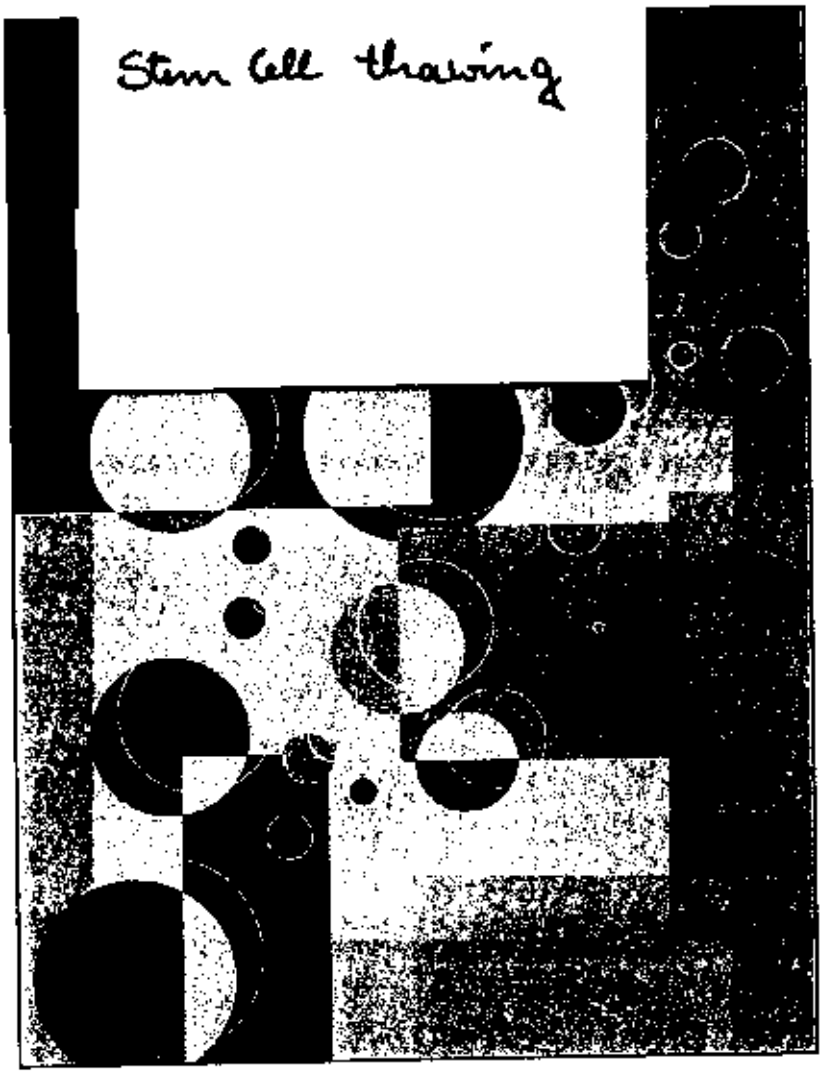
Stem Cell thawing



Guide to the preparation, use and quality assurance of blood components

7th edition

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In the absence of substitutes, the use of blood components remains essential in therapy. This guide contains a compendium of measures designed to ensure safety, efficacy and quality of blood components and is particularly intended for all those working in blood transfusion services. In accordance with the approach recommended by the Council of Europe in this field, it is based on the premise of voluntary, non-remunerated blood donation. It describes the different blood components and gives information on their clinical indications and possible side effects.

Adopted in 1995 as a technical appendix to Recommendation No. R (95) 15 of the Council of Europe, the guide continues to be the "golden standard" for European blood transfusion services and forms the basis for many national guidelines.

In this 7th edition, Chapter 1 on selection of donors has been completely overhauled in its presentation to make it more user-friendly. Where necessary, chapters have been revised to take into account what can be achieved with new technology. For example, in Chapter 3 a paragraph has been added on leucocyte-depleted components. A new Chapter 28 on statistical process control has also been introduced.

