with PAGGS-M or PAGGG-M

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Hemoglobin [g/unit]							
PAGGS-M	52·3 ± 6·0	–	–	–	–	–	–
PAGGG-M	54·4 ± 6·1	–	–	–	–	–	–
Hematocrit [%]	*	*	*	*	*	*	*
PAGGS-M	63·6 ± 2·2	65·8 ± 4·2	65·7 ± 2·9	67·7 ± 3·3	66·3 ± 3·8	67·5 ± 2·8	68·5 ± 5·1
PAGGG-M	57·2 ± 2·9	58·2 ± 3·1	59·6 ± 3·2	60·3 ± 3·7	60·1 ± 4·3	64·1 ± 4·0	66·0 ± 4·1
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
Glucose [mM]							
PAGGS-M	24·5 ± 1·3	21·1 ± 1·7	17·3 ± 2·0	13·6 ± 2·4	11·3 ± 2·6	9·2 ± 2·7	7·3 ± 2·8
PAGGG-M	26·0 ± 1·1	20·6 ± 2·2	15·7 ± 3·4	11·9 ± 3·7	8·6 ± 3·7	6·2 ± 3·7	4·2 ± 3·3
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
Potassium [mM]	*	*	*	*	*	*	*
PAGGS-M	4·5 ± 0·3	19·4 ± 2·1	30·4 ± 2·4	37·6 ± 2·7	44·6 ± 3·3	49·7 ± 3·8	52·9 ± 3·8
PAGGG-M	3·5 ± 0·3	14·9 ± 1·8	23·6 ± 2·6	30·3 ± 3·3	35·8 ± 3·5	40·6 ± 3·7	49·1 ± 6·1

*$P < 0·05$.

of the units in PAGGG-M increased to a greater extent than for the units in PAGGS-M. At day 28, the difference was no longer statistically significant. The pH decreased similarly in units from both sets during the first 2 weeks of storage time, after which the decrease in the mean pH in units in PAGGS-M slowed down. By day 42, the difference in pH between the two sets was statistically significant. Glucose consumption and lactate production were also higher in PAGGG-M than in PAGGS-M. However, the difference was only statistically significantly different for lactate. The concentration of potassium ions was higher in the units stored in PAGGS-M already from day 1 and throughout storage.

The levels of 2,3-DPG were comparable at day 1 (PAGGS-M, 0·78 ± 0·17 mol/mol Hb, PAGGG-M, 0·84 ± 0·18 mol/mol Hb; mean ± SD; Fig. 1). The 2,3-DPG content of the units stored in PAGGS-M decreased rapidly, and from day 21 on there was practically no 2,3-DPG left. Half of the units stored in PAGGG-M showed a similar decay, whereas the 2,3-DPG content of the other half of units increased during the first week of storage, stayed at a high level until day 21, and then decreased.

The ATP content also developed differently depending on the additive solution (Fig. 2). The units in PAGGS-M started with a mean of 6·1 ± 0·7 μmol/g Hb that continuously decreased during storage down to 2·3 ± 0·6 μmol/g Hb at day 42. The units in PAGGG-M had a mean ATP content of 5·7 ± 0·5 μmol/g Hb at day 1, which then increased and stayed at levels of 6·5 μmol/g Hb until day 28 and then decreased to 4·6 ± 0·6 μmol/g Hb at day 42.

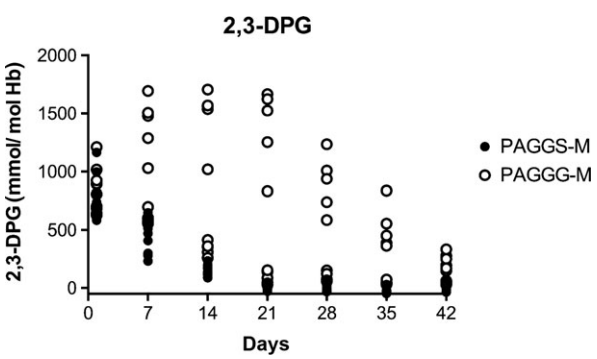

Fig. 1 Comparison of 2,3-DPG content in RBC units during storage in PAGGS-M and PAGGG-M. Each dot represents one RBC unit.

