



Cord blood is a valuable source of haematopoietic stem cells for bone marrow transplantation - particularly in paediatric patients. It has advantage of lower HLA requirements and is readily available.

The minimum dose of nucleated cells (CD45+), which includes the haemopoietic stem cells needed for a bone marrow transplant, is  $> 10\text{-}20 \times 10^6 / \text{kg}$ .

A cord blood unit can have anywhere between 100,000,000 and 1200,000,000 cells and this is dependent on the volume collected.

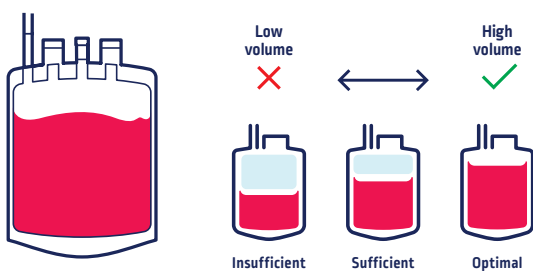
The standard or mean collection volume of cord blood is about 80 – 100ml including the 29ml of anticoagulant in the bag. This is the volume is necessary to achieve adequate cell counts for bone marrow transplants.

As each birthing situation is unique, cord blood collections vary greatly from patient to patient. The following protocol is a guide to attempt to achieve optimal cord blood collection:

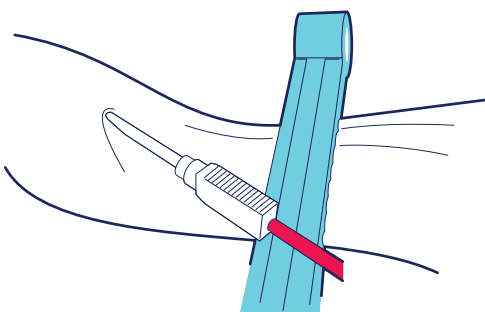
### Cord Blood collection Protocol

1. A sterile cord blood collection bag is supplied by Netcells.

2. Approximately 80-100ml of blood needs to be collected for successful storage.



3. Collection takes place after the baby is delivered, and the cord is clamped and cut.

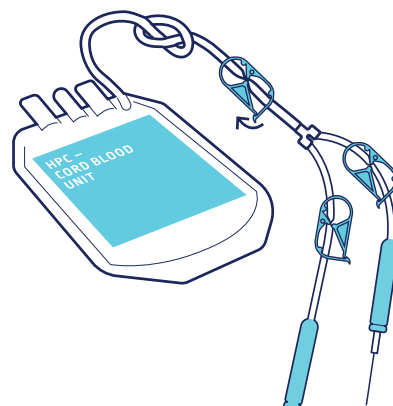


4. The attending obstetrician or midwife does the collection.

5. Once the baby is separated from the cord, the needle attached to the blood bag, is inserted into the umbilical vein. It is advisable for the surgical assistant to milk the cord toward the needle – this will increase the blood flow into the lab. If the flow of blood stops, the needle can be reinserted further up the cord towards the placenta.

6. Collection can take place during a caesarean section or a normal vaginal delivery.

7. After the collection has been done, all 3 clamps are closed and a knot tied in the main line, and the cord blood collection bag is placed in a temperature-controlled kit, ready for transport.



### **Cord Blood Processing**

- Once received in the laboratory, the cord blood unit is reduced by centrifugation and the plasma and red blood cells are separated out from the buffy coat.
- The final product contains white blood cells and haematopoietic progenitor cells (HPC).
- Final storage bag is 25ml in capacity with a 80:20 split.
- Once the cryopreservative has been added, the cord blood undergoes freezing at a controlled rate using Liquid Nitrogen (LN<sub>2</sub>) and stored in Liquid Nitrogen vapour at -1800C

### **Testing**

- Several tests on the cord blood unit are required before storage:
  - Sterility testing – aerobic, anaerobic and fungal,
  - Flow cytometry – cell count and viability,
  - BO/Rh blood grouping,
- Maternal blood tests for HIV, Hepatitis B, Hepatitis C, HTLV, Syphilis and CMV are conducted
- Multiple segments of continuous tubing are created before storage to be used for future testing (integrally attached samples).
- 2 extra sample vials are retained during storage for future HLA and viability testing.

### **Flow Cytometry**

- Flow Cytometry | 2 cell populations are tested for:
  - CD34+ (Haematopoietic Stem Cells)
  - CD45+ (Leukocytes – total nucleated cells)
- Total nucleated cell count is considered as the most standardised criteria for determining the potency of cord blood products
- Minimum cell dose required for transplant >10-20 x 10<sup>6</sup>/kg CD45+ and >17x10<sup>4</sup> for CD34+
- Cord blood units are assessed according to FC data and a decision is made as to whether the unit should be stored or not
- Emerging therapies, such as treatment for cerebral palsy, are showing that units containing smaller numbers of cells may be useful
- Criteria: CD45+ >100,000,000 and CD34+ >200,000, lower counts are deemed suboptimal, and the client is given the option of whether they would like to continue storage or not. Clients are refunded if they choose not to store their babies stem cells.