

Stem Cell Processing

The role of the Stem Cell &
Immunotherapy Department in
Birmingham

NHS Blood and Transplant

- Provision of Blood Products
- Tissue Typing
- Stem Cell Processing, Testing and Cryopreservation

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Guidelines

- JACIE – Joint Accreditation Committee of ISCT & EBMT - 2013
- Human Tissue Authority – HTA - 2015
- MHRA – Medicines and Healthcare products Regulatory Agency - 2016

Transplant Centres

- New Queen Elizabeth Hospital Birmingham
- Birmingham Heartlands Hospital
- Birmingham Children's Hospital
- University Hospital Coventry & Warwickshire
- Royal Stoke University Hospital
- Russell Hall Hospital

Why Transplant?

- To replace stem cells which have been 'knocked out' following the intensive Chemotherapy and/or Radiotherapy
- This replaces the patients bone marrow and restores the function of the immune system

Source of Stem Cells

- Bone Marrow (HPC-M)
- Haematopoietic stem Cells (HPC-A)
- Umbilical Cord Blood (HPC-C)

Types of stem cell transplant

- Autologous
(patients own cells)
- Allogeneic
(related or unrelated)
- Stem Cell
engraftment
- Stem Cell
engraftment
- Cells 'rescue' patient
after chemotherapy
- Donor cells attack
tumour cells (GVL)

Testing, Processing and Cryopreservation

- Analysis of Stem Cells
- Processing
- Specialist freezing for storage prior to Transplantation

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graph TD; A[HOSPITAL BEDSIDE] --> B[HOSPITAL WARD]; B --> C[HOSPITAL BLOOD BANK]; C --> D[NBS BLOOD BANK]; D --> E[STEM CELL LAB  
Analyse stem cells]; E --> F[+ 4.0°C FRIDGE  
storage of stem cells prior to processing]; F --> G[CLEAN ROOM  
Processing of Stem cells]; G --> H[CRYOBIOLOGY  
Cryopreservation  
And longterm storage of stem cells]; H --> I[Specialised transport  
of frozen stem cells]; I --> J[Thawing of frozen stem cells]; J --> A;
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The flowchart illustrates the stem cell processing and transport cycle. It begins at the **HOSPITAL BEDSIDE**, where stem cells are collected. The process then moves to the **HOSPITAL WARD**, represented by a blood bag icon, and then to the **HOSPITAL BLOOD BANK**, represented by a car icon. From there, the cells are transported to the **NBS BLOOD BANK**. The next step is the **STEM CELL LAB**, where stem cells are analyzed. Following analysis, the cells are stored in a **+ 4.0°C FRIDGE** for storage prior to processing. The process then moves to the **CLEAN ROOM** for processing of stem cells. The processed cells are then sent to **CRYOBIOLOGY** for cryopreservation and long-term storage. Finally, the cells are transported via **Specialised transport of frozen stem cells** to the **Thawing of frozen stem cells**, which then feeds back into the **HOSPITAL BEDSIDE** to complete the cycle.



Stem Cells and plasma are transported to NBS in an insulated porter box.



Details are checked and the cells are quarantined in a labelled container. Processing paperwork is prepared.

