REMI GROUP

# Blood Bank Chronicles

The Transfusion Medicine Update

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## 'Transfusion Medicine - Expanding Horizon'

## Editorial

### From blood transfusion to Cellular Therapies & Transplantation

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The last decade has witnessed a phenomenal and mind blowing achievements in blood transfusion, stem cell transplantation and stem cell therapies.

Apheresis platforms have revolutionised blood component delivery systems. It is now possible to collect desired blood components in good quantity and quality and in a timely manner. This is in absolute contrast to collecting whole blood in citrated bottles! We will also witness blood donor data bases split into respective component donors. Providing ABO and Rh compatible, leucoreduced and irradiated blood components should be the nation's priority and privilege.

Stem cell transplantation utilising allogeneic, autologous, cord blood and unrelated stem cell sources are finding increasing applications to treat haematopoietic malignancies, haemoglobinopathies, bone marrow failure syndromes, metabolic and immune deficiency disorders.

Early institution of therapy is cornerstone for achieving good results. The concept of small nuclear families has made unrelated cord blood stem cells and matched unrelated donor stem cell transplants a viable option. The financial burden and increased incidence of acute GVHD have been limiting factors. Modifying GVHD needs considerable research utilising mesenchymal stem cells or drug therapy.

Cellular therapies have been the focus of the past years and will remain so in the ensuing years. The plasticity of embryonic stem cells can be used to treat a variety of diseases. In contrast to gene therapy, a vector is not required to make stem cells differentiate to a specific cell type. Growth factors and interleukins induce stem cell differentiation into neurons, cardiac myocytes, beta cells of pancreas, skin and cartilage. If in reality this could prove a major breakthrough for treatment of diabetes, myocardial infarction, osteoarthritis and burns, then many patients will reap the benefit.

Limbal stem cells from cornea have indeed helped to treat corneal blindness without fear of rejection. Cloning of skin stem cells enables organs like functional kidney and liver to be grown in vitro.

It may sound as a fiction story. The future may see patients ordering for liver and kidney from stem cell clinics!

*For References : Contact the author on Email Id: lakshmanin@gmail.com

# Best Practices

## Critical Issues In Stem Cell Collection And Transplantation

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**Background:** The advent of aphaeresis technology has catapulted the role of blood bankers from being merely providers of blood to performing complex therapeutic procedures. Thus today Transfusion medicine consultants are also transplant physicians. The modern day transfusion specialists are a good blend of lab medicine and bench side physician. TM physicians perform a wide array of procedures on donors and patients. The most important of these is of course the MNC / Peripheral Blood stem cell collection.

Almost always Peripheral Blood Stem Cell transplant is a second line of treatment when the conventional treatment for cancer fails. More common than Allo- transplants are auto transplants as HLA matched donors are sparse in our country. While infection in the post-transplant setting is a common cause of morbidity and mortality in both types of transplants, GVHD is a complication of allotransplant only. However this GVHD can have a beneficial effect , where the Graft versus leukemia effect kills the residual cancerous cells. Engraftment period is shorter in auto transplants and therefore chances of infection are less and recovery more.

**Methodology :** Peripheral Blood Stem cell(PBSC) or Hematopoietic stem cell(HSC) transplants is a therapeutic procedure wherein the stem cells mobilized by drugs from the bone marrow into the peripheral blood are harvested using a cell separator, cryopreserved, and given to the patient post bone marrow ablative therapy in the form of high dose chemotherapy and /or radiotherapy. PBSCT is a multi-disciplinary activity in which the blood centre plays the key role. Besides the blood bank, the treating oncologist/hematologist, the infection control team and most importantly the nursing team play a very important role in the success or failure of a transplant. The activities leading up to a transplant and the role played by each can be summarized as below:

| PBSCT/HSCT Activity : Summary | |
|---|---|
| **Activity** | **By** |
| HLA Lab | Blood Centre |
| Mobilization of Stem cells | Oncology |
| Collection of PBSC | Aphaeresis Blood Centre |
| Cryopreservation of Stem cells | Blood Centre |
| Transfusion/Transplantation of stem cells post CT/RT | Blood Centre + Oncology |
| Transfusion support | Blood Centre |

Let us now examine the critical issues/factors in PBSCT / HSCT.

