Optimization of blood component preparation

Processing and donor influence

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CHAPTER 5

A new preparation method for red blood cells
for intrauterine transfusion enabling reduction of
donor exposure

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ABSTRACT

Background
Severe hemolytic anemia of the fetus, caused by maternal red blood cell alloantibodies, is treated with intrauterine transfusion (IUT) of red cell concentrates (RCC). Because IUT is associated with additional antibody formation, RCC with the closest match between donor and mother are preferred. Because one fetus needs a median of 3 IUT finding such RCC is complicated. Collection of repeated low volume donations from one selected donor during the entire IUT treatment period would reduce donor exposition and possibly IUT-associated alloimmunization.

Study design and Methods
Whole blood donations of 100 and 200 ml were diluted with saline, filtered, centrifuged and separated to prepare experimental RCCs. Before and after gamma irradiation, the RCC were sampled for comparison of in vitro quality with standard RCC for IUT. An additional washing procedure was investigated to remove anti-A/B antibodies.

Results
Experimental RCCs were leukoreduced to levels conforming with current guidelines and had final volumes of 44 (n=12) and 84 (n=8) mL with hematocrits between 0.80-0.88 L/L. Hemolysis was lower (0.12 vs 0.42%), potassium leakage comparable, ATP levels lower (4.8 vs 6.1 µmol/g Hb) and 2,3-DPG levels were higher (10.3 vs 7.7 µmol/g Hb) at 6 hours after irradiation (product expiration time) compared to standard RCC for IUT (n=3). Anti-A/-B titers decreased substantially by the washing procedure.

Conclusion
Red cell concentrates for IUT can be prepared from 100 or 200 ml whole blood donations, showing the potential of this new blood product to reduce donor exposure. A washing procedure is recommended to remove anti-A/-B.
Severe fetal anemia from hemolytic disease of the fetus or newborn (HDFN), caused by maternal antibodies against paternally inherited RBC antigens on the fetal cells, is treated with intrauterine transfusion (IUT) of red cell concentrates (RCC)\(^1\). Depending on the gestational week when starting the anemia treatment, a fetus receives 1 to 8 (median 3) intrauterine transfusions\(^2\) prepared from an equivalent number of donors. Although perinatal survival has improved to more than 90% with this treatment, the procedure is associated with some feto-maternal blood exchange and, despite Rhesus and K matching between mother and donor, additional maternal antibody formation against fetal or donor RBC antigens in more than 20% of cases\(^3,4\). To reduce maternal antibody formation at least against IUT donor antigens, either extended recipient-donor RBC antigen matching or minimizing donor exposure is essential. However, due to the special requirements of the IUT, it is not always possible to completely match the IUT donor with clinically relevant maternal RBC antigens (results submitted for publication).

The current routine in the Netherlands for the preparation of a RCC for IUT is as follows: a fresh (≤3 days old) O RhD negative standard leukoreduced (LR) RCC in saline-adenine-glucose-mannitol (SAGM), cytomegalovirus-seronegative and Parvo B19-safe, is selected from the inventory, based on compatibility with maternal antibodies and the best possible match with the extended blood group typing (Rh, K, Fy, Jk and S) of the mother. The selected RCC is centrifuged at high speed (30 min at 3,835 x g), SAGM is removed, the hematocrit (Hct) adjusted to 0.80-0.85 L/L by adding 0.9% saline, and the unit is then gamma irradiated. A disadvantage of this procedure is that for every following IUT, a RCC from another donor has to be selected because the interval between subsequent IUTs is typically 14 (interval between 1\(^{st}\) and 2\(^{nd}\) IUT) to 28 days (for subsequent IUTs), whereas a regular 500 ml donation interval is at least 56 days. Because only small volumes (mostly between 10-100 mL) are transfused intrauterine, a substantial part of the selected RCC is discarded. In addition, for every IUT treatment there is a chance that the RCC selected from inventory deviates in blood group typing of the mother.

