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**ORIGINAL PAPER**

# Separation of whole blood into plasma and red cells by using a hollow-fibre filtration system

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Vox Sanguinis

**Background and Objectives** The aim of this study was to assess the separation of whole blood into red cells and plasma by using the Sangofer® device, which is a gravity-fed, hollow-fibre system. The components would then be compared with those produced by the use of more elaborate technical equipment.

**Materials and Methods** Ten whole-blood units were leucoreduced by using a WBF2 filter and immediately separated into red cells and plasma by using the Sangofer® blood-separation device. Red cells were stored in additive solution and tested on days 1 and 42. The plasma was assayed for levels of various coagulation factors and for markers of both coagulation and complement activation.

**Results** The red-cell parameters were similar to those obtained when routine centrifugation methods were used. The filter did not cause haemolysis. Levels of plasma factor VIII and factor XI were lower than those seen in routinely produced leucoreduced plasma units but there was no evidence of activation of the coagulation and complement systems.

**Conclusions** The Sangofer® device is simple and straightforward to use and eliminates the need for both centrifugation and automated separation steps during the processing of whole blood into red cells and plasma components. Minor changes are required to make the procedure easier to incorporate into routine use.

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## Introduction

The routine separation of whole blood into red cells and plasma requires the use of elaborate technical equipment (e.g. programmable centrifuges and automated blood-component preparation systems). Trained staff are also required. Donated blood therefore often has to be transported some distance to a central facility before it can be processed. A simple filtration method, such as the Sangofer® device, could be of value in hostile situations (e.g. war zones) or in countries where expensive equipment is not available.

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Sekiguchi *et al.* [1] described the use of hollow fibres to separate blood into red cells and plasma without centrifugation. This methodology has been developed by Heim, Medizintechnik (Gladbeck, Germany) to produce the Sangofer® blood-separation device with an integral leucoreduction filter (WBF2; Pall Biomedical, Portsmouth, UK). The device is gravity fed and the prototype is being developed by the EMS Medical Group Limited (Stonehouse, Gloucestershire, UK).

By using this device, 10 whole-blood units were separated in the Components Laboratory, Scottish National Blood Transfusion Service (SNBTS) (Edinburgh, UK). The red cells in additive (SAGM) were tested on day 1 and then stored until day 42. Various parameters were measured, including 2,3-diphosphoglyceric acid (2,3-DPG), which influences haemoglobin's affinity for oxygen. The plasma was assayed for levels of various coagulation factors as well as for markers of both coagulation and complement activation.

The components produced were compared with those processed routinely, with reference being made to the validation procedures in the Guidelines for the Blood Transfusion Services in the UK [2].

## Materials and methods

### Blood collection and processing

Ten whole-blood units were processed. Donations were collected into NPBI Compoflex WB system packs, which are used routinely within the SNBTS. Following donation, the packs were docked on to the Sangofer® modules by using the Compodock sterile-connecting device (NPBI). At the same time, the dry packs attached to the Sangofer® modules for red cell collection were replaced with NPBI Compoflex SAGM bags containing 100 ml of additive solution.

Processing of the whole-blood units was carried out within 2 h of donation. The units were not, however, placed on the cooling trays that are used during routine processing.

The Sangofer® system allows plasma to be separated from cells by gravity-fed passage through a 0·5-μm pore size hollow-fibre filter. The hollow-fibre filter consists of 650 capillary membranes made from polyethersulphone with a wall thickness of 100 μm ± 25 μm and a pore size of 0·5 μm ± 0·1 μm. The diameter of the capillaries was 300 μm ± 40 μm, and the total free length of the fibres was 175·5 m. The fibres were enclosed in a plastic casing in the shape of a stirrup, which was separated, by polyurethane, into compartments for plasma (outside the capillaries) and red cells (within the fibres). The red cell inlet and outlet were situated on the upper part of the Sangofer® module. There were two openings for sodium chloride and plasma outflow. The blood inflow took place at a height of 60 cm above the upper edge of the Sangofer® via a leucocyte filter (WBF2; Pall). The blood flow was regulated via a roller clamp (75–100 drops/min), and the flow rate was regulated via a drip chamber.

The system was primed before use by manually forcing sodium chloride into the plasma compartment and, after 1 min of residence time, allowing it to return passively back to the initial bag.

Plasma processing began as soon as the whole blood entered the hollow-fibre filter. The red cells ran into the additive solution (SAGM). Following separation, the device was vented to optimize red cell and plasma yield.

The time, from the start of leucofiltration to the end of processing, was noted and volumes of the whole-blood units, red cells in additive and plasma were measured. The red cells in additive were immediately stored at +4 °C for 42 days.

