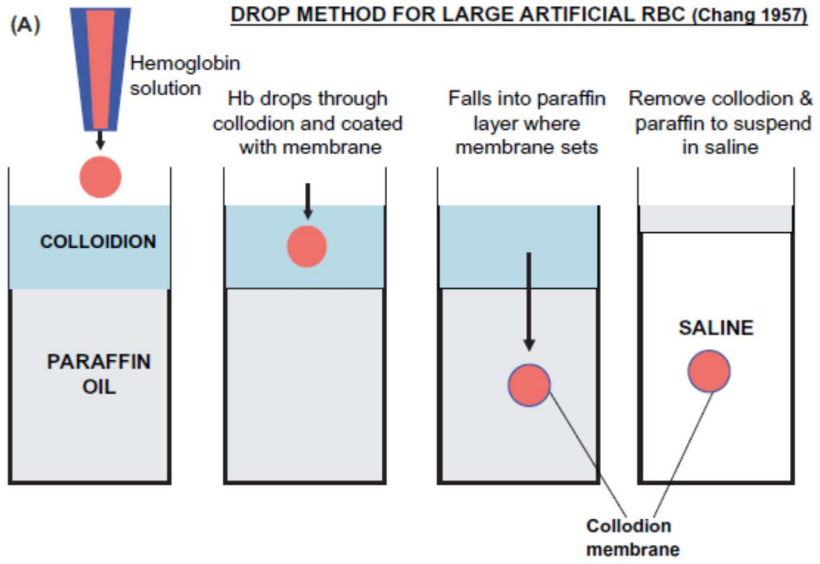
Exam Questions

1. Chapter 1 of the text, Artificial Cells, is truly amazing. Please describe and expand upon the "drop method" for creating artificial RBC's.

Answer: The drop method for creating Artificial RBCs is to create a solution of haemoglobin with enzymes and pass it through a colloidal layer of coating membrane (eg: nylon,polyglycol) which then drops through a layer of paraffin oil and helps the drops set.

Colloid and paraffin is removed and is suspended in saline.



This method's drawback was the weakening of membrane due to thin covering and hence breakage of membrane and exposure of Artificial cells(ACs) content, leading to in-vivo immune-rejection . A double coating method was later modified to ensure this phenomenon was prevented and long circulating times were ensured for coated ACs.

The double coating drop method – First initial small microspheres were made using conventional drop method for AC formation.
These microspheres were then coated with a larger microsphere. This ensures no cell exposure. Once encapsulated, the inner gel membrane of smaller microspheres is dissolved, leading to more robust larger ACs with intact membrane.

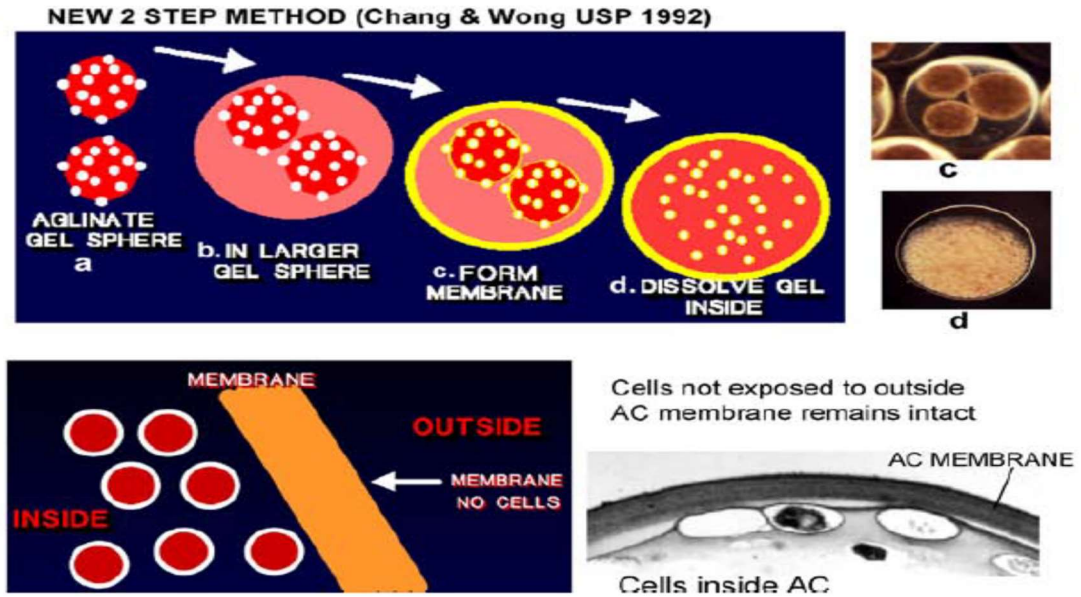
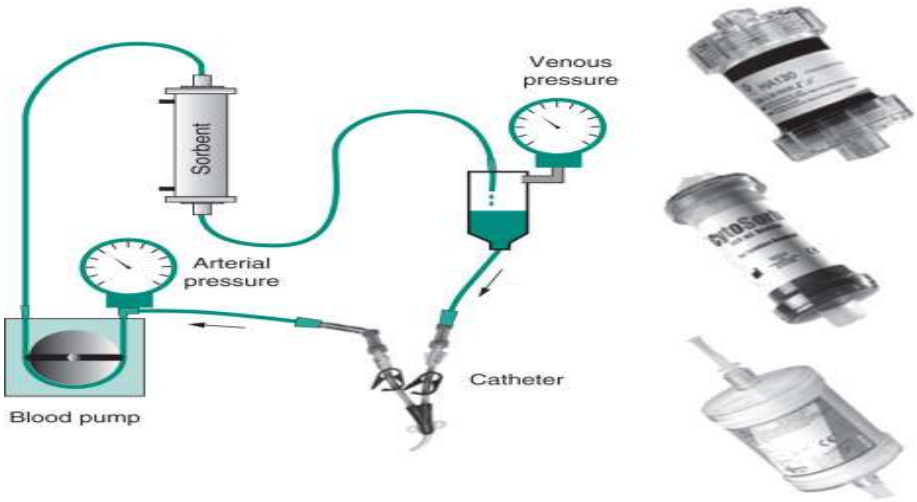