For (preterm) neonates, but not for fetuses, transfusion programs to minimize donor exposure have already been developed and reducing donor exposure and red cell wastage is reported\(^5,6\). This was realized by splitting normal size RCCs in series of pedipacks which are stored and designated for one patient. While IUT needs fresh RCCs, a strategy to improve matching and reduce donor exposure, would be to select a compatible donor (for C, c, D,
E, e, K, Fy\textsuperscript{a}, Fy\textsuperscript{b}, Jk\textsuperscript{a}, Jk\textsuperscript{b}, M, S and s antigens) from the large donor pool for the whole IUT treatment period of a fetus, instead of matching within a limited number of available fresh RCC on the shelf. This selected donor would then donate small volumes of whole blood (WB) 1 to 2 days prior to each planned IUT.

It was our aim to minimize donor exposure to one or two donors at most. To show the potential of such an approach, a production process was developed and validated for the preparation of RCC for IUT from small (100 and 200 mL) WB donations with at least the same quality as the currently prepared RCC for IUT.

**MATERIALS AND METHODS**

**Preparation of RCCs for IUT**

RCCs for IUT were prepared according to the current process from selected RCCs (reference) or from small WB volumes, as depicted in Figure 1. Low-volume WBs were either mimicked using fractions from a standard 500-mL donation (experimental processing) or using low-volume donations (feasibility test of the complete process). Two small donation volumes were used, 100 mL to obtain 40 to 50 mL of RCC for IUTs early in pregnancy and 200 mL to obtain RCCs with larger volumes (80-90 mL) for transfusion later in pregnancy.

**Reference process**

Five-hundred mL WB donations were collected and processed into LR-RCCs as described earlier\textsuperscript{7}. LR-RCCs for IUT were prepared from routine LR-RCCs in SAGM 60 h after collection to reflect current routine practice. The LR-RCC units were centrifuged for 30 minutes at 3835 x g (maximum acceleration, slow stop 2, Sorvall RC12BP, Thermo Fisher Scientific, Asheville, NC) resulting in an accumulated centrifugal effect (ACE) of approximately 21x10\textsuperscript{7}. The SAGM was removed using a plasma extractor, and 0.9% NaCl (Ref. 8001895, Fresenius, Emmer-Compascuum, The Netherlands) was added to obtain a Hct of 0.80 to 0.85 L/L. The RCCs were gamma irradiated with a minimum of 25 Gy (Gammacell 3000 Elan, MDS Nordion, Ottawa, Canada) and stored for 24 hours at 2-6°C.

**Experimental processing**

To develop the new process, RCCs were prepared from 100- or 200-mL WB fractions obtained from standard 500-mL WB collections. The 500-mL WBs were collected (with consent of the donors), cooled as described above, and held at room temperature (RT) for 2-24 h.
Bag systems (Composelect PQ31451, Fresenius) with a WB filter were modified by removing the CPD from the WB collection containers and the SAGM container, leaving the systems entirely with empty containers and filter. After sterile docking, the collection containers were filled with 100 or 200 mL WB. Subsequently, 300 mL or 400 mL 0.9% NaCl was added, respectively. The diluted WB was mixed gently by inversion of the bag and filtered to remove leukocytes and platelets. Then, the LR-WB was then centrifuged at the standard WB centrifugation run (maximum acceleration; 4790 x g up to ACE = 9.00 x 10^7; slow stop 3; Sorvall RC12BP) and units were separated in a RCC and supernatant using an automatic blood component
extractor (CompoMat G5; lower press to 50.0 mm; upper press to detector A7 [100-mL units] or A6 [200-mL units]). The experimental RCCs were gamma irradiated as described above.

**Feasibility test of new process with small WB donations**

To obtain small WB units of 100 (n=2) and 200 mL (n=2), four collection systems (CQC3941) were modified by removing part of the CPD into the RCC bag using a scale. Taking into account a WB sample of 5-10 mL before processing, 15 mL of the CPD in two collection systems intended for 110 mL and 29 mL of the CPD in two collection systems intended for 210 mL was left behind. Regular WB donors (of blood group O and A) were asked to give their written consent for these experimental collections. A collection mixer (CompoGuard, Fresenius) was adjusted to the desired blood volume. The small WB units were cooled as described above and held overnight at RT. The following day, the collection bags of CQC3941 containing the small WB units were docked to PQ31451 systems, thereby replacing the empty collection bags of that system. The WB units were processed into RCCs for IUT as described for the experimental processing.