The *Guide to the preparation, use and quality assurance of blood components* will be of interest to blood transfusion centres, legislators, health personnel and all those working in the field of blood transfusion.

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"Stem cells"

Chapter 20: Haematopoietic progenitor cells

Definition

Haematopoietic progenitor cells (HPC) are primitive pluripotent cells capable of self renewal as well as differentiation and maturation into all haematopoietic lineages. They are found in bone marrow [bone marrow cells (BMC)], fetal liver, in the mononuclear cell fraction of circulating blood [peripheral blood stem cells (PBSC)] and in umbilical cord blood [umbilical stem cells (USC)].

HPC preparations (from all four sources) are intended to provide a successful engraftment of haematopoietic stem cells leading to a restoration of all types of blood cells to a normal level and function in the recipient. The infused HPC can originate from the recipient or from another individual.

Properties

The size and specific gravity of HPC from different sources are similar to those of mononuclear cells (MNC) in whole blood. HPC are recognised by their colony-forming capacities in different *in vitro* cell culture assays and by surface antigen markers. The membrane marker CD34 is a common tool for the successful isolation/purification of HPC and is routinely used as an indicator in the quality control of the preparations.

HPC are short lived in culture medium *in vitro*; cryopreservation (below -120 °C) allows storage for prolonged periods of time.

The yield of mononuclear cells should be sufficient to facilitate successful engraftment in the recipient.

Methods of collection and preparation

All treatment of the donors required to obtain an effective HPC preparation should comply with the relevant medical ethical codes and be performed with informed consent of the donor.

A. Allogeneic transplantation:

All requirements for donor selection and laboratory testing are applicable as for a normal whole blood and cytapheresis donor with addition of full HLA typing. If the donor does not meet these criteria, deviation is permissible only after the documented approval of the donor's and recipient's physician.

B. Autologous transplantation:

If there is any positive marker for transfusion-transmitted diseases all personnel involved in the testing, collection, processing and storage of HPC preparation should be informed prior to their involvement. Storage should ensure that there is no possibility of contamination of other components. All containers and material which have been in direct contact with the biomaterial involved in the HPC preparation should then be labelled as a biohazard or disposed as hazardous waste.

C. Types of preparation

Purification/manipulation of components, if indicated, can include: removal of granulocytes and erythrocytes, reduction/elimination of malignant cells contaminating in the autologous HPC preparations or of the number of T-lymphocytes in the allogenic HPC preparations to minimise Graft Versus Host disease.

Purging and *in vitro* expansion are techniques which are sometimes employed to obtain higher purity or increased numbers of progenitor cells in the final component.

- Bone marrow

Bone marrow is harvested by aspirating the cells from the cavities of hollow bones. Further purification consists of removal of bone fragments by filtration and isolation of the "buffycoat" cells after centrifugation.

- Peripheral blood stem cells

PBSC are collected as mononuclear cells by cytapheresis. The number of HPC recovered from peripheral blood is usually only sufficient for a successful autologous engraftment when the patient is treated with growth factors prior to collection. A minimum dose for successful engraftment is generally accepted to be 2×10^6 CD 34+ cells/kg weight of recipient.

- Umbilical cord blood

Umbilical stem cells are extracted from fresh placentae via the vein of the umbilical cord.

- Fetal liver

Fetal liver is excluded from consideration.

Cryopreservation and thawing

Cryopreservation is commonly a part of the preparation of HPC due to an interval between HPC collection and transfusion needed for the clinical treatment of the recipient.

The cells are suspended in a medium containing a cryoprotectant (DMSO) and protein (autologous plasma/albumin) and are frozen in cryobags at a controlled-rate of -1 °C per minute and stored in the vapour phase of the liquid nitrogen freezer. A secure labelling system is essential for the identification of units in frozen storage.

The frozen HPC preparation is thawed by mixing in a +37 °C to +42 °C, in continuous agitation and transfused immediately. Washing, ultrafiltration of the thawed product or the use of other cryopreservatives may be future developments in order to minimise DMSO toxicity.