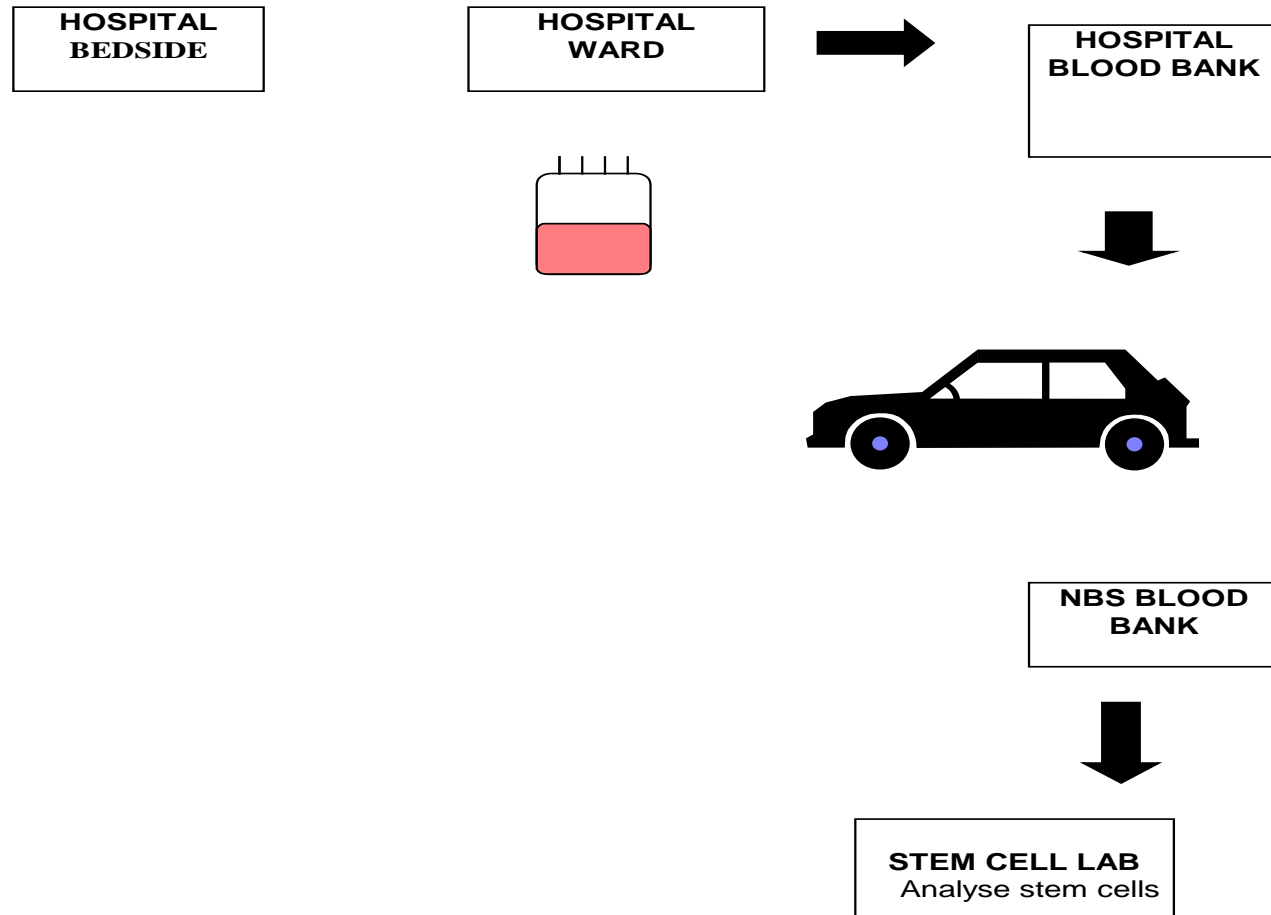


All stem cell harvests received are assigned a barcode



If necessary harvested are quarantine overnight at 4°C prior to cryopreservation/processing

STEM CELL JOURNEY *Blood and Transplant*



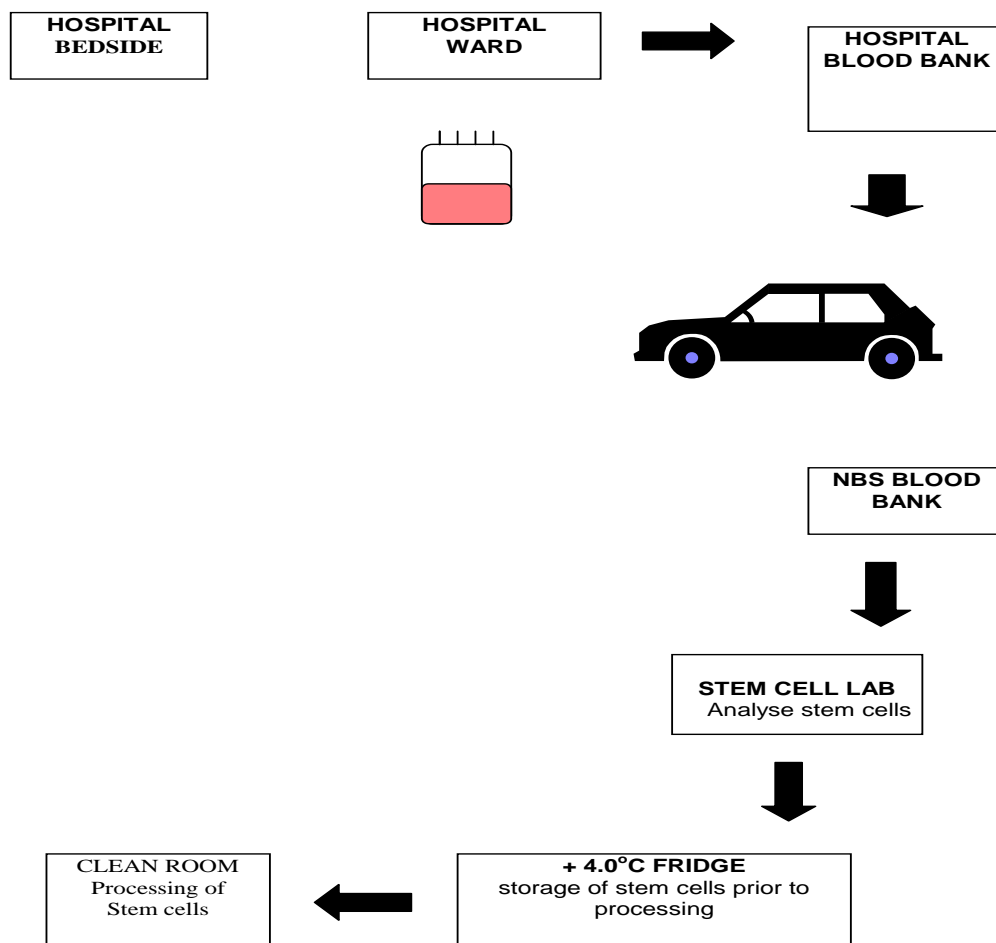


Flow Cytometer for quantitating the stem cell number in the apheresis harvest, by virtue of the fluorescently labelled antibodies to the CD34 marker on stem cells

Numbers of cells required for transplant

- CD34: $2 - 6 \times 10^6/\text{Kg}$ patient weight
- wbc: approx. $2 \times 10^8/\text{Kg}$
- CFU: $>1 \times 10^5/\text{Kg}$

STEM CELL JOURNEY





A stem cell harvest bag is transferred to the clean room via a hatch.