**In vitro measurements**

WB units for preparation of the reference RCCs for IUT were not sampled. WB units used for the experimental RCCs were sampled by using the remainder after separation into fractions, and small WB donations were sampled by using a sample pouch (5-10 mL). These samples were analyzed for complete blood count using a hematology analyser (Sysmex XT2000i, TOA, Tokyo, Japan) and for determination of anti-A/-B antibody titers using the standard saline indirect antiglobulin test.

RCCs were weighed to determine the volume and samples were taken using a sample site coupler and a syringe, just before gamma irradiation at t=0, and at t=6 and 24 hours after irradiation. Only in the t=0 sample, Hct was determined using a capillary centrifuge (Hettich Mikro 12-24, Hettich Zentrifugen, Tuttingen, Germany) and low level leukocytes were counted with a counting kit (LeucoCount, Ref 340523, BD Biosciences, San Jose, CA) on a flowcytometer (FACSCalibur, BD Biosciences).

Immediately after each sampling, the RCCs were analyzed for pH at 37°C, glucose, lactate and potassium levels (ABL705, Radiometer, Copenhagen, Denmark). Red cell counts, hemoglobin (Hb) concentrations and platelet counts were measured using the cell analyser (Sysmex, TOA). Hemolysis was determined by measuring the amount of free Hb with a low-Hb analyzer (HemoCue, Angelholm, Sweden). Adenosine triphosphate (ATP) and 2,3 diphosphoglycerate
A new intrauterine transfusion product

(2,3-DPG) levels were only measured at t=0 and 6 h as described elsewhere. Anti-A and anti-B titers were only determined in the last sample (t=24 h) as described above.

**Statistical analyses**
The *in vitro* quality of reference and new RCCs was compared with an unpaired t-test. A two-sided p value of less than 0.05 was considered significant. The effect of storage was analyzed by comparing parameter results of t=0 hour versus t=6 hours and versus t=24 hours with a paired t test. A two-sided p value of less than 0.01 was used to indicate a significant difference to correct for multiple comparisons.

**RESULTS**

**Filtration conditions of low-volume WB units**
To study the filtration efficacy of the PQ31451 WB filter for much smaller volumes than for which the filter was designed, different filtration conditions were investigated after consulting the research and development department of the manufacturer. To minimize loss of red blood cells caused by the dead volume of the filter, WB (n=4) was diluted two or four times with 0.9% NaCl and leukoreduced at different time points to find optimal conditions. These time points were 2 hours after collection (either cooled down to 4°C or left at RT) or after overnight storage (cooling to <25°C within 2 hours after donation and overnight hold at RT). All conditions resulted in adequate leukocyte removal with less than 0.2x10⁶/unit remaining. The highest red blood cell recovery of 80% was found for the units which were 4 times diluted. Therefore, routine WB storage conditions (cooling to <25°C within 2 hours after donation and overnight hold at RT) were considered applicable and used for further investigations.

**RCCs from 100 and 200 mL WB**
RCCs prepared from 100 and 200 ml WB had a final volume ranging from 39-47 mL and 78-92 mL and contained 10-14 g and 20-25 g of Hb, respectively. Red cell recovery and contaminating leukocytes were slightly higher in RCCs prepared from 200 mL as compared to 100 ml WB (Table 1). Median anti-A titers were 16 and anti-B titers 8 in RCCs prepared from 100 mL WB (n=8) and up to 2 titer steps higher in the RCC prepared from 200 mL WB (n=6), probably due to a lower prefiltration dilution. After washing, antibody titers decreased on average 3 titer steps (range 1-7) and even antibody titers of 512 were reduced to less than 4 in the final product.
Table 1: Composition of RCC for IUT (mean ± SD) prepared from 100 and 200 mL WB.

