Cells4Life
the experts in stem cell processing

Technical information - TotiCyte

TotiCyte is a CE marked reagent used to isolate the buffy coat (stem cell fraction) from umbilical cord blood. TotiCyte retains 97% of haematopoietic stem cells post-processing and 63% of haematopoietic stem cells post-thaw. This means that it recovers 2.1 times more stem cells post-thaw than the current industry leading processing method at the point of use.

Stem cells isolated using TotiCyte also perform 20% better than the industry leader for cell growth (colony forming units or CFU). CFU is the way that treating facilities evaluate how well stem cells will perform in treatment.(1)

Taking into account both total number of cells retained post-thaw, and how well each of these cells grows, TotiCyte delivers 3 times more cells in treatment than competing systems in the UK.

Efficacy

We have conducted in-house testing of our own system and the two systems used by other private umbilical cord blood banks in the UK. For the purpose of this study, we have called these systems 'industry leader' and 'low-cost' based on their current market positions in the global blood processing industry.

All experiments were conducted according to the manufacturers' recommended protocols by technicians who had received training by the manufacturers of each system.

Pre- and Post- thaw cell recovery

- CD34+ is the marker for haematopoietic stem cells, which are the cells currently used in cord blood therapies.
- Post-processing means the number of viable cells remaining in a sample after it has undergone processing, but prior to freezing.
- Post-thaw means the number of viable cells remaining after a sample has undergone processing, freezing and then thawing.

Table.1 *Post-processing and post-thaw viable CD34+ recovery for TotiCyte, industry leader and low-cost systems*

	Average Viable CD34+	
	Post-Processing	Post-Thaw
TotiCyte	96.9%	63.2%
Industry Leader	89.8%	29.8%
Low-cost	87.6%	26.0%

Fig.1 *Bar chart comparison of post-processing viable CD34+ recovery for TotiCyte, industry leader and low-cost*

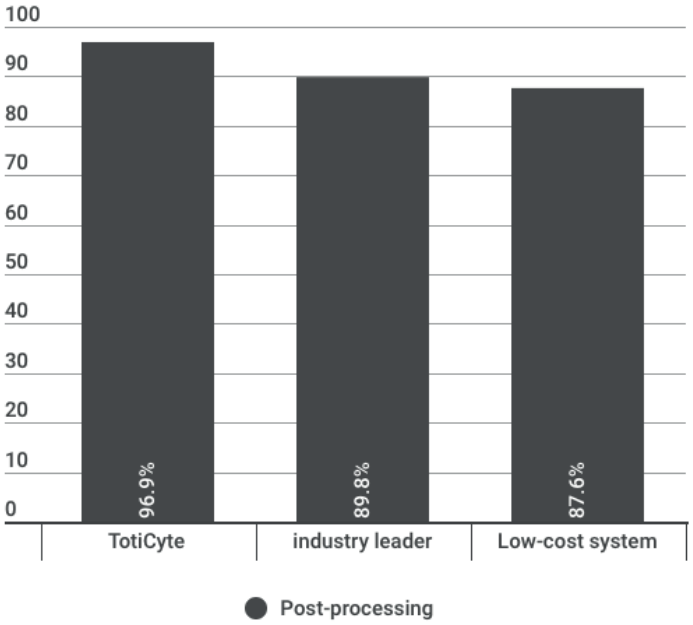

Fig.2 *Bar chart comparison of post-thaw viable CD34+ recovery for TotiCyte, industry leader and low-cost*

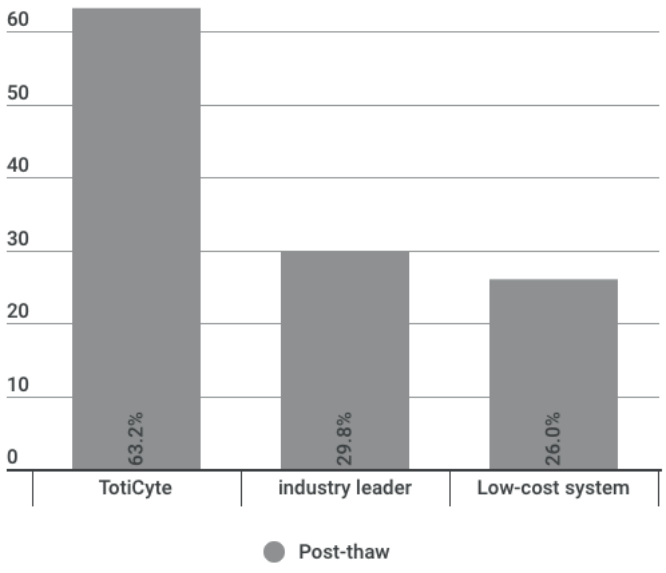

Table.2 *Increase in viable CD34+ post-thaw recovery provided when samples are processed using TotiCyte compared to samples processed using Industry leader and Low-cost system. 95% confidence level has been applied to give the range of the possible uplift that TotiCyte delivers*

	Industry leader	Low-cost
Post-thaw comparison TotiCyte uplift (average)	2.1	2.4
95% confidence level range	1.6 - 2.9	1.8 - 3.5

TotiCyte outperforms both the industry leader and the low-cost system post-processing, delivering 8% more viable CD34+ cells than the industry leader and 9% more viable CD34+ cells than the low-cost system.