The difference in haemolysis during storage was not statistically significant except for day 28 when haemolysis was significantly lower in PAGGG-M (Fig. 3). Levels of haemolysis approaching 0·8% were noticed already at day 21 in PAGGS-M and in PAGGG-M at day 28 (0·7 ± 0·2%; mean ± SD).

Discussion

This is a first evaluation of a new blood bag system manufactured of plastic materials without plasticizers. The new blood bags were designed with the objective to avoid leakage of chemical substances from the plastic material

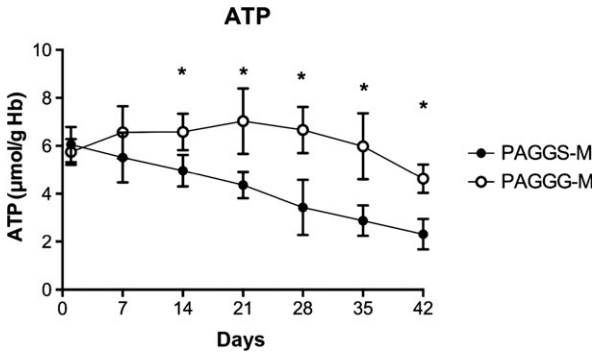

Fig. 2 Comparison of ATP content in RBC units stored in PAGGS-M and PAGGG-M. Data are presented as mean of ten units ±SD. Statistical significance was tested using a *t*-test for each time point and the Holm-Sidak method to correct for multiple comparisons. $*P < 0{\cdot}05$.

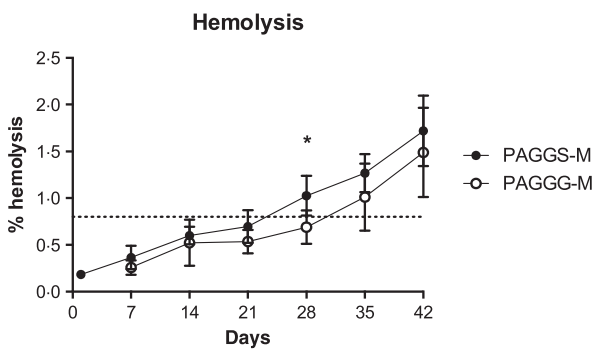

Fig. 3 Comparison of hemolysis in RBC units stored in PAGGS-M and PAGGG-M. Data are presented as mean of ten units ±SD. Values for the units in PAGGG-M at day 1 were below the detection limit. The dotted line represents the European maximum limit of 0·8%. Statistical significance was tested using a *t*-test for each time point and the Holm-Sidak method to correct for multiple comparisons. $*P < 0{\cdot}05$.

into blood components. Storage of RBC in blood bags manufactured of plastic materials that do not include plasticizers will be associated with new problems, different from what we generally experience in RBC storage studies. Plasticizers, in particular DEHP, have a stabilizing effect on the erythrocyte membrane as the plasticizer will be incorporated into the interior and membrane fractions of erythrocytes and thereby reduce osmotic fragility and in a second step haemolysis [6–10]. For this reason, we started this evaluation of a new blood bag system manufactured of plastic materials without plasticizers with RBC storage studies with a special focus on haemolysis. We used two different RBC additive solutions, viz. PAGGS-M and PAGGG-M.