### Samples

Whole-blood samples were taken for leucocyte and platelet counts plus estimations of haematocrit, total haemoglobin, potassium and lactate dehydrogenase. Extra samples (5 × 1 ml) were stored at +4 °C for 24 h until virology results were available. The samples were then assayed (day 1) for adenosine-5′-triphosphate (ATP), 2,3-DPG and plasma haemoglobin. The samples for plasma haemoglobin and fibrinogen estimations were prepared by centrifuging 1-ml aliquots of whole blood in a microcentrifuge for 1 min and then freezing the supernatants.

Red cells in additive were sampled on day 1 and day 42 when measurements and assays were performed as for the whole blood.

Fresh plasma samples were taken for leucocyte and platelet counts. Eight 0·5-ml aliquots were frozen at −40 °C for factor (F)VIII, FXI, fibrinogen, plasma haemoglobin, prothrombin fragments 1+2 and FXIIa assays. A further two 0·5-ml aliquots were frozen in EDTA tubes for use in the C3a des Arg assay.

### Cell counts, haematocrit and total haemoglobin

Cell counts (except for leucocyte counts in red cells in additive or in plasma), haematocrits and total haemoglobin levels were determined by using a Sysmex KX-21 Haematology Analyser (Sysmex, Milton Keynes, UK). Residual leucocytes, following leucoreduction, were enumerated by using a Becton Dickinson FACS Calibur flow cytometer (Becton Dickinson, Cowley, Oxford, UK) with Leucocount reagents.

### ATP, 2,3-DPG and plasma (or supernatant) haemoglobin

Plasma haemoglobin levels were measured by colorimetric determination at 600 nm by monitoring the catalytic action of haemoglobin on the oxidation of $3,3^1,5,5^1$-tetramethylbenzidine by $H_2O_2$.

Percentage haemolysis was calculated from the following formula:

$$\% \text{ Haemolysis} = \{[\text{plasma Hb} \times (100 - \text{Hct})] \div [\text{total Hb}]\},$$

where the haemoglobin (Hb) level is expressed in g/dl and Hct represents the haematocrit.

ATP and 2,3-DPG were assayed by enzymatic determination at 340 nm.

All three methods used reagent kits from Sigma-Aldrich (Poole, UK) and a Spectronic Unicam UV/VIS scanning spectrophotometer (Unicam Ltd, Cambridge, UK).

### Potassium and lactate dehydrogenase

The concentrations of potassium and of lactate dehydrogenase were measured in the Department of Clinical Biochemistry, Lothian University Hospitals NHS Trust (Edinburgh, UK) by using routine methods, which included an ion-selective electrode for potassium and a dry slide method for lactate dehydrogenase.

### Coagulation factors

FVIII and FXI were assayed on a Coag-A-Mate X2 analyser (Organon Teknika Ltd, Cambridge, UK) by one-stage clotting assays. Fibrinogen was measured by the Clauss assay with Fibriquik reagents (Organon Teknika Ltd) using an Amelung KC 4A micro analyser (Sigma Diagnostics, Poole, UK).

FVIII assays were standardized using the British standard from the NIBSC (South Mimms, Herts, UK). All other assays were standardized with Coagulation Reference plasma 100% (Technoclone Ltd, Dorking, Surrey, UK). A control plasma of known potency was assayed, on each occasion, for all coagulation assays.

### Enzyme-linked immunosorbent assays

Commercially available enzyme-linked immunosorbent assays (ELISA) were used to determine the levels of prothrombin fragment 1+2 (Sysmex) and FXIIa (Axis-Shield, Dundee, UK). C3a des Arg was assayed by radioimmunoassay (Amersham Pharmacia Biotech UK Ltd, Bucks., UK).

### Statistics

Results are presented as mean ± standard deviation.

## Results

### Whole-blood units

The whole-blood units were routinely collected whole-blood donations with a mean volume (including 63 ml of anti-coagulant) of 522 ± 3 ml and a mean haematocrit of 38 ± 3%. Plasma haemoglobin levels were 7·5 ± 3·8 mg/dl with percentage haemolysis calculated as 0·04 ± 0·02%. The mean fibrinogen concentration was 2·83 ± 0·60 mg/ml.

Mean ATP and 2,3-DPG levels were 4·70 ± 0·60 μmol/g of Hb and 13·78 ± 1·59 μmol/g of Hb, respectively. The potassium concentration was 3·8 ± 0·3 mM and lactate dehydrogenase, 291 ± 32 units/l.

The time taken from the beginning of leucoreduction to the end of the process ranged from 35 to 55 min (mean: 44 ± 7 min).