**Factor 1 - HLA matching between donor and Patient**

It is a well-documented fact that precise HLA matching between donor and patient significantly reduces chances of GVHD, improves rate of engraftment and improves overall survival. As per NMDP 1987 guidelines (USA) HLA matching is traditionally done at three HLA loci (HLA-A, -B, and -DR). For a successful outcomes a 5/6 match is a minimum requirement. Recent research has shown matching at HLA-C in addition, improves the outcome. Many centers now prefer DNA-based tissue typing which is more accurate and specific and allows for more precise HLA matching compared to conventional serologic techniques.

**Factor 2-Transplantation Timing**

It is well known that high-resolution DNA matching for HLA-A, -B, -C, are associated with higher rates of survival. A single mismatch at HLA-A, -B, -C or -DRB1 (7/8 match) is associated with higher mortality and mismatching at 2 or more loci compounded the risk. Single mismatches at HLA-B or HLA-C appear better tolerated than mismatches at HLA-A or HLA-DRB1. However lack of a fully matched donor (8/8 matched, i.e., matched at HLA-A, -B, -C, -DRB1) does not preclude transplantation as a possible treatment option. In fact 6/8 matched patients transplanted early in the disease do better than fully matched 8/8 patients transplanted late in advanced disease stage.

**Factor 3: Non-HLA factors**

Cytomegalovirus (CMV) sera-negative, for patients with CMV-negative serology are always preferred. Female donors with multiple pregnancies are associated with higher risk of GVHD. Based on age we can say that younger the age betters the prognosis. ABO compatibility between patient and donor in case of allotransplant is desirable but not essential for a PBSCT. Patients with larger body weight have been shown to mobilize better, possibly because they receive larger doses of Granulocyte-Colony stimulating factors (G-CSF). Transplants are also better tolerated when the patient and donor are matched for race.

**Factor 4-Choice of Mobilization:** Chemotherapy/Growth Factors or both

Good mobilization of stem cells from marrow to blood is a factor that is not often under the control of the blood centre, but is very essential for a good collection/transplant. Stem cells can be mobilized by Chemotherapy or by use of growth factors-most commonly G-CSF although GM-CSF and Interleukin-3 are also used by some. The dose of G-CSF used varies widely from 5-10ug/Kg. Some centers use 16ug/Kg.

**Factor 5- Timing of PBSC collection and Volume processed**

While receiving G-CSF the WBC count and CD34 count which is the marker for HSC peaks by 5-6 days. Elevated WBC count does not always reflect an elevation of CD 34 count. CD34 normally plateaus by 8 days. Normally the collection begins on the 5th day of mobilization. However it is a good practice to begin collection when

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# Transfusion Medicine Chronicles

**1950 : Carl Walter and W.P. Murphy Jr. develop the plastic bag for blood collection.**

## Blood Bank Chronicles
## Important Links

| | |
|---|---|
| International Society of Stem Cell Research (ISSCR) | www.isscr.org |
| International Consortium of Stem Cell Networks(ICSCN) | www.stemcellconsortium.org |
| International Stem Cell Forum | www.stem-cell-forum.net/ISCF |
| European Human Embryonic Stem cell Registry | www.hescreg.eu |
| Indian Council of Medical Research (ICMR) | http://icmr.nic.in |

## Quiz

**Q. Select the benefit of dry thawing process (for Stem cell) over conventional thawing process?**

a) Potentially reduced risk of cross contamination
b) Better Good Clinical Practices
c) Both a & b
d) None

**To enroll yourself for the lucky draw, Send us the Mail to us on supportggn@remilabworld.com**

**you have to type the following**

1. Mention the subject = Lucky Draw Registration
2. Type the correct option in the mail
3. Mention your mobile no., Blood Bank Name & Contact details

**Send the Answer for the question to us to win lucky draw (5 Nos) : - *Last date of enrollment : 31st Aug 13***

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the pre collection CD 34 count is about 10 X $10^6$ cells/L (10 cells/ul ) . Many oncologists and transfusionists prefer to give a loading dose of 20ug/Kg on the morning of collection and begin the collection within 2 hours after this. The process volume in case of adults (Auto-transplants) should be in the range of 2-3 blood volumes over 4-5 hours. Many centers safely process 15-20 liters in one sitting and get adequate cells in 2-3 sittings. Probably half this volume is enough in case of allo transplant from healthy donors.

