The challenge of producing a novel split frozen/thawed & washed red cell component for intrauterine transfusion (IUT) in the National Frozen Blood Bank (NFBB)

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Introduction

Blood management to cover pregnancies is becoming more complex with the requirement for multiple antigen and/or rare antigen negative red cells becoming more common.

In this case the National Frozen Blood Bank (NFBB) reviewed its ability to support pregnancy management in the context of maternal alloimmunisation and very rare maternal red cell types. The feasibility of maternal blood for splitting and freezing prior to manufacturing multiple intrauterine transfusions (IUT) was investigated, to ensure blood availability for IUT's throughout a pregnancy. Maternal blood is not normally considered due to the transfusion associated risk of Graft vs Host Disease (GvHD).

The challenge was to determine how well the Haemonetics ACP215 machine (figure 1) would process small volume units during de-glycerolisation; and if, after further processing to increase the haematocrit (Hct) suitable for an IUT (70-85%), and irradiation to further reduce the risk of GvHD, a product of acceptable safety and quality could be produced.

Methods

The NFBB selected 6 non-rare red cell (RBC) units, 5 days post bleed to represent the latest we freeze standard RBC units, centrifuged them to remove the SAGM, glycerolised (to preserve the cells once frozen) on a Haemonetics ACP215 (Haemonetics 2017), then split into 3 smaller packs prior to freezing. The frozen RBC packs were stored in a -80°C freezer.

One split from each original donation was then thawed at 37°C and deglycerolised using the ACP215 to wash off the cryopreservative and resuspend in SAGM. A haematocrit suitable for an IUT (70-85%) was then achieved by centrifuging the unit at 3,840 rpm for 15 minutes (following NHSBT DAT14), and pressing off a specific amount of SAGM (figure 2) determined using a series of (linear regression) calculations incorporating the weight, Hct, MCHC and target Hct.

The thawed split packs were irradiated immediately post processing, then transferred to NHSBT Component Development Laboratory (CDL).

Testing for haemoglobin and supernatant haemoglobin was carried out by NFBB immediately post processing, with further testing for haemolysis and supernatant potassium completed by CDL at 8 and 24 hours post irradiation to determine the clinically acceptable shelf life of this novel product.

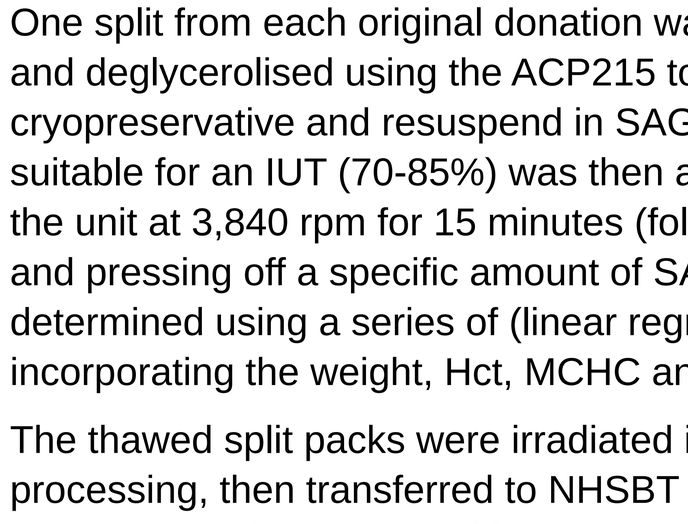

Figure 2. Donation on a manual plasma press extracting SAGM to increase Hct.

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Reference

- Haemonetics (2017) ACP215 Operations Manual P/N 85271-30(EC)
- NHSBT DAT14/6.5 – Centrifuge Parameters
- Meli A et al 2022 A comparison of the effect of X and gamma irradiation on red cell storage quality. Vox Sanguinis; 117:39-48

