Technical information - TotiCyte

TotiCyte is a CE marked reagent used to isolate the buffy coat (stem cell fraction) from umbilical cord blood. TotiCyte retains 63% of haematopoietic stem cells post-thaw, which is 1.5 times more than our industry standard processing method, the AXP.

Stem cells isolated using TotiCyte also perform 110% better 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 3.3 times more cells in treatment than the AXP system.

The AXP system is regarded as an industry leader for cord blood processing and is used by both the NHS and most American cord blood banks. The data we present for the AXP is consistent with other published studies that have evaluated this system. Published papers also show that the AXP is comparable, if not slightly superior, in performance to both the Sepax and Macopress, which are the other two most widely used cord blood processing systems.\(^{(2,3)}\) This indicates that the uplift shown for TotiCyte is significant on an industry-wide level.

Efficacy

We have conducted extensive in-house testing of the two cord blood processing systems that are available at Cells4Life. These are our proprietary processing method, TotiCyte, which is the CellsPlus service, and the AXP system, which is used for our entry level service, Cells.

These experiments were conducted in accordance with our standard operating procedures, by technicians who were experienced in the operation of both systems.

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 and the AXP.

<table>
<thead>
<tr>
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<th>Average Viable CD34+</th>
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<tbody>
<tr>
<td></td>
<td>Post-Processing</td>
</tr>
<tr>
<td>TotiCyte</td>
<td>96.9%</td>
</tr>
<tr>
<td>AXP</td>
<td>96.9%</td>
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</tbody>
</table>

Fig.1 Bar chart comparison of post-processing viable CD34+ recovery for TotiCyte and the AXP.

Fig.2 Bar chart comparison of post-thaw viable CD34+ recovery for TotiCyte and the AXP.
Toticyte and the AXP system perform equally well for CD34+ cell recovery post-processing, both delivering 96.9%.

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 in therapy. Here Toticyte delivers a significant uplift, recovering 63.2% of CD34+ cells compared to the AXP at 40.87%.

Comparatively, and normalising for starting CD34+ positive viability pre-processing, Toticyte recovers 1.5 times more viable stem cells post-thaw than the AXP.

### CFU growth
We have also compared Toticyte against the AXP 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 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 2. Increase in viable CD34+ post-thaw recovery provided when samples are processed using Toticyte compared to samples processed using AXP**

| Post-thaw comparison | Toticyte uplift (average) | 1.5 |

On a like-for-like basis, cells isolated using Toticyte grow on average 110% more than those processed using AXP.

**Table 3. Colony forming unit growth per CD34+ cell plated for Toticyte, and AXP**

<table>
<thead>
<tr>
<th>Number of colonies per CD34+ plated (average)</th>
<th>Toticyte</th>
<th>AXP</th>
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<tbody>
<tr>
<td></td>
<td>0.74</td>
<td>0.35</td>
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Taking into account both pre-freeze and post-thaw CD34+ cell recovery and the CFU growth of the cells recovered, Toticyte provides on average 3.3 times more stem cells than our industry standard AXP method.

### References

### Toticyte

**Raw materials:**
- PBS 95%
- Dextran 2.5%
- DMSO 2.5%

**Certifications:**
- CE marked
- ISO13485
- HTA authorised

**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.\(^{(5)}\)