PAGGS-M is an isotonic solution (285 mOsm/l), in contrast to the hypertonic solution SAG-M (376 mOsm/l) widely used in Europe. PAGGS-M has been shown to reduce the spontaneous haemolysis rate and osmotic fragility during red cell storage [19]. As haemolysis was reduced in conventional blood bags, PAGGS-M could be an appropriate solution to test in a polyolefin blood bag environment. On the other hand, PAGGS-M is not different from SAG-M with regard to degree of echinocytosis, decreasing RBC deformability and aggregability and increasing blood viscosity [19]. In a previous study, storage of RBCs in PAGGS-M using blood bags manufactured of PVC with a new plasticizer (DINCH) gave excellent results in an *in vitro* study including a low degree of haemolysis [13]. However, the results of our study using PAGGS-M were not very encouraging. Increased haemolysis with levels exceeding the European maximum limit of 0·8% was noted already after day 21 [17]. In addition, low ATP levels below 3 umol/g Hb were noticed by the end of storage ($2{\cdot}9 \pm 0{\cdot}6$ and $2{\cdot}3 \pm 0{\cdot}6$ umol/g haemoglobin at day 35 and day 42, respectively; mean ± SD). ATP concentration in RBCs is correlated with *in vivo* recovery and negative consequences in terms of 24-h RBC recovery below 75 per cent in healthy volunteers will be found at levels below 2–3 μmol/g haemoglobin [20–23]. The level of 2,3-DPG decreased continuously to almost zero after day 14. The rate of synthesis of 2,3-DPG is associated with the intracellular RBC pH with breakdown favoured below pH 7·2 [21]. The levels of extracellular pH showed a continuous fall from $6{\cdot}86 \pm 0{\cdot}02$ (day 1) to $6{\cdot}32 \pm 0{\cdot}05$ (day 42; mean ± SD).

PAGGG-M was developed to maintain stable product characteristics during RBC storage [24]. It is an alkaline RBC additive solution based on the same ideas as the previously described Erythro-Sol additive solution [25, 26]. PAGGG-M has been shown to maintain ATP and 2,3-DPG at high levels during storage for up to 42 days combined with a very limited rate of haemolysis [13, 24]. We observed the same pattern regarding ATP in this study, with ATP levels varying between 6·5 and 4·6 μmol/g Hb. Our results also suggested higher rates of glycolysis as evidenced by higher consumption of glucose and higher production of lactate. This is in compliance with earlier results [13]. Increasing haematocrit during storage also indicated a continuous change in cellular volume of red cells. Regarding 2,3-DPG, results were inconsistent. Half of the units stored in PAGGG-M showed low 2,3-DPG levels similar to those in units stored in PAGGS-M, whereas 2,3-DPG content in the other half of units increased during the first week of storage, stayed at a high level until day 21 and then decreased in the same way as was described previously [24]. All the RBC units were prepared in the same way. We do not know the explanation of this phenomenon. However, haemolysis levels below the European maximum limit of 0·8% were observed only up to day 28, in contrast to the previously reported very low levels even after RBC storage for 42 days [13, 24].

To summarize, the physical properties of the new polyolefin plastic are different from those of PVC-DEHP. The sealing conditions are a somewhat different, implicating that the present sterile connecting devices probably can be used, but new sealing equipment for closing blood bag tubing will be needed. This is a first preliminary study of RBC storage in a new type of blood bags without plasticizer. Only two different RBC additive solutions were tested. Those studies should be repeated to confirm our results and, in addition, 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 haemolysis. There will be room for further development of this solution or other RBC additive solutions to address the haemolysis problems. At the end, we may have to make a balanced judgment based on the combination of the environmental and toxicological benefits and clinical and laboratory parameters, of which an accepted rate of haemolysis and shelf life are only two important factors, before selecting the future approach. 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 device processes in this context needs to be validated.

Acknowledgement

We thank Dr. D. de Korte, Sanquin Research, Amsterdam, The Netherlands, for his generous supply of the PAGGG-M RBC additive solution and also for his valuable comments on the manuscript.