### Factor 6: Mobilizing PBSC in G-CSF non responders: (Alternatives choices)

Plerixafor (Dose 0.24 mg/Kg) given on the 4th day after G-CSF administration can significantly improve mobilization in G-CSF non-responders. This is particularly of proven value in lymphoma and multiple myeloma but needs to be procured from outside India.

Using Plerixafor minimum cell yield ($\geq 2x10^6$ CD34+ cells/kg) was harvested in 98% myeloma and 80% non-Hodgkin's lymphoma patients in a median of 1 aphaeresis procedure according to a study. The optimum cell dose ($\geq 5x10^6$ CD34+ cells/kg for non-Hodgkin's lymphoma or $>6x10^6$ CD34+ cells/kg for myeloma) was harvested in 89% myeloma and 48% non-Hodgkin's lymphoma patients.

### Factor 7 - Monitoring Stem Cell Collection

The access vein used, the type of machine used, the method adopted, the skill of the operator all play an important role in collection. Continuous flow cell separators are faster and give a better yield in case of MNC collection. While most blood centers would like to collect from Hemodialyisis catheter in the neck( sublcavian vein) collection from the peripheral vein is probably safer.Both manual and automated method maybe b employed with nearly the same results depending on the cell separator used.

### Factor 8: Use of a Controlled rate freezer (CRF)

Freezing of PBSC in a controlled rate freezer (CRF) is always better than snap freezing. Rapid freezing without the use of a CRF leads to crystallization of water within the cells which destroys the cells on thawing.

### Factor 9 - Composition of cryoprotectant

There are various types of cryoprotectants but a 10% DMSO is most commonly used as a cryoprotectant. Some centres use a 5% DMSO to reduce toxicity due to DMSO.10% DMSO reduces the osmolality between the interior of the cell and its environment from 19000 mOsm to 1350 mOsm thereby maintaining better viability of cells.

### Factor 10-Critical factors in Engraftment

Dose of CD 34 cells and their viability coupled with patient factors like the extent of previous chemotherapy or radiotherapy, use of post-transplantation growth factors are some critical factors affecting engraftment marked by neutrophil recovery ( >500/uL) and in Platelet recovery( >20,000/uL). Normally engraftment takes 9-11 days (up to 15 days). Engraftment could be slower in case of mismatched transplants and could take as much as 16-33 days.

### Factor 10-

Finally post-transplantation support with blood components that are leukoreduced and irradiated, granulocyte transfusion in case of severe neutropenic sepsis and donor lymphocyte infusion in case of GVHD all play an important role in the success of a peripheral blood/hematopoietic stem cell transplant.


### Conclusion:

Good infrastructure, good aseptic techniques using trained nurses and doctors are a pre-requisite for a successful transplant. Cautious optimism is needed while using stem cells for non-traditional applications. Team work –with the team principally comprising of the Transfusion Consultants and Hemato-Oncology consultants is essential for successful PBSCT.

*References are not printed due to space constrain, for details please contact the author on Email Id: shivaram@manipalhospitals.com

**1958 : Jean Dausset & Rose Payne discovers blood compatibility due to HLA on the blood cell.**

# PBSC harvest by large-volume leukapheresis - An analysis of collection efficiency

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## INTRODUCTION

Harvest and transplantation of Hematopoietic Progenitor Cells (HPCs) is used increasingly in the treatment of several blood disorders, malignancies, and genetic abnormalities . These stem cells are collected by leukapheresis and the adequacy of a collection is measured by the number of CD34+ cells per kilogram of recipient body weight. Successful engraftment has been observed with counts ranging from 2 to 5 × $10^6$ CD34+ cells/kg [6,7; Krause, Bender]. These levels of circulating cells are achieved between 5 and 7 days after initiating mobilization with growth factors [8, 9; Grigg, Perez] and this is considered an appropriate time to initiate harvest. Apart from outcome, Collection Efficiency (CE) is one of the objective quality parameters which can be used to assess a cell separator's potential for generating high yields of extracted cells and hence facilitating successful transplants. However, data on the CE of cell separators is limited, especially with reference to CD34+ cell collection [11; Ford].

We analyzed various aspects of the collection efficiency of 52 consecutive leukapheresis procedures in 40 donors between December 2010 and July 2012. Collections were carried out in both autologous and allogenic donors (33 and 7 respectively). There was a retrospective comparison of CE and analysis of various patient and procedural factors which may affect CE. Donors underwent mobilization with hematopoietic Granulocyte Colony Stimulating Factor and were harvested on the fifth day of mobilization. CD34+ cell counts were determined pre-procedurally in the donor's peripheral blood, and in the leukapheresis product, by flow cytometry. All procedures were carried out using the Fresenius ComTec cell separator which was calibrated and worked on its default settings. The desired yield was set at a mean value of $4x10^6$ cells/kg.