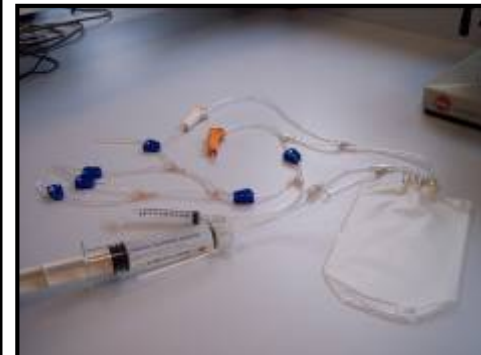
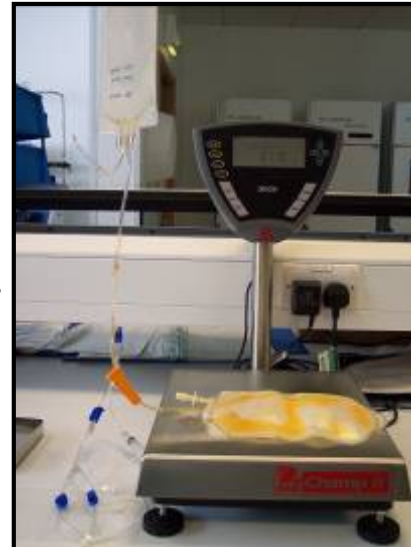
Stem Cell dept. staff process the stem cells in laminar flow cabinets wearing sterile garments thereby providing a sterile working system.

Cryo-Doc

Open system processing



Closed system processing



Autologous Processing

- CD34 analysis
- Prepare for cryopreservation
- Cryopreservation
- Storage
- CFU assay if stored over 5 years

Allogeneic Processing HPC-A

- CD34 & CD3 analysis and issue
- TC-T doses
- CD34 Selection
- CD3/19 Depletion
- TCR $\alpha\beta$ CD19 Depletion
- T cell depletion with Campath
- Directed Cord blood storage
- CFU assays before and after process

Allogeneic Processing HPC-M

- CD34 analysis and issue
- Bone Marrow filtration
- Plasma depletion or volume reduction
- Buffy coat preparation (BC)
- Red Cell Depletion (RCD)
- Clinimacs procedure only after BC or RCD
- CFU assay before and after process
- Frozen VUD Cord blood storage