<table>
<thead>
<tr>
<th></th>
<th>100 mL WB (n=12)</th>
<th>200 mL WB (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Blood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume including CPD, mL</td>
<td>110 ± 16</td>
<td>209 ± 17</td>
</tr>
<tr>
<td>Hematocrit, L/L</td>
<td>0.38 ± 0.03</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td><strong>Red Cell Concentrate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>44 ± 4</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>Hematocrit, L/L</td>
<td>0.84 ± 0.01</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>Hemoglobin, g</td>
<td>11.5 ± 1.1</td>
<td>22.8 ± 1.7</td>
</tr>
<tr>
<td>RBC recovery, %</td>
<td>84 ± 3</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>WBC, x10⁶</td>
<td>0.01 ± 0.03</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>PLT, x10⁹</td>
<td>0.5 ± 0.2</td>
<td>1.1 ± 0.4</td>
</tr>
</tbody>
</table>

During the 24-hour storage period at 2 to 6°C, pH and glucose decreased and lactate, potassium and hemolysis increased as expected (Table 2). At routine product expiration time (6 hr after irradiation), the washed RCCs showed slightly higher hemolysis (but well below the limit of 0.8%), lower glucose and lactate levels and comparable potassium levels compared to the non-washed RCCs. Increasing lactate levels in washed units, up to 24 hours after irradiation, demonstrated that sufficient glucose was still available for the red cells.

**Comparison of experimental and reference RCC for IUT**

The mean final volume of experimental RCCs from 100 ml of WB was approximately four times lower than the reference RCCs (44±4 vs.160±6 mL). Mean Hb contents were 11.5±1.1 and 41.5±2.2 g, and the mean Hct was 0.84±0.01 and 0.80±0.01 L/L for experimental and reference RCCs, respectively. Both experimental and reference RCCs for IUT fulfilled European guidelines for residual leukocytes (<1.0x10⁶) and platelets (<50x10⁹/L)¹⁰. Reduction of anti-A/-B titers in experimental RCCs depended on application of the wash procedure, while anti-A/-B was not detectable in reference RCCs.

Storage measures (Table 2) show a slightly higher pH, lower glucose and lactate levels, slightly lower potassium leakage, lower hemolysis, slightly lower ATP but higher 2,3-DPG in the experimental RCCs as compared to reference RCCs.

**RCCs for emergency IUT procedures**

Parallel to the new process using overnight held WB, two emergency situations were simulated. First, an urgent IUT request was simulated by preparing RCCs on Day 0 from 100 mL of WB (n = 2), 2 hours after collection and cooling to RT. Second, a postponed IUT treatment was mimicked by preparing RCCs on Day 1 from overnight-held 100-mL WB units.
Table 2: Storage measures (mean ± SD) of RCC for IUT prepared from 100 or 200 ml WB, with or without a washing procedure, and from reference leukoreduced RCC stored at 2-6°C, before (t = 0h) and 6 and 24h after gamma irradiation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling time (h)</th>
<th>Reference RCC (n=3)</th>
<th>100 mL WB non-washed (n=8)</th>
<th>100 mL WB washed (n=4)</th>
<th>200 mL WB non-washed (n=6)</th>
<th>200 mL WB washed (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (37 °C)</td>
<td>0</td>
<td>6.85 ± 0.02</td>
<td>6.94 ± 0.03*</td>
<td>6.96 ± 0.03</td>
<td>6.93 ± 0.04</td>
<td>6.96 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.83 ± 0.02†</td>
<td>6.92 ± 0.04**</td>
<td>6.92 ± 0.04</td>
<td>6.90 ± 0.04†</td>
<td>6.93 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6.81 ± 0.02</td>
<td>6.89 ± 0.05*†</td>
<td>6.90 ± 0.04†</td>
<td>6.88 ± 0.03†</td>
<td>6.90 ± 0.01†</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0</td>
<td>22.4 ± 0.1</td>
<td>5.1 ± 0.3*</td>
<td>0.2 ± 0.1</td>
<td>0.02 ± 0.1</td>
<td>0.01†</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>22.4 ± 0.1</td>
<td>4.8 ± 0.6*†</td>
<td>0.1 ± 0.1</td>
<td>6.1 ± 0.4†</td>
<td>0.1†</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>21.8 ± 0.1†</td>
<td>3.9 ± 0.7†</td>
<td>0.1 ± 0.1†</td>
<td>5.2 ± 0.4†</td>
<td>0.1†</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>0</td>
<td>7.0 ± 0.6</td>
<td>2.4 ± 0.6*</td>
<td>0.6 ± 0.1</td>
<td>3.1 ± 0.9</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.8 ± 0.6†</td>
<td>3.3 ± 0.8†</td>
<td>1.7 ± 0.1†</td>
<td>4.4 ± 0.9†</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.8 ± 0.7†</td>
<td>5.0 ± 0.9†</td>
<td>2.8 ± 0.2†</td>
<td>6.3 ± 0.8†</td>
<td>4.9 ± 1.3</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>0</td>
<td>3.0 ± 0.6</td>
<td>2.1 ± 0.1*</td>
<td>1.5 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>14.3 ± 1.6†</td>
<td>12.4 ± 1.7†</td>
<td>13.0 ± 2.7†</td>
<td>12.5 ± 1.3†</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>473 ± 4.4†</td>
<td>39.0 ± 6.6†</td>
<td>45.6 ± 8.1†</td>
<td>45.4 ± 4.1†</td>
<td>52.1 ± 5.7</td>
</tr>
<tr>
<td>Hemolysis (%)</td>
<td>0</td>
<td>0.31 ± 0.05</td>
<td>0.11 ± 0.05*</td>
<td>0.17 ± 0.05</td>
<td>0.06 ± 0.03</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.42 ± 0.03</td>
<td>0.13 ± 0.05*</td>
<td>0.20 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.45 ± 0.05</td>
<td>0.17 ± 0.06*†</td>
<td>0.23 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>ATP (µmol/g Hb)</td>
<td>0</td>
<td>5.9 ± 1.0</td>
<td>4.4 ± 0.8*</td>
<td>4.8 ± 0.2</td>
<td>5.4 ± 0.5</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.1 ± 1.1</td>
<td>4.4 ± 0.9*</td>
<td>4.5 ± 0.3†</td>
<td>5.2 ± 0.5†</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>2,3-DPG (µmol/g Hb)</td>
<td>0</td>
<td>7.9 ± 2.7</td>
<td>10.8 ± 3.0*</td>
<td>12.5 ± 4.3</td>
<td>9.7 ± 3.5</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.7 ± 2.8</td>
<td>10.8 ± 3.2*</td>
<td>12.4 ± 4.5</td>
<td>9.8 ± 3.7</td>
<td>9.6 ± 0.7</td>
</tr>
</tbody>
</table>