However, the more crucial measure of cell recovery is post-thaw, as this is the number of cells the sample will contain when it is used. The low-cost system loses over 74% of viable CD34+ and the industry leader loses just over 70%. TotiCyte recovers the highest number of viable CD34+ post-thaw, at 63.2% viable cell recovery. Comparatively, and normalising for starting CD34+ positive viability pre-processing, TotiCyte recovers 2.1 times more viable stem cells post-thaw than the industry leader and 2.4 times more than the low-cost system.

With a confidence interval of 95%, comparing the lowest performance of TotiCyte against the highest performance of the other two systems, TotiCyte delivers between 1.6 - 2.9 times more viable CD34+ cells post-thaw than the industry leading system, and between 1.8 - 3.5 more viable CD34+ cells post-thaw than the low-cost option.

CFU growth

We have also compared TotiCyte against the industry leader and low-cost system for CFU growth. CFU stands for colony forming unit and is the measure of how well the cells grow and divide. CFU growth is the best indicator of how the stem cells will perform in treatment; the better they grow, the more efficacious they are likely to be. This is the gold standard test used by treating facilities to measure the quality of cells recovered.[1]

Table.3 *Colony forming unit growth per CD34+ cell plated for TotiCyte, Industry leader and low-cost system*

	TotiCyte	Industry leader	low-cost
CFU growth (average)	0.74	0.61	0.55

On a like-for-like basis, cells isolated using TotiCyte grow on average 20% more than those processed using the current industry-leading and low-cost systems.

Table.4 *Total increase in CD34+ when taking into account viable CD34+ post-thaw recovery and CFU growth of the recovered cells when samples are processed using TotiCyte compared to samples processed using Industry leader and Low-cost system. 95% confidence level has been applied to give the range of the possible uplift that TotiCyte delivers.*

TotiCyte uplift vs industry leader (post-thaw + CFU comparison)	2.5
95% confidence level range	1.4 - 5.3

TotiCyte uplift vs low-cost system (post-thaw + CFU comparison)	3.3
95% confidence level range	1.7 - 18.5

Taking into account both pre-freeze and post-thaw CD34+ cell recovery and the CFU growth of the cells recovered, TotiCyte provides on average 2.5 times more stem cells than the current industry leader and 3.3 times more stem cells than the low-cost system.

Method of action

When added to cord blood at a 1:1 ratio, TotiCyte causes the erythrocyte fraction (red cells) to rouleaux and fall to the bottom of the sample. The white cell fraction remains suspended in the plasma, which is then expressed into a separate bag. This leaves over 99% of the waste red cells behind with minimal loss of the white cells. The plasma is then centrifuged at low speed to isolate the buffy coat and reduce the storage volume down to 25ml. It is hypothesised that the low spin speed on centrifugation is responsible for superior cell recovery and CFU growth post-thaw.

Safety

TotiCyte is formulated of low concentration solutions routinely transfused in blood therapy.

The final concentration of DMSO in a sample processed using TotiCyte is 7.5%. The final concentration in samples processed using all other methods is between 10% - 12%. These levels of DMSO are routinely transfused into human patients as part of cord blood transfusion.[2]

The final concentration of Dextran in TotiCyte is 1.25%. This concentration of Dextran is routinely transfused into human patients without adverse effect.[3]

Phosphate buffered saline is also routinely transfused into human patients without adverse effect.[4]

Most treating facilities operate a washing protocol meaning that there would be no DMSO or Dextran remaining in a cord blood sample at the point of treatment.[5]

TotiCyte

Raw materials:

- PBS 95%,
- Dextran 2.5%,
- DMSO 2.5%

Certifications:

- CE marked
- ISO13485
- HTA authorised

References

1. Page et al., 2011. Total Colony-Forming Units are a strong, independent predictor of neutrophil and platelet engraftment after unrelated umbilical cord blood transplantation: a single center analysis of 435 cord blood transplants. Biology of Blood and Marrow Transplantation: Vol.17 (9), 1362-74.
2. Fry et al, 2015. Assessing the toxic effects of DMSO on cord blood to determine exposure time limits and the optimum concentration for cryopreservation. International Society of Blood Transfusion: Vol. 109 (2), 181-90.
3. National Center for Biotechnology Information. PubChem Compound Database; CID=105075, https://pubchem.ncbi.nlm.nih.gov/compound/105075 (accessed Nov. 28, 2017).
4. Annual Reports in Medicinal Chemistry, Volume 43 Academic Press, 17 Dec 2008.
5. Nagamura-Inoue et al., 2003. Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank, Transfusion Vol 43: 1285-94.