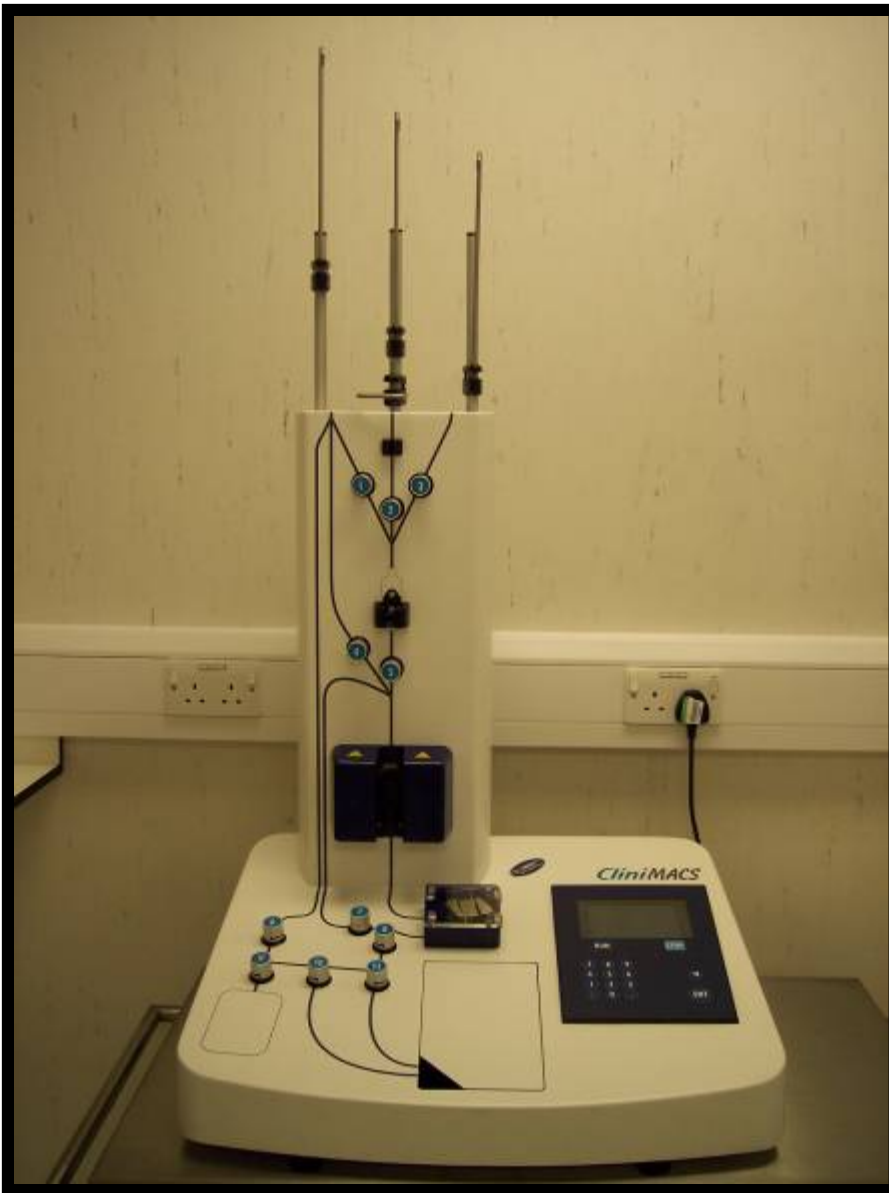
Specialist Procedures

COBE 2991 + CliniMACS

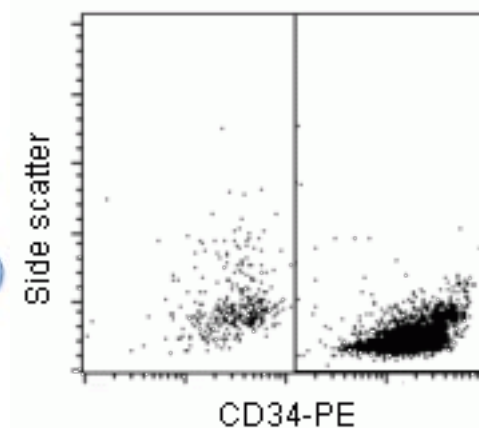
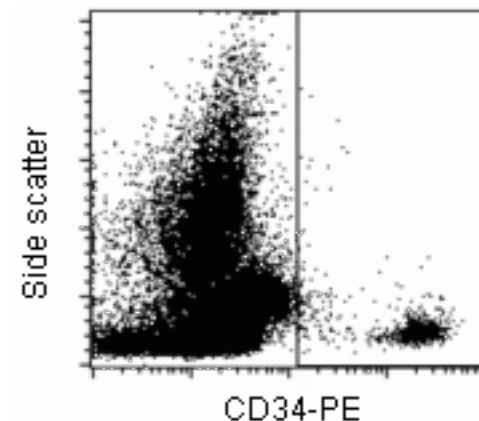
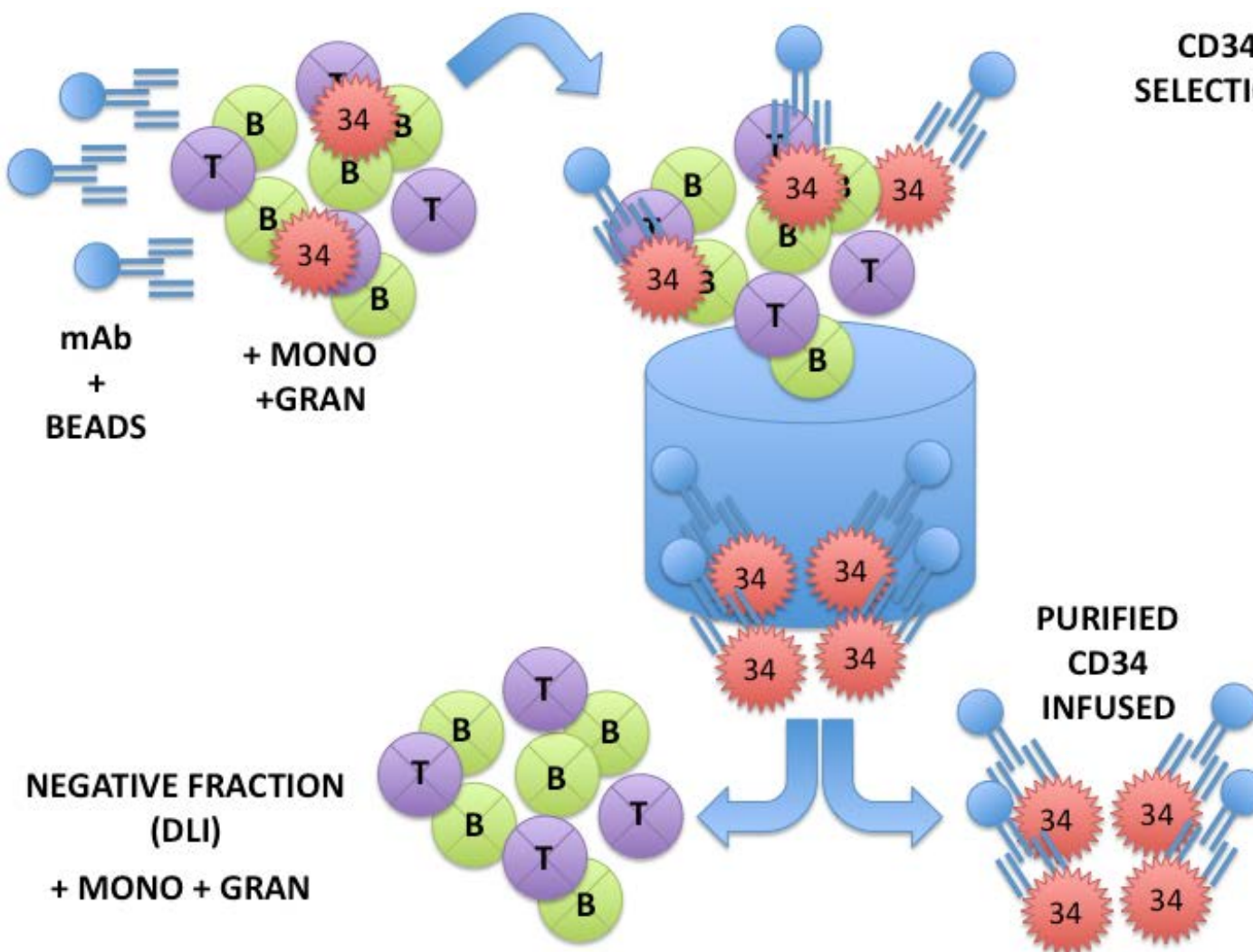
CD34+ selection