Reference samples of HPC preparations should be cryopreserved and used for quality control. An inventory control system should allow the allocation and retrieval of any components and its reference samples.

Labelling

The label of the thawed unit shall have at least the following information:

- the name of the component, bone marrow, peripheral blood progenitor cells, or umbilical cord blood unit;
- the producer's name and address (text or code);
- date of collection;
- the approximate volume in the container;
- the names and volumes of anticoagulants or other derivatives;
- the donor identifier;
- the intended recipient's name or identifier;

and before issue for infusion, to the previous data shall be added:

- identification and date of processing procedures used when applicable;
- storage temperature;

- in cases of allogenic transplantation, ABO and Rh type of donor and results of irregular antibody tests when positive;
- in cases of autologous transplantation, the label should include "For autologous use only";
- a biohazard label, if the donor has tested positive for any test of infectious disease markers.

Storage and stability

HPC are commonly stored frozen within a temperature range -80 °C to -196 °C as determined to be appropriate for the cryoprotectant used.

An inventory control system shall be able to locate any component or the quality control vials from that component.

PBSC that test positive for any infectious disease markers must be stored separately.

Quality assurance

As indicated in table 20.

Table 20: Quality control

Parameters to be checked	Quality requirements	Frequency of control	Control executed by
ABO Rh(D) Allogenic	grouping	all donations	grouping lab
HLA allogenic	typing	all donations	HLA lab
anti-HIV 1&2	negative by approved screening test	all donations	screening lab
ALT (when required)	not elevated as specified by national authorities	all donations	screening lab
HBsAg	negative by approved screening test	all donations	screening lab
anti-HBc (when required)	negative by approved screening test	all donations	screening lab
anti-HCV	negative by approved screening test	all donations	screening lab
Syphilis (when required)	negative by approved screening test	all donations	screening lab
anti-CMV (when required)	negative by approved screening test	all donations	screening lab
anti-HTLV I&II (when required)	negative by approved screening test	all donations	screening lab
leucocyte viability	>80 %	all donations	haematology lab
Sterility	sterile	all donations	screening lab

Time of engraftment is defined as the number of days taken to achieve counts in the peripheral blood of the patient of $> 0.5 \times 10^9$ granulocytes/l and $> 20 \times 10^9$ platelets/l.

Review of the cellular counts and leucocyte viability, together with a review of the engraftment times, will be a component of quality control for HPC collection, processing and cryopreservation of the facility.

For any of the procedures mentioned as part of the HPC processing a relevant and validated assay has to be performed.

Transport

During transport of cryopreserved HPC preparations the temperature should remain below −120 °C; therefore the units should be shock and spill proof surrounded with adsorbing material soaked in sufficient liquid nitrogen; the primary container should be wrapped into a leakproof secondary container not interfering with the cooling. The container should bear the labels reading "Do not X-ray", "Keep frozen", "Labile unique human transfusion material" and "Rush". The address of the transport recipient should be clearly readable on at least two sides of the package.

Indications for use

Aplasia of the haemopoietic tissue by disease or after high dose chemotherapy and/or radiotherapy and some congenital diseases.

Precautions in use

HPC components shall not be irradiated and leucocyte-reduction filters should not be used during its administration.

Side effects

- non haemolytic transfusion reactions (fever, chills, urticaria);
- risk of viral or bacterial disease transmission;
- toxicity of reagents used in its processing and cryopreservation e.g. DMSO, animal antigens;
- graft versus host disease in allogenic transfusions;
- sepsis due to inadvertent bacterial contamination;
- haemolytic transfusion reaction.

Records

Records to be maintained include:

- donor, family of donor and patient pre and post transplant;
- processing, procedures and protocols;
- compatibility tests;
- quality tests;
- storage and transport;
- infusion and possible adverse reactions;
- final disposition of the product, facilities involved and personnel.