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 63.2% of haematopoietic stem cells post-thaw. This means that it recovers 2.2 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 2.0 times 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]

Efficacy

We have conducted in-house testing of all three systems used by umbilical cord blood banks in the UK, meaning that we are able provide a meaningful comparison of TotiCyte against both other methods currently available. These are the current industry leader and the current lowest cost system.

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

Pre- and Post- thaw cell recovery

The below data compares pre- and post- thaw viable CD34+ cell recovery.

- CD34+ is the marker for haematopoietic stem cells, which are the cells currently used in cord blood therapies.
- Pre-freeze 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 *Pre-freeze and post-thaw viable CD34+ recovery for TotiCyte, Industry leader and Low-cost system*

	Average Viable CD34+	
	Pre-Freeze	Post-Thaw
TotiCyte	96.9%	63.2%
Industry Leader	89.9%	32.1%
Low-cost	93.7%	18.3%

Fig.1 *Bar chart comparison of pre-freeze and post-thaw viable CD34+ recovery for TotiCyte, Industry leader and Low-cost system*

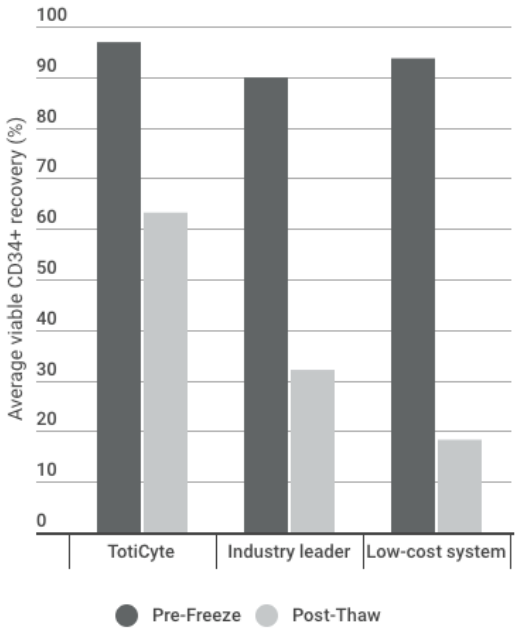

Table.2 *Increase in viable CD34+ post-thaw recovery provided when samples are processed using TotiCyte compared to samples processed using Industry leader and Low-cost system. 95% confidence level has been applied to give the range of the possible uplift that TotiCyte delivers*

	Industry leader	Low-cost
Post-thaw comparison TotiCyte uplift (average)	2.24	4.30
95% confidence level range	1.43 - 3.05	2.35 - 6.26

All three systems perform well pre-freeze; recovering 90% of viable CD34+ or higher. TotiCyte slightly outperforms both the industry leader and the low-cost system by 7% and 3.2% respectively.

The most significant differences in recovery between the systems occur post-thaw. The low-cost system loses over 80% of viable CD34+ and the industry leader loses just under 70%. TotiCyte recovers the highest number of viable CD34+ post-thaw, at 63.2% viable cell recovery. Comparatively, TotiCyte recovers 2.2 times more viable stem cells post-thaw than the industry leader and 4.3 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 1.4 and 3.1 times more viable CD34+ cells post-thaw than the industry leading system, and between 2.4 and 6.3 more viable CD34+ cells post-thaw than the low-cost option.

CFU growth

We have also compared TotiCyte against the industry leader 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.[1]

Table.3 *Colony forming unit growth per 1.5x10^5 cells for TotiCyte and Industry leader*

	TotiCyte	Industry leader
CFU growth (average)	49.9	26.5

On a like-for-like basis, cells isolated using TotiCyte grow on average 2.0 times more than those processed using the current industry-leading system.

Table.4 *Increase in viable CD34+ post-thaw and CFU provided when samples are processed using TotiCyte compared to samples processed using Industry leader. 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)	4.86
95% confidence level range	2.02 - 7.70

Taking into account both pre-freeze and post-thaw CD34+ cell recovery and CFU growth, TotiCyte provides on average 4.9 times more stem cells than the current industry leader. With a confidence interval of 95%, the range is 2.0 to 7.7 times more stem cells.

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]

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.