CD3/CD19 depletion

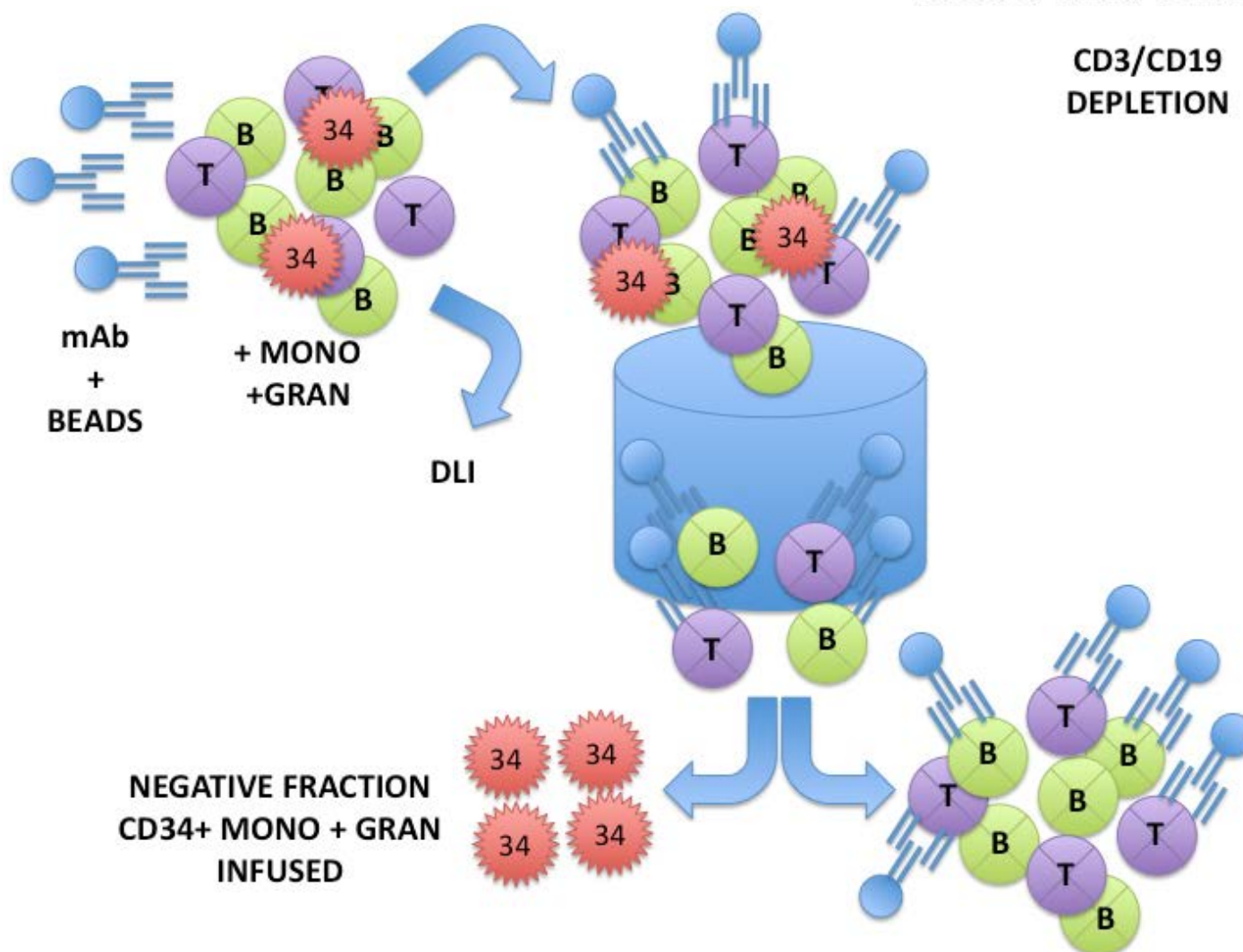
TCR ab / CD19 depletion



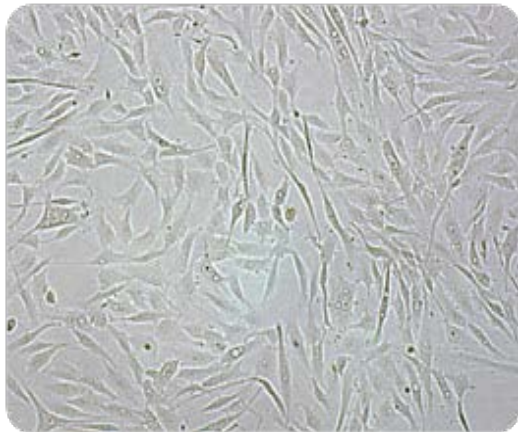
**CD34
SELECTION**



**CD3/CD19
DEPLETION**

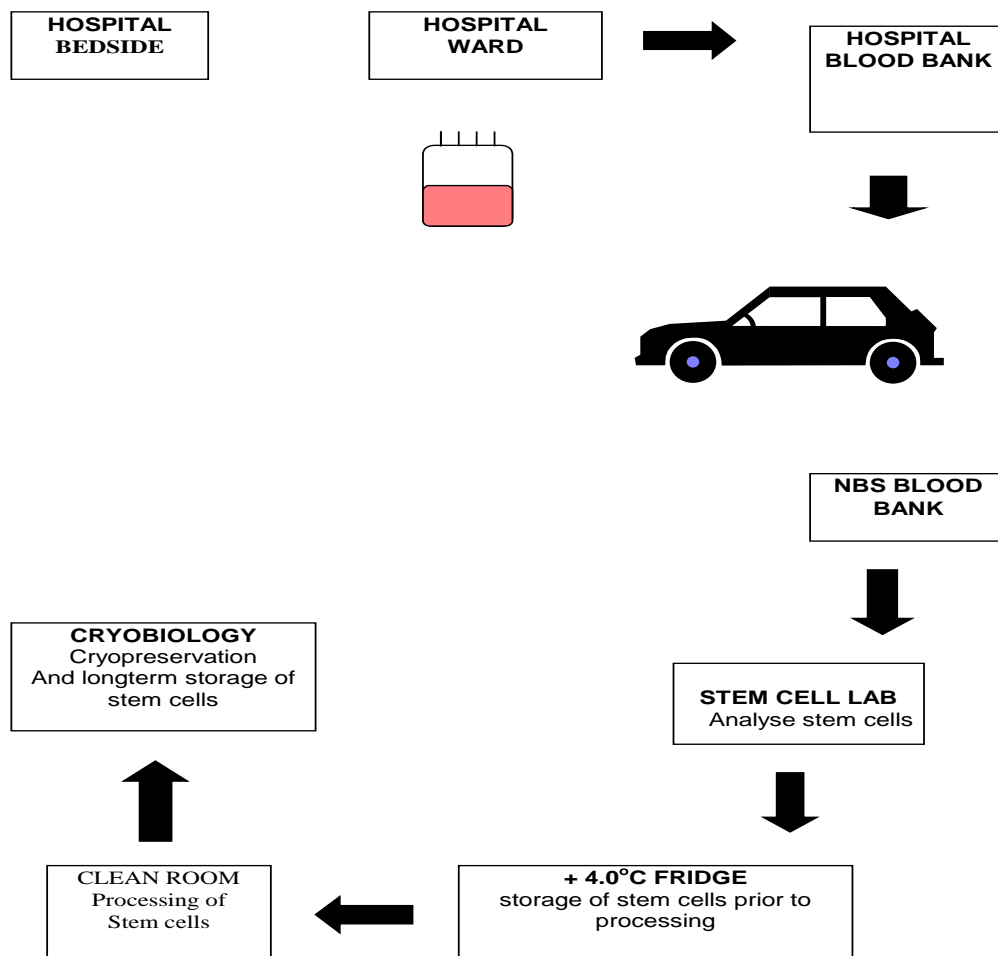


Mesenchymal Stem cells (MSC)



- Adult Stem cell
- Stromal cells
- Ability to differentiate:
 - Cartilage
 - Bone
 - Muscle
 - Hepatocyte like cells
 - Neural like cells
- Resides primarily in bone marrow
- Tissue regenerative and Immuno-suppressive effect
- Expanded *Ex-vivo*

STEM CELL JOURNEY



Cryopreservation

Prepare cryoprotectant:
Autologous plasma or 4.5% HAS/
20% DMSO
* put on ice *

Slowly add equal volume of cold cryoprotectant to equal volume of stem cells
* put on cold packs *

aliquot into cryocyte bags
and ampoules
* put on cold packs *

place cryocyte bag into outerbag, and place in
stainless steel cassettes

Freeze in a
Controlled Rate Freezer
using programme

-160 °C

transfer cryocyte bags to
storage Vat
- stored in vapour phase at -150 °C



The cell/cryoprotectant mixture is aliquoted into equal volumes into cryocyte bags made of special plastic able to withstand extremely low temperature.



Cryocyte bags are vacuum packed into another plastic bag for extra security then placed in a stainless steel cassette for support during freezing