### Collection Efficiency (CE)

Collection efficiency, a measure of the cell separator's ability to extract maximum number of CD34+cells from the cells available in the donor's blood was calculated as follows:

$$CE = \frac{\text{Absolute CD34 count of the product X volume of the product}}{\text{Pre-procedural absolute Cd34 count of the donor X (Total blood volume processed - ACD)}}$$

Regression analysis was performed to evaluate the impact of donor age, weight, disease status, hematocrit, Total Leukocyte Count (TLC), CD34+ cell count and apheresis processed volume on CE1. Correlation analysis was carried out to find the relationships of pre-procedure CD34+ cell count with the collected cells and yield/kg.

The mean collection efficiency calculated using the formula CE was 35.7% in autologous donors (range 7.41% to 114.56%). Both leukocytes and CD34+ cells were concentrated in the product many times their initial number in the peripheral blood. Leukocytes showed an average five-fold increase in number, in both autologous (range 1.6-18.21) and allogenic donors (range 1.26-7.58). However, differences were seen between the two groups in the extent of concentration of CD34+ cells. In autologous donors, CD34+ cells were concentrated 11-fold on an average (range 3.1-91.07). In allogenic donors, the cells showed a larger increase of 18 times the initial number on an average (range 2.38-28.23). These differences in concentration were a reflection of differences in the CE between the two groups. While the average CE in autologous donors was 35.7% as previously mentioned, the allogenic donors showed a significantly higher mean CE of 67.5% at $p < 0.01$.

Pre-procedural TLC and post-procedural CD34+ cell counts were also significantly higher in allogenic donors, resulting in achievement of a significantly higher yield/kg in these donors (mean 8.42 x $10^6$ cells/kg vs. 4.0 x $10^6$ cells/kg) in spite of a significantly lower blood volume processed (mean 6.98L vs. 18.97L).

### Factors affecting CE

Out of all the factors analysed by multiple regression, only pre-procedural CD34+ cell count showed a significant relationship with CE. The pre-procedural CD34+ cell count was also found to be strongly correlated with both post- procedural CD34+ cell count in the product ($r = 0.83$) and the yield (per kilogram recipient body weight) ($r=0.34$).

## DISCUSSION

The CD34+ cell yields obtained through leukapheresis are partly determined by the efficiency of collection, making CE an important parameter for successful harvests. Collection efficiency values are highly variable with mid-values as low as 30% [12; Mehta] and as high as 85% [13; Rowley]. Apart from donor characteristics, the type of collection device, cell separation mechanism, program and operator settings all contribute towards this variability. The collection efficiency CE in the present analysis ranged from 7.41% to 114.5% in autologous donations. The values above 100% may be explained by the intra-collection mobilization phenomenon, which causes fluctuation of peripheral CD34+ cell concentration

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## Transfusion Medicine Chronicles

**1960 : Alan Solomon & John L. Fahey develop plasmapheresis, for separating plasma and RBC**

## Transfusion News Track

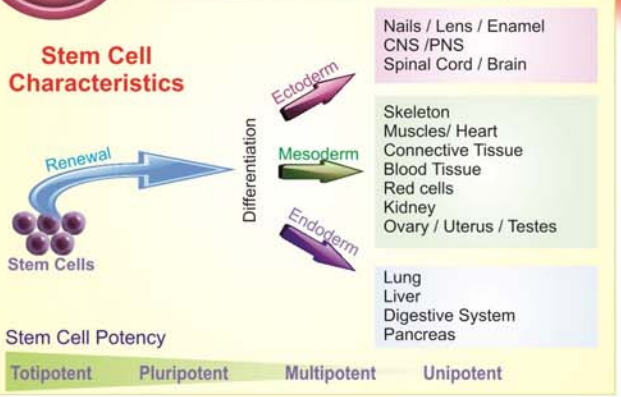
### Study reveals more versatile approach for creating Human iPSCs (Induced pluripotent stem cells)

For the first time, they have created human iPSCs without using OCT4 and SOX2.