* RCC from 100 ml WB versus reference RCC: p<0.05; † versus t=0: p< 0.01; ‡ individual data
(n = 3) followed by a 24-hour cold storage (2-6°C) before irradiation on Day 2. Both ‘urgent’ and ‘postponed’ RCCs showed comparable *in vitro* quality during 24-hour storage after irradiation (data not shown), as compared to the RCCs prepared and irradiated on Day 1 (Table 2). After the 24-hour cold storage period in saline before irradiation, postponed RCCs had higher initial potassium levels (6-9 mmol/L).

**DISCUSSION**

A new method for the preparation of RCCs for IUT from low-volume WB collections is described. The method is based on a repeatedly returning donor for the entire IUT treatment period, instead on the use of different RCCs from multiple donors from the blood bank stock. This new approach aims to achieve two major advantages for patient blood management; 1) extended donor-mother RBC antigen matching for clinically relevant antigens (i.e., Rhesus, K, Duffy, Kidd and S) is more feasible from the large donor pool than from RCC inventory, and 2) donor exposure for mother and fetus is reduced. From 100- and 200-mL donations, RCCs of approximately 40 and 80 mL with Hct levels of 0.80 to 0.88 L/L, respectively, were prepared with comparable or better *in vitro* storage properties than reference RCCs for IUT prepared from LR-RCCs. The preparation process was further improved by avoiding the removal of adenine and mannitol (from SAGM) from the LR-RCCs, substances that have possible renal toxicity in fetuses and pre-term infants.

Variables of special attention were hemolysis, extracellular potassium and anti-A/-B antibody titers, which all should be as low as possible. Hemolysis was always well below the limit of international guidelines (<0.8% in Europe) during the 6- and 24-hour storage period. In some cases, there was a modest increase (<0.1%) due to irradiation, but maximum values remained within specifications between 0.1 and 0.4% even after 24 hours, far beyond the product expiry time. Despite low numbers tested, the reference RCCs for IUT showed the highest hemolysis. This was probably caused by the two centrifugation steps, of which the second step to remove SAGM exposes red cells to a long and hard centrifugation with an ACE more than two times the ACE of the WB centrifugation run that is used for the new process. Upon application of a washing procedure, the experimental RCCs showed also slightly more hemolysis, but, despite the absence of nutrients and protecting compounds like plasma proteins and/or mannitol, well within limits.