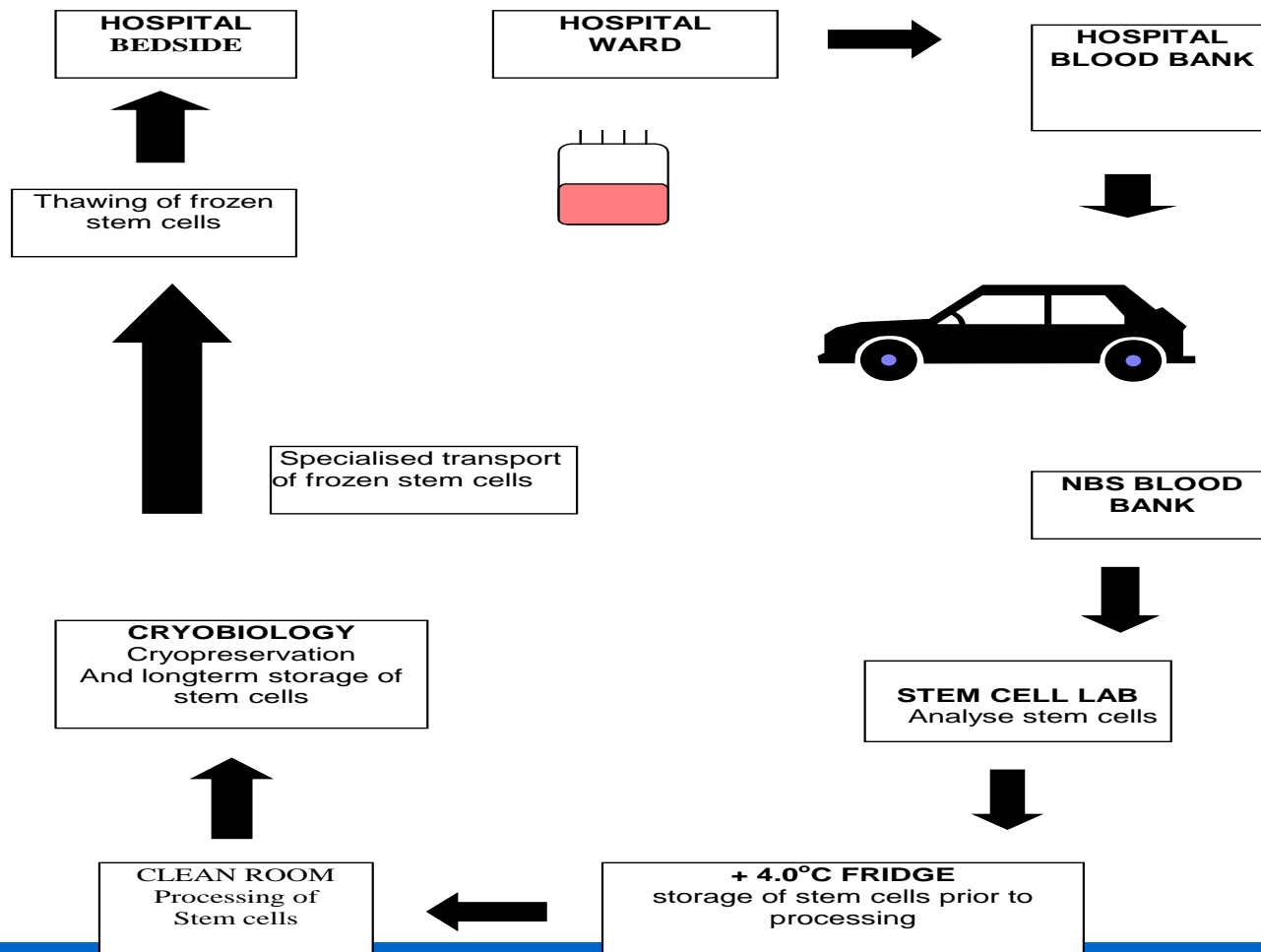


A controlled rate freezer with control unit and dewar of liquid nitrogen attached to provide slow controlled freezing of stem cells over 1hr 15 mins to a temperature of -160°C



The Vat store facility at the National Blood Service Birmingham

STEM CELL JOURNEY



Thawing

Transferred from storage Vat
to Dry Shipper
at -150 o C

Just before transfusion

Thaw rapidly at 37 oC
in waterbath set at 39 oC
(use sterile water)

manipulate cryocyte bag until all volume has JUST THAWED

Try infuse within
10 minutes of thawing

Thaw next bag as above

Repeat as necessary

SAHARA-TSC — Standardised thawing of cryogenically preserved stem cells



- The frozen stem cells are defrosted one at a time between an upper adaptation compress & a lower aluminium heating dish, which is actively heated by means of a hotplate. The continuous & gentle rotating action of the heating dish ensures gentle & homogeneous temperature stabilisation of the cells. The SAHARA-TSC machine:
- Records temp using an infrared sensor.
- Documents the temperature & systems test via protocol printer.
- Agitates the cells gently for homogeneous tempering
- Carries out visual & sensorial verification of the cells during the entire thawing process
- Allows fast availability of preparations due to 'free of ice' indication
- Carries out integrated system test for checking the device functionality
- Is easy to clean
- Module cart allows mobility & offers storage space for accessories



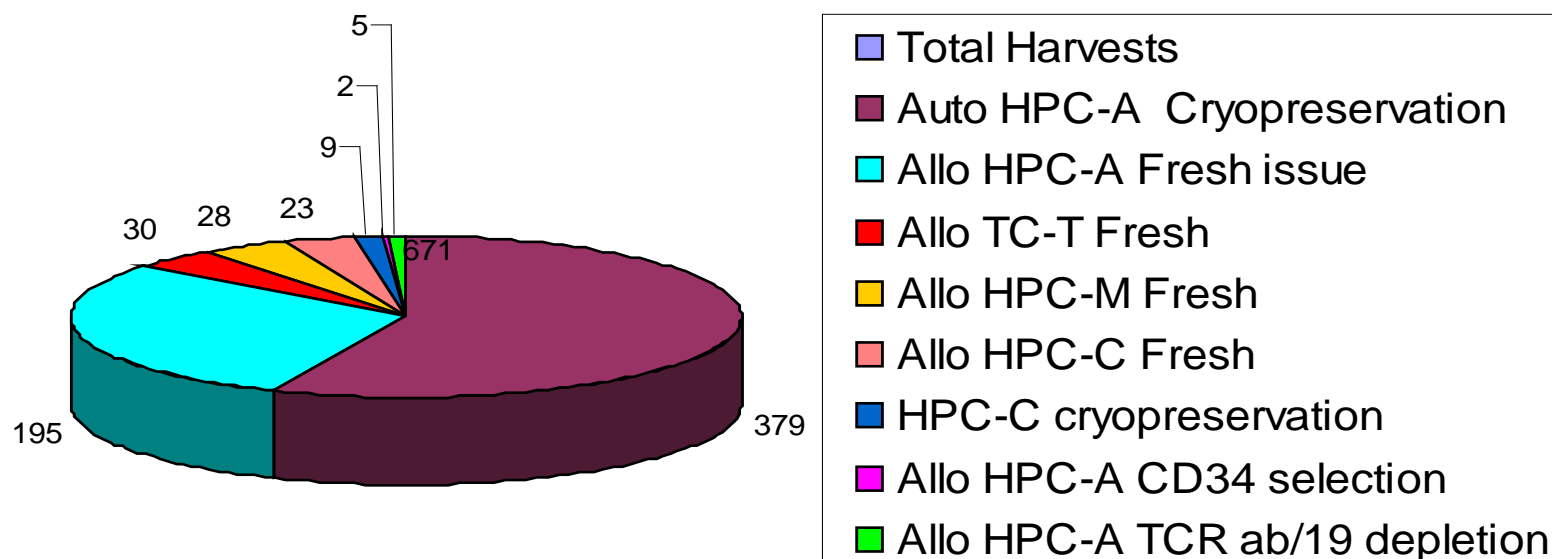
An open stem cell storage vat with nitrogen vapour. The vat contains numbered boxes to provide an inventory for stored bags of stem cells.