Dr Izpisua Belmonte and his colleagues discovered that pluripotency can be accomplished by balancing the genes required for differentiation. These genes code for lineage transcription factors—proteins that start a stem cell down the path to differentiate, first into a particular cell lineage and then into a specific cell. The researchers believe their work should help to overcome one of the major hurdles to the widespread adoption of iPSC therapies: the original 4 genes used to reprogram stem cells have been implicated in cancer development.

Ref : http://www.hematologytimes.com/p_article.do?id=3405&sid=-147083418

## Facts Compendium

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by recruiting additional cells from the bone marrow during the leukapheresis procedure.

The mean CE1 of 35.7% in the present analysis was lower than the values in most other studies, including those done on the same cell separator. This may be due to differences in operation, as well as the fact that the average leukapheresis volumes at authors' institute were higher than the blood volumes processed at most other centers. Larger volumes were processed in an attempt to harvest an adequate dose in a single procedure to minimize expense and patient discomfort, even at the cost of a lower CE.

The CE of the cell separator is also reflected in its power to extract and concentrate the cells of interest. Matic et al [14; Matic] observed that CD34+ cells were enriched 38-fold in the apheresis product when less than one total blood volume was processed, but the efficiency decreased as higher volumes were processed. In cases where TLC lay between 45,000 and 50,000 cells/$\mu$L, the final CD34+ concentration averaged eight times the number in the peripheral blood [14, Matic]. In the present study, 4.4 blood volumes were processed on an average resulted in an average 11-fold concentration in the number of CD34+ cells, which falls within the range of values observed by Matic et al.

As expected, healthy allogenic donors showed higher CE and yields of CD34+ cells than autologous donors. It was observed that both mean CE and yield in allogenic donations were approximately twice the mean values obtained in autologous donations. The disease-free condition of the allogenic donors, coupled with their younger age (34.42 years Vs. 50.03 years) may have contributed to their high pre-procedural cell counts, which in turn facilitated the extraction of larger numbers of cells. Although the individual impact of each of these parameters is not known, it can be concluded that age, state of health and lower processed blood volumes led to the differences seen in autologous and allogenic donors, an observation supported by the literature.

Optimization of CD34+ cell collection efficiency requires the identification of factors impacting this parameter. Although various studies have been conducted, no factor has yet been identified which uniformly and individually predicts collection efficiency. Multiple regression analysis carried out in the present analysis to evaluate the impact of age, weight, disease status, hematocrit, TLC, CD34+ cell count and processed volume identified pre-procedural CD34+ cell number as the sole significant predictor for collection efficiency. Sarkodee-Adoo et al [15; Sarkodee-Adoo] also found that circulating CD34+ count had a modest effect on CE, although in their case an inverse correlation was seen [36 Sarkodee]. Both these results are further at odds with the findings of Ford et al, who stated that peripheral CD34+ count is not associated with collection efficiency [Ford;15]. This underlines the fact that jury is still out on relationship between CD34+ cells and CE and possibly a larger study would finally establish an association-none, directly proportional or inversely proportional.

Collection efficiency is affected by a wide range of procedural factors and donor characteristics and shows a lot of variability in the clinical setting. Not only does the average collection efficiency value vary considerably among different centres, there is also no uniform consensus on which factors impact CE and result in this variability. In the present analysis, the average CE was 35.7% and pre procedural CD34+ cell count was the only significant predictor for CE. However, CD34+ cell count also showed a strong correlation with the acquired yield of product, a relationship which has uniformly been established across multiple centres. This leads to the suggestion that pre-procedural CD34+ cell counts have a more important role to play than CE in determining the success of a harvest, and should be used as the reference mark for deciding the settings and timing of leukapheresis. Hence, a low CE should not be a limiting factor for carrying out apheresis, and will only be a cause for concern in the event where peripheral CD34+ counts are also low

*References are not printed due to space constrain, for details please contact the author on Email Id: aseemtwr@yahoo.co.in

**1964 : Judith Pool discovered a method of concentrating clotting factors from FFP for hemophilia patient.**

## Expert Speaks

# Indian Standards of Collection & Processing of Stem Cells

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Stem cell therapy has been instrumental in treatment and cure of thousands of hematological and non hematological malignant disorders. However, the procedure still carries significant morbidity and mortality. It is also at risk of commercial exploitation and unethical practices. Therefore, collection and processing of stem cells must be strictly regulated and periodically monitored. ICMR and the Department of Biotechnology (DBT) have together laid down "Guidelines for Stem Cell Research and Therapy". All stem cell therapy other than Bone marrow transplantation should be treated as experimental and should be conducted after approval of IC-SCRT (Institutional committee for Stem Cell Research and therapy) and DCGI (Drug Controller General of India). All experimental trails should be registered with the NAC-SCRT (National Apex committee for stem cell research and therapy). Indian standards provide guidelines for collection, processing and storage of cells for clinical use. Each stem cell collection and processing unit should maintain an effective quality system to ensure that adequate and appropriate standards of work are maintained.