References

1 Walter CW, Murphy WP: A closed gravity technique for the preservation of whole blood in ACD solution utilizing plastic equipment. *Surg Gyn Obst* 1952; **94**:687–692
2 Carmen R: The selection of plastic materials for blood bags. *Transfus Med Rev* 1993; **7**:1–10
3 Marcel YL, Noel SP: Contamination of blood stored in plastic packs. *Lancet* 1970; **1**:35–36
4 Jaeger RJ, Rubin RJ: Di-2-ethylhexyl phthalate, a plasticizer contaminant of platelet concentrates. *Transfusion* 1973; **13**:107–108
5 Valeri CR, Contreras TJ, Feingold H, *et al.*: Accumulation of di-2-ethylhexyl phthalate (DEHP) in whole blood, platelet concentrates and platelet-poor plasma. I. Effect of DEHP on platelet survival and function. *Environ Health Perspect* 1973; **3**: 103–108
6 Stern IJ, Carmen RA: Hemolysis in stored blood: Stabilizing effect of phthalate plasticizer. 18th Congress International Society Hematology and 16th Congress International Society Blood Transfusion (joint meeting) Montreal, Quebec, Canada August 16–22, 1980, p 151 (abstract)
7 Estep TN, Pedersen RA, Miller TJ, *et al.*: Characterization of erythrocyte quality during the refrigerated storage of whole blood containing di(2-ethylhexyl) phthalate. *Blood* 1984; **64**:1270–1276
8 Rock G, Tocchi M, Ganz PR, *et al.*: Incorporation of plasticizer into red cells during storage. *Transfusion* 1984; **24**:493–498
9 Horowitz B, Stryker MH, Waldman AA, *et al.*: Stabilization of red blood cells by the plasticizer, diethylhexylphthalate. *Vox Sang* 1985; **48**:150–155
10 Labow RS, Card RT, Rock G: The effect of the plasticizer di (2-ethulhexyl) phthalate on red cell deformabilty. *Blood* 1987; **70**:319–323
11 Högman CF, Eriksson L, Ericson Å, *et al.*: Storage of saline-adenine-glucose-mannitol-suspended red cells in a new plastic container: polyvinylchloride plasticized with butyryl-n-trihexyl-citrate. *Transfusion* 1991; **31**:26–29
12 Seidl S, Gosda W, Reppucci AJ: The in vitro and in vivo evaluation of whole blood and red cell concentrates drawn on CPDA-1 and stored in a non-DEHP plasticized PVC container. *Vox Sang* 1991; **61**:8–13
13 Lagerberg JW, Gouwerok E, Vlaar R, *et al.*: In vitro evaluation of the quality of blood products collected and stored in systems completely free of di(2-ethylhexyl)phthalate-plasticized materials. *Transfusion* 2015; **55**:522–531
14 Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR): Opinion on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015 update). European Union 2015; ISSN: 1831-4783; ISBN: 978-92-79-35606-3
15 Sampson J, de Korte D: DEHP-plasticized DEHP: relevance to blood services. *Transfus Med* 2011; **21**:73–83
16 Shaz BH, Grima K: Hillyer CD. 2 (Diethylhexyl)phthalate in blood bags: is this a public health issue? *Transfusion* 2011; **51**:2510–2517
17 Guide to the preparation, Use and Quality Assurance of Blood Components, 17th edn. Strassbourg, European Directorate for the Quality of Medicines & Healthcare, Council of Europe, 2013 and the Directive of the European Union 2004/33/EG
18 Lundin A: Use of firefly luciferase in ATP-related assays of biomass, enzymes and metabolites. *Methods Enzymol* 2000; **305**:346–370
19 Zehnder L, Schulzki T, Goede JS, *et al.*: Erythrocyte storage in hypertonic (SAGM) or isotonic (PAGGS-M) conservation medium: influence on cell properties. *Vox Sang* 2008; **95**:280–287
20 Hess JR, Greenwalt TG: Storage of red blood cells: new approaches. *Transfus Med Rev* 2002; **16**:283–295

21 Hess JR, Hill HR, Oliver CK, *et al.*: Alkaline CPD and the preservation of RBC 2,3-DPG. *Transfusion* 2002; **42**:747–752
22 Högman CF, Meryman HT: Storage parameters affecting red blood cell survival and function after transfusion. *Transfus Med Rev* 1999; **13**:275–296
23 Heaton WA: Evaluation of posttransfusion recovery and survival of transfused red cells. *Transus Med Rev* 1992; **6**:153–169
24 de Korte D, Kleine M, Korsten HGH, *et al.*: Prolonged maintenance of 2,3-diphosphoglycerate acid and adenosine triphosphate in red blood cells during storage. *Transfusion* 2008; **48**:1081–1089
25 Högman CF, Knutson F, Lööf H, *et al.*: Improved maintenance of 2,3-DPG and ATP in RBCs stored in a modified additive solution. *Transfusion* 2002; **42**:824–829
26 Gulliksson H, Vesterinen M, Payrat J-M, *et al.*: Extended storage of whole blood before the preparation of blood components: in vitro effects on red cells in Erythro-Sol environment. *Vox Sang* 2009; **96**:199–205