Problems occurred during the processing of the first four units. In unit 1 there was no air in the top bag to clear the filters. A mistake was made when the bypass lines were set up in unit 2. Unit 3 had narrow connections on the red cell collection bag, and there were break valve problems in unit 4. However, the processing times for these units were similar to those seen with the remaining six units.

### Red cells in additive

The results for days 1 and 42 are shown in Table 1. These are compared with the results obtained for red cells stored in SAGM at SNBTS in 1998/99 following whole-blood leucoreduction by Pall WB filters. Platelet counts were either undetectable or at the lower limit of detection for the Sysmex KX-21.

### Plasma

Approximately 5 ml of saline remained in the hollow-fibre filter after priming and this mixed with the plasma.

Results are shown in Table 2, together with results for plasma produced from whole blood that had been leucoreduced using Pall WB filters in 1998/99.

**Table 1** Leucoreduced red cells in additive solution

| | **Sangofer® device (*n* = 10)** | | **Pall WB filter (*n* = 10)** | |
|---|---|---|---|---|
| | **Day 1** | **Day 42** | **Day 1** | **Day 42** |
| Volume (ml) | 321 ± 11 | 296 ± 11 | **NA** | |
| WBC (× $10^6$/unit) | 0·01–3·05 | | **0·04–0·30** | |
| Hct (%) | 53 ± 2 | 56·0 ± 3 | **57 ± 3** | 60 ± 2 |
| Total Hb (g/unit) | 54·7 ± 4·2 | 49·9 ± 3·7 | **NA** | NA |
| Plasma Hb (mg/dl) | 22·0 ± 6·1 | 85·2 ± 30·8 | **19·7 ± 8·6** | 304·4 ± 217·2 |
| Haemolysis (%) | 0·06 ± 0·2 | 0·22 ± 0·06 | **0·05 ± 0·02** | 0·64 ± 0·48 |
| ATP (μmoles/g of Hb) | 4·71 ± 0·48 | 2·59 ± 0·49 | **4·16 ± 0·32** | 2·61 ± 0·24 |
| 2,3-DPG (μmoles/g of Hb) | 10·82 ± 1·61 | 0·60 ± 0·15 | **11·36 ± 2·02** | 1·05 ± 0·15 |
| Potassium (mM) | 3·2 ± 0·3 | 31·9 ± 2·8 | **NA** | NA |
| LDH (units/l) | 131 ± 9 | 189 ± 43 | **NA** | NA |

ATP, adenosine-5′-triphosphate; 2,3-DPG, 2,3-diphosphoglyceric acid; Hb, haemoglobin; Hct, haematocrit; LDH, lactate dehydrogenase; NA, not available; WBC, white blood cells.

Results are expressed as mean ± standard deviation (except for WBC, where the range is given) for units produced using the Sangofer® blood-separation device, compared with units produced from whole blood leucoreduced by using Pall WB filters.

**Table 2** Plasma, leucoreduced

| | Sangofer® device ($n$ = 10) | Pall WB filter ($n$ = 10) |
|---|---|---|
| Volume (ml) | 291 ± 14 | NA |
| WBC ($\times 10^6$/unit) | < 0·01 | NA |
| Platelets ($\times 10^9$/l) | < 1·0 | NA |
| FVIII (U/ml) | 0·87 ± 0·21 | 0·99 ± 0·32 |
| FXI (U/ml) | 0·70 ± 0·16 | 0·76 ± 0·14 |
| Fibrinogen (mg/ml) | 1·91 ± 0·44 | 2·65 ± 0·28 |
| Plasma Hb (mg/dl) | 4·8 ± 3·8 | NA |
| Prothrombin fragment 1+2 (nM) | 1·01 ± 0·14 | 0·67 ± 0·14 |
| FXIIa (ng/ml) | 1·8 ± 0·8 | 2·4 ± 1·3 |
| C3a des Arg (ng/ml) | 484 ± 355 | 238 ± 194 |

Hb, haemoglobin; FVIII, FXIIa, FXI, factor VIII, factor XIIa, factor XI; NA, not available; WBC, white blood cells.
Results (mean ± standard deviation) for units produced using the Sangofer® blood-separation device compared with plasma produced from whole blood leucoreduced using Pall WB filters.

Three of the 10 units (30%) had FVIII levels of < 0·7 U/ml.

## Discussion

### Whole-blood units

Recent data [3] suggest that 2 h is sufficient for free bacteria to be removed from blood by phagocytosis. The whole-blood units in this study were processed as soon as possible. However, it may be preferable to store them at room temperature for at least 2 h prior to leucoreduction. Rapid removal of the red cell concentrates to 4 °C, after processing, is also effective in the control of bacterial growth [3].