·**Premises: -** A designated area for appropriate donor examination, collection and processing, preparation and storage of the reagents and equipments.

**Personnel: -** There should be a qualified physician with appropriate experience for the scope of stem cell collection and processing activities. Adequate number trained personnel should be available at the facility

**Quality management: -** The collection and processing facility Incharge should maintain a quality management plan and develop, implement, and document procedures for the validation of processes, equipment, reagents, labels, containers, packaging materials, and computer systems. Procedures for production of in-house reagents should be validated. The facility should have a system for detecting, evaluating, documenting and reporting errors, adverse reactions, biological product deviations and complaints. Documentation and clinical outcome after cell therapy should be reviewed regularly. The reliability, accuracy, precision and performance of laboratory test procedures and instruments should be monitored. All open cell handling procedures must be performed in class 100 environment. There should be a written request from the recipient's physician before processing is initiated. Methods for processing should be validated to result in acceptable cell viability and recovery. The objectives and acceptable end-points for each procedure should be specified. Notification and appropriate remedial actions, if taken, should be documented in the processing record. There should be a policy and procedure to cover the processing of ABO incompatible products, when applicable

**Policies and procedures: -** Policies and procedures should be available for donor screening, consent, collection, treatment, emergency and safety procedures, donor and patient confidentiality, quality management and improvement, errors, accidents and adverse reactions; biological product deviations, corrective actions, personnel training, competency assessment, outcome analysis, audits, labeling, storage, transportation, expiration dates, release and exceptional release, disposal of medical and bio-hazard waste, equipment and supplies, maintenance and monitoring, cleaning and sanitation procedures and a disaster plan. Format for procedures, including worksheets, reports and forms and a system of numbering of individual procedures should be maintained.

**Donor evaluation, selection and management: -** Prospective donors should be evaluated by medical history, physical examination and laboratory testing for the risks of the collection procedure and should be documented ensuring both confidentiality of donor and patient health information. Each donor should be tested for viral markers. A complete blood count should be performed before each apheresis. Informed consent from the donor or patient (Autologous collection) should be obtained and documented.

**Stem cell collection: -** Collection should be performed according to facility's SOP manual using aseptic techniques. Procedures for transportation of the collected product should be designed to protect the integrity of the product and the health and safety of facility personnel.

**Cryopreservation: -** Facility's SOPs should describe: The name and freezing criteria of the cell product, the cryoprotectant solution and its final concentration, Cryopreservation container, acceptable range of product volume, acceptable range of nucleated cell concentration of the final product after cryopreservation, Cooling rate, and acceptable storage temperature.

**Product identification: -** The facility Incharge should ensure accuracy regarding identity, content, and conformity of container labels. Unique alphanumeric identifier, collection date and time, product identity, donor and recipient information on the original container should be maintained. A sufficient area of the container should remain uncovered to permit inspection of the contents. Modifications made to the product subsequent to collection and prior to cryo-preservation should be noted. A bio-hazard label should be applied to each product if any test shows evidence of infection due to communicable disease agent(s). Any container used during, at the end of processing and at the time of distribution should contain at a minimum the information required.

**Issue of products prior to distribution: -** Each product issued for infusion should be inspected by two trained personnel immediately before release to verify appropriate labeling and integrity of the product container. Instructions for administration- For each type of product, the laboratory should maintain a current document containing the following - The use of the cell product, indications, contraindications, side effects and hazards, dosage and administration recommendations. The instructions for administration should be available to the clinical staff caring for the recipient.