A dry shipper with integral temperature logger

SCI Processing 2014/15

SCI Birmingham processing activity 2014/15



Summary

- HPC-A (PBSC), HPC-M (BM) and
- HPC-Cord (Cord blood) are all sources of stem cells used for transplantation
- Processing and cryopreservation of the stem cells allows a transplantable product to be available to the patient following their period of conditioning

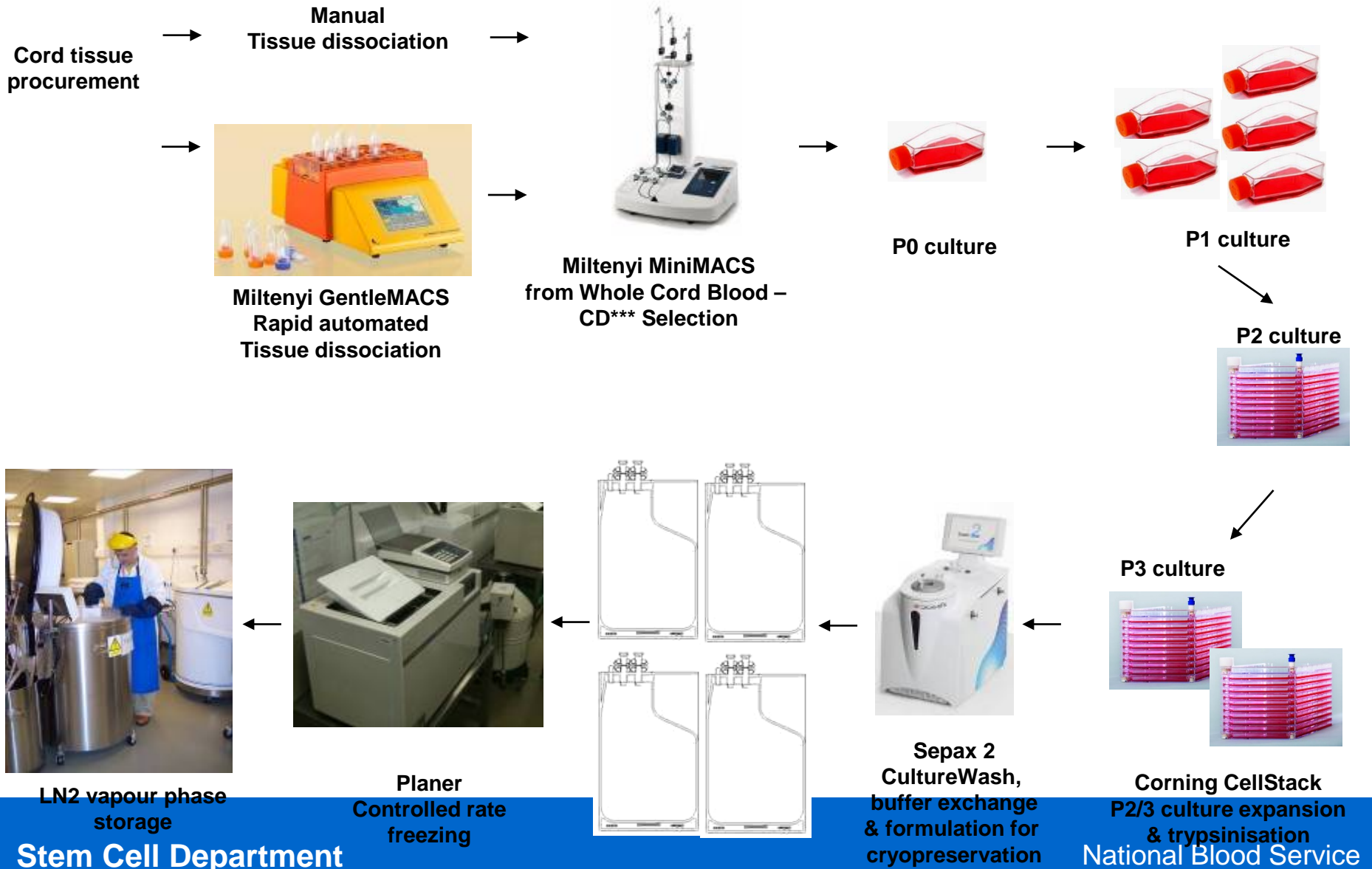
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GMP manufacturing scheme

NHS

Blood and Transplant



The Future

Semi-automated
Open process



Fully automated
Closed process