Extracellular potassium levels of RCC suspended in saline increased rapidly after irradiation. Calculated rates of potassium leakage after irradiation were 33 up to 56 µmol/mL/24h,
A new intrauterine transfusion product showing a small range for all investigated variations in volumes, processes and processing times. Extracellular potassium levels were 2 to 4 µmol/mL of RCC at t=6 hours (current product shelf life) and 8 to 10 µmol/mL of RCC at t=24 hours, the latter comparable with data reported by Bashir and colleagues\textsuperscript{12} for gamma irradiated RCCs for IUT in plasma. Because potassium levels of new RCCs were similar to those of reference RCCs, no complications during the IUT treatment due to potassium load are expected because there is continuous placental exchange of potassium\textsuperscript{13,14}. Regardless, considering that potassium levels increase very fast after irradiation of the RCC, and avoiding posttransfusion peak levels in the fetus that may cause transfusion-associated hyperkalemic cardiac arrest (TAHCA)\textsuperscript{15}, the shelf life of the new product was maintained at 6 hours.

Because the ABO blood group of the fetus is often unknown before the first IUT treatment, blood group O RhD negative donors are routinely selected. Notwithstanding low A and B expression on fetal red blood cells, possible minor ABO donor-fetus incompatibility, in theory, can cause additional fetal cell hemolysis and hemolytic transfusion reactions. As is known from ABO hemolytic disease of the newborn, the IgG fraction of these antibodies will not lead to severe hemolysis during pregnancy, because complement is seldom activated\textsuperscript{16-18}. It is unlikely that the passively transferred IgM A and B antibodies are capable of activating the fetal complement system, because as estimated from CH50 lytic potential, this is impaired during fetal life and still relatively immature at birth\textsuperscript{19}. Because the washing procedures of the reference RCC leads to undetectable A and B antibodies, a wash procedure was introduced to guarantee at least an extra two step lower titer or even undetectable anti-A/-B antibodies. Additionally, with the clinical introduction of the product, donors (to prevent donor deferral as much as possible male donors only\textsuperscript{20}) will be selected with anti A or B titers below 128. The combination of all measures, donor selection, dilution and washing, will guarantee anti-A/-B titers less than 4 which is considered to be sufficiently low.

Analysis of historical data of IUT treatments showed that in 27% of treatments, 40 mL and in 52% of treatments, 80 mL of RCC sufficed. For 15% of treatments, 80 to 100 mL and for 6% more than 100 mL of RCC was used (unpublished data). Because small donations of 200 mL of WB resulted in RCC volumes of 85 mL, it is estimated that more than 90% of IUT treatments can be covered by 100- or 200-mL WB donations from a single donor. According to EU, US and Canada regulations, the minimal interval between two 500-mL WB donations is 56 days (8 weeks). Based on the typical IUT intervals of 2 (interval between first and second IUT) or 4 weeks (for subsequent IUTs), permission to give 60 mL per week, instead of a blockade for 8 weeks after a 500 mL donation, will be introduced in our blood
bank information system to allow for a donor to donate multiple smaller volumes with a maximum of 500 mL during an 8-week period.

Once this new product has passed all Dutch regulations regarding new blood products, a clinical study on the effects of this new product will be performed, with donor exposure and maternal antibody formation against IUT donor antigens as primary endpoints. Secondary endpoints will include safety issues for fetus and donor, period between subsequent IUTs, total number of IUTs, extended typed donor availability, and costs. The results from this study will be compared with historical data, which are readily available from the department of Obstetrics of the Leiden University Medical Center database, in which data on HDFN patients have been documented since 1987.

In conclusion, RCCs for IUT can be prepared from WB donations of 100 and 200 mL, showing the potential of this new method to minimize donor exposure to one or two donors at most. This is achieved by matching a patient to a donor instead of to a donation. The in vitro quality of the products obtained with the new procedure was similar or even better than the reference standard IUT products.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

There are no conflicts of interest.
REFERENCES