**Conditions for Storage: -** Policies for the duration and conditions of storage and indications for discard will be established. For products immersed in liquid nitrogen, procedures to minimize the risk of

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## Transfusion Medicine Chronicles

**1968 : Rh Immune Globulin, discovered to address the differences between -ve & +ve blood types**

# Good Clinical Practices for Stem Cell from Storage to Transplant

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In the setting of autologous BM or PBSC transplantation, the time interval between collection of hematopoietic stem cells (HPCs) and transplantation can be from a few days to several weeks and years. Therefore HPCs may need to be stored for weeks to years prior to transplant. To allow these prolonged storage times, a cryopreservative is added and HPCs are subjected to a controlled rate freeze followed by storage in a mechanical freezer maintaining -80°C, or a freezer containing liquid or vapour nitrogen to ensure a steady temperature of -196°C to -135°C, is typically used for storage of stem cell products after cryopreservation. The process of storage involves a series of steps which follow the harvest procedure.

## Centrifugation and Cryopreservation

The pre-cryostorage processing entails the actual collection procedure, the removal of cell bulk, volume reduction with concentration of the stem cells and addition of the cryomedium. The process is performed in a strictly sterile environment

Most cell processing laboratories use the cryoprotectant DMSO, commonly 10% concentration along with a source of plasma protein for cryopreservation of the hematopoietic stem cells. Some people add hydroxyl ethyl starch (HES), which allows for decreased concentration of DMSO (e.g. 5% DMSO/6 % HES). DMSO is a colligative cryoprotectant which rapidly diffuses into the cell, reducing the osmotic stress on cell membrane. It acts by preventing dehydration injury by moderating the non penetrating extracellular solutes that increase during ice formation. It also slows extracellular ice crystal formation. HES is an extracellular, macromolecular cryoprotectant. It is a high molecular weight polymer that likely protects the cell, retarding the movement of water outside the cell and into the extracellular ice crystals.

Freezing of HPCs may be controlled rate or non-controlled rate (i.e. simply transferring HPCs into freezer bag and placing at -80°C mechanical freezer. Controlled rate freezing utilizes computer programming to incrementally decrease HPC product temperature in a controlled manner. The initial cooling rate is 1°C/minute and this proceeds to (liquid to solid) phase change (roughly between -14 and -24°C) at which time a rapid, super-cooling occurs. Following warming of the chamber to the end phase change temperature, cooling continues at the rate of 1°C/minute until the temperature of the product has reached -60°C. At this point, the product is cooled at 3°C/minute until -100°C is reached. A slow controlled-rate freezing can minimize the effects of thermal shock, phase transition time and post transition freezing rate. Mechanical or uncontrolled-rate freezing is a viable and cost effective alternative to controlled rate freezing. The mechanical freeze produces a freezing rate of 3°C/min. This sudden drop in temperature can result in thermal injury to the stem cells and reduced viability post thawing. Freezing bags are the most frequently used long term storage containers for stem cells and ethylene vinyl acetate(EVA) based products represent the majority in the market.

## Storage

The minimal requirements for a long term storage temperature are technical feasibility and a successful clinical outcome. The storage temperature for hematopoietic stem cells varies between different centers. Temperatures range from -196°C to -80°C. The initially used storage temperatures of -196°C, reflecting the liquid phase nitrogen storage, have largely been replaced by temperatures of -156°C to -135°C, reflecting the vapor phase storage. A recent study by Mc Cullough et al. compared 5 different protocols for five year PBSC storage. Along with other variables, liquid phase nitrogen storage (-196°C) was also compared to a mechanical freezer temperature at -135°C. No significant outcome differences were observed between both techniques. Point of importance during storage of HPCs is that even slight temperature variation can lead to devitrification of the metastable cryopreservate with crystal formation and corresponding cell damage (Baicu, M. J. Taylor, Z. Chen, & Rabin, 2008). HPCs can be stored in a mechanical freezer no longer than 6 months. If however HPCs have to be stored for longer periods then freezer containing liquid or vapour nitrogen has to be used.

## Thawing

The frozen bags of stem cells are ideally thawed at the bedside of the patient. The thawing procedure is stressful to frozen cells, and using good technique and working quickly ensures that a high

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microbial cross-contamination of products should be employed. Refrigerators and freezers for product storage should have an active alarm system and a continuous temperature monitoring system. A mechanism to ensure the levels of liquid nitrogen in freezers should be maintained. There should be written instructions to be followed if the storage device fails. The inventory control system records include: Donor, Patient and Product details, Date of collection, storage device identifier, Dates of issue or Disposition.