Images taken by NHSBT NFBB staff

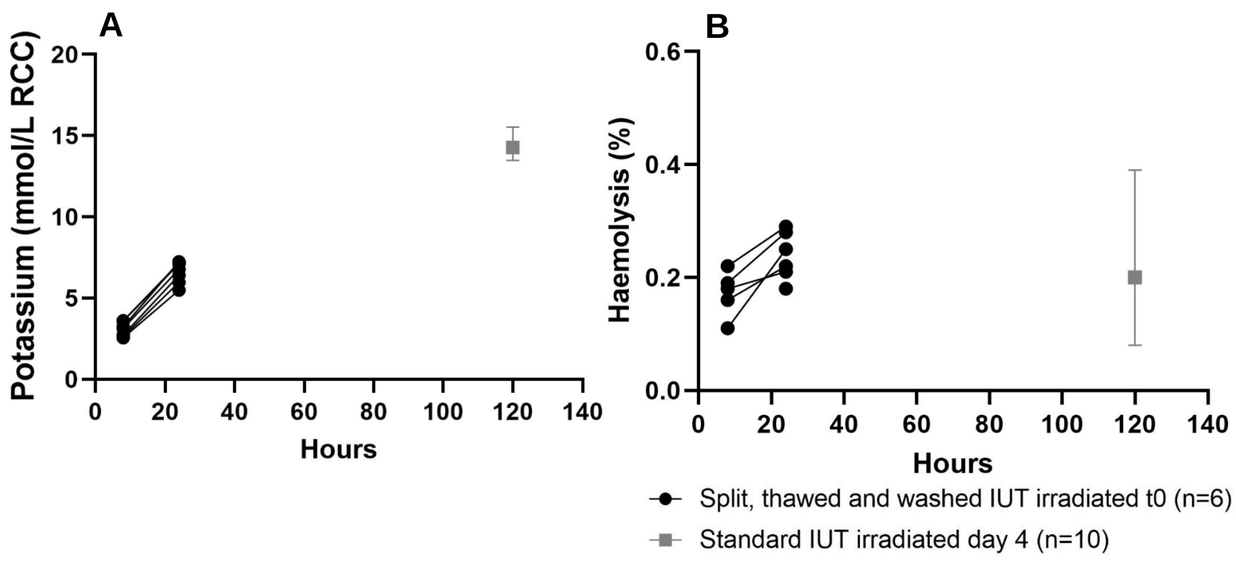
Figure 1. Haemonetics ACP215 used to glycerolise and deglycerolise frozen/thawed & washed red cells

Results

Table 1. NFBB component specification results, mean (range); n=6:

Thawed split units prior to washing on the ACP215	
Volume (mL)	137.5 (128 - 151)
Haematocrit (%)	59.1 (53.4 - 62.9)
Thawed split units post washing on the ACP215	
Volume (mL)	291.7 (290 – 294)
Haematocrit (%)	18.2 (17.0 – 20.5)
Thawed split units after processing into IUT components	
Volume (mL)	77.8 (73 – 84)
Haematocrit (%)	73.2 (67.4 – 78.8)
Total haemoglobin (g/unit)	23.3 (21.7 – 25.2)
Supernatant haemoglobin (g/unit)	0.066 (0.04 – 0.11)

Figure 3. CDL potassium and haemolysis results



A. Supernatant potassium concentration (mmol/L Red Cell Concentrate) from the 6 units tested at 8 and 24 hours post-manufacture (black lines).

B. Percentage haemolysis from the 6 units tested at 8 and 24 hours post-manufacture (black lines).

The grey square shows historic CDL data from standard IUT irradiated on day 4 and tested on day 5 of storage (n=10; Meli A *et al* 2021).

Discussions / Conclusions

Despite some technical challenges, we were able to produce a split IUT unit that met Hct specifications for IUT in 5 of 6 units, and supernatant haemoglobin specifications for thawed and washed red cells for all units (Table 1). Potassium and haemolysis levels were checked in CDL, 8 and 24 hours post irradiation, and found to be no higher than standard IUT components at the end of shelf-life 24 hours post-irradiation (figure 3), suggesting acceptable quality and safety for use under concessionary release when there is no alternative.

Further work processing more units to give us a better statistical analysis may allow us to freeze rare split units as a novel component more regularly for small volume fetal and neonatal transfusions thereby helping to conserve rare blood stocks, and allowing the use of maternal donations where they are the only alternative.