Two units were processed at the same time, taking between 35 and 55 min. Although problems occurred during the processing of units 1 to 4 (see the Results), the parameters obtained were similar for all units.

### Red cells in additive

The UK specification for leucoreduction is that a minimum of 99% of leucoreduced components tested should contain $< 5 \times 10^6$ leucocytes within 95% confidence limits [2]. (The European Union directive states that leucocytes should be reduced to $< 1 \times 10^6$/unit. Nine of the 10 units analysed in the present study reached this level). Further specifications for red cells, leucoreduced in additive, state that 75% of the units tested should have a volume of 280 ± 60 ml and a total haemoglobin level of > 40 g/unit.

All units satisfied these specifications and the haemoglobin range was 48·9–61·3 g/unit. The percentage haemolysis calculated was low (0·06 ± 0·02%) and therefore it can be concluded that the filtration caused no stress to the red cells. Unit 1 had the lowest haematocrit, of 49%, and it also had the highest leucocyte count of $3{\cdot}05 \times 10^6$/unit. However, this was the first unit processed and various problems were encountered.

Hogman *et al.* [4] demonstrated that there is a rapid loss of 2,3-DPG if the blood is held at room temperature for > 4 h. In this study, the 2,3-DPG levels on day 1 were 10·82 ± 1·61 μmol/g Hb, indicating reasonable retention of red-cell 2,3-DPG despite the brief hold without cooling.

The leucoreduction filter, used as part of this Sangofer® blood-separation device, was produced by Pall. When Williamson *et al.* [5] tested Pall WB filters (WBF1) under several processing conditions, the red cells in SAGM met the UK specifications. During 1998/99, the SNBTS tested various leucoreduction filters, and the results of testing red cells in additive and plasma leucoreduced using Pall WB filters are shown in Tables 1 and 2. In this current study, residual leucocyte counts ranged from 0·01 to $3{\cdot}05 \times 10^6$/unit which, although higher than expected when using a Pall whole-blood filter, is still below $5 \times 10^6$/unit. Very little haemolysis was observed after 42 days of storage (range 0·15 to 0·30%). The ATP, 2,3-DPG and potassium concentrations seen were very similar to those observed in the 1998/99 work.

### Plasma

The UK specifications for fresh-frozen plasma state that, in 75% of units tested, platelet counts should be $\leq 30 \times 10^9$/l and that the FVIII concentration should be ≥ 0·7 U/ml. Leucocyte counts should be below $5 \times 10^6$/unit. In this study, leucocytes and platelets were undetectable and were therefore well below $5 \times 10^6$/unit and $30 \times 10^9$/l respectively.

Three of the 10 units (30%) had FVIII concentrations of < 0·7 U/ml and therefore this did not meet the UK specification. FXI levels were 0·45–0·99 U/ml. Although Cardigan *et al.* [6] did not test Pall WB filters, they did observe losses of FVIII and FXI when plasma was leucoreduced by using Pall plasma filters. The losses seen in this work may therefore be partly owing to leucoreduction and partly as a result of the use of the Sangofer® device.

The whole-blood samples contained fibrinogen concentrations of 2·10 to 3·52 mg/ml. Fibrinogen levels in the plasma units ranged from 1·33 to 2·68 mg/ml. It is not known why these levels are so low. The plasma produced from whole blood leucoreduced using Pall WB filters contained 2·25 to 3·1 mg/ml of fibrinogen and therefore the Sangofer® device may be responsible for these low levels. It is, however, unlikely to have been selectively removed by the 0·5-μm pore size.

Prothrombin fragments 1+2 and FXIIa levels (0·76 to 1·18 nM and 1·0 to 3·1 ng/ml respectively) were within the normal ranges and there was no evidence of activation

of the coagulation system. Values for C3a des Arg varied within the range 110–1295 ng/ml, with a median value of 455 ng/ml. The normal range for C3a des Arg is quoted as 179–387 ng/ml when samples are taken immediately into EDTA. However, the plasma aliquots were not stored in EDTA until 1·5 to 2 h postdonation and therefore the levels seen did not give cause for concern.

## Conclusions

It can be concluded that the Sangofer® blood-separation device produces red cells in additive that are similar to and store as well as those prepared by the SNBTS using routine centrifugation methods.

The plasma FVIII and FXI concentrations were lower than desirable. However, there was no evidence of either coagulation or complement activation.

The Sangofer® blood-separation device is simple and straightforward to use and eliminates the need for both centrifugation and automated separation steps during the processing of whole blood into red cells and plasma components. For routine use, it would be preferable for an integral pack system containing anticoagulant and an additive solution to be attached to the device. *In vivo* recovery studies in volunteers may then be required.

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