**Transportation: -** The non-frozen products should be placed in a secondary plastic bag and sealed to prevent leakage and should be transported in a thermally insulated outer shipping container. Cryopreserved products should be shipped in validated liquid nitrogen "dry shipper" with a temperature monitor and appropriate label. The products should not be passed through X-Ray irradiation devices designed to detect metal objects. Transport records should permit tracing of the product from one facility to another. Transport records should identify the date and time product is shipped and received, the source and receiving facility, and the personnel responsible for shipping and receiving the product

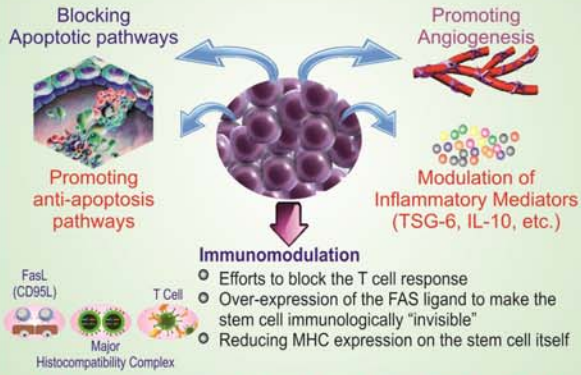
**Records: -** All records related to the collection, processing, storage and distribution, compatibility testing etc should be maintained with adequate safeguards and retained for the period required by government laws and regulations

*For References : Contact the author on Email Id: deepti.vij@gmail.com

**1969 : Scott Murphy and Frank Gardner develop a method for storing platelets at room temperature.**

## Blood Bank Chronicles Gallery

### Strategy to Enhance the Ability for In-vivo Stem Cell Survival**




- Efforts to block the T cell response
- Over-expression of the FAS ligand to make the stem cell immunologically "invisible"
- Reducing MHC expression on the stem cell itself

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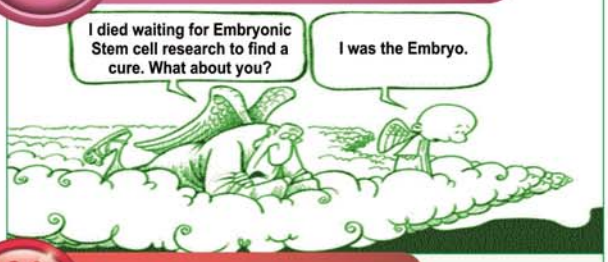
## Humor




## Last Quiz Winners

Dear Customer,
We are happy to announce that lucky draw winners of last issue quiz are as follows: -

1. Dr. Jyoti Priyadarshini Srivastava, Gwalior,
2. Dr. Y Sudhakar, Nalgonda, A.P
3. Dr. Ashu Dogra, Jammu
4. Dr. Lincy Jacob, Mumbai
5. Dr. Aslam Jamkhana Karnool A.P.

**Congratulations to All the Winners!!!**
Your gift will reach to you in next 15 days.

....Continue from Pg No. 7

proportion of the cells survive the procedure. The bag must be removed as soon as the last of the ice has melted. The cells must not be allowed to reach the temperature of the water bath i.e. 37°C. Once thawed, the stem cells are immediately re-infused into the bloodstream, similar to a blood transfusion. Thawed HPCs are infused rapidly if the patient's condition permits, for example, at 5 to 20 mL/ minute with or without an in-line 170 to 210-micron screen filter. If filters are used, the staff must be carefully trained to ensure that the proper filter is selected and that leukocyte reduction filters are not used, because they may remove HPCs and could result in graft failure. Sometimes HPCs may be thawed and also washed in the laboratory and then transported to the bedside for infusion. The advantage of laboratory thawing and washing is reduction of DMSO infusion toxicity, but laboratory thawing and washing require skills and excellent communications with the bedside care-givers to ensure that the thawed cells are infused without undue delay and without loss of cell viability. Several techniques for the thawing procedure have been proposed. The standard method is warming in a water bath at 37°C until all ice crystals disappear. Lately there is a shift of trend towards thawing of frozen HPCs in an electric dry-warming device containing warmed gel pads. Various studies have compared the thawing of cryopreserved units in a warm water bath with dry heat applied by gel pads at 37°C. The viability and clonogenic potential were comparable, with a trend towards less infectious contamination in the dry method. Dry thawing has demonstrated a potentially decreased risk of bacterial contamination of either the cell product or the patient rooms, and guidelines of good clinical practice (GCP), favor the use of the dry warming procedure.

*For References :

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