Fig. 8.3. Standard drop method of preparing artificial cells to encapsulate cells results in weakening of the membrane and exposure of cells, resulting in immuno-rejection. New 2-step method solves this problem.

2. From any source, including course texts please summarize the current state of artificial RBC's for clinical use on patients.

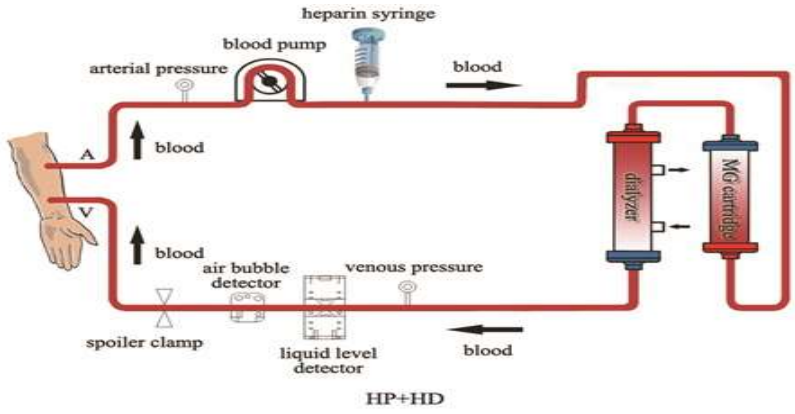
Answer :

Hemoperfusion technique is an extra corporeal therapy used when endo or exogenous intoxication(poisoning) occurs and hemodialysis is ineffective in its clearance.
Hemoperfusion devices made of artificial cells with activated carbon or other adsorbents are used clinically to treat patients with drug poisoning or sepsis . It allows for the small molecules of the toxins to be absorbed while the blood cells pass unhindered through the extra-corporeal device.



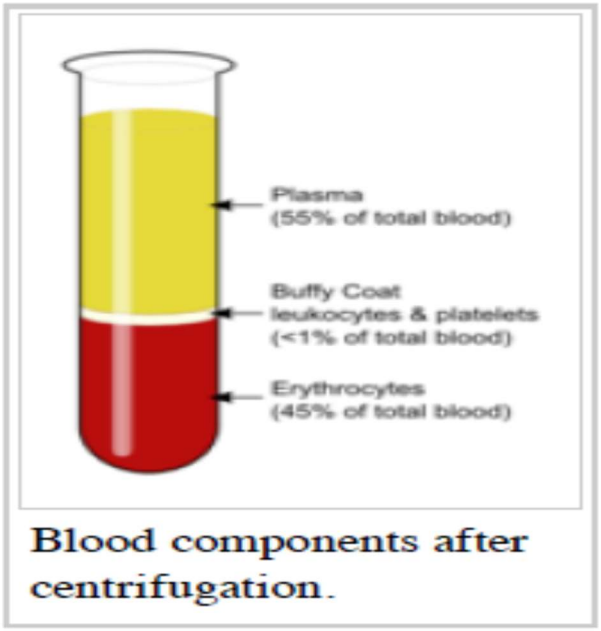
Ref - Guido Filler, CHAPTER 68 - Extracorporeal Therapies for Poisoning, Editor(s): Denis F. Geary, Franz Schaefer,Comprehensive Pediatric Nephrology, Mosby,2008,Pages 1045-1052, ISBN 9780323048835, https://doi.org/10.1016/B978-0-323-04883-5.50074-X.
(http://www.sciencedirect.com/science/article/pii/B978032304883550074X)

Hemoperfusion devices are being used with patients of renal failure in conjugation with hemodialysers .The hemoperfusion removes the molecules of 300-15000 mW but not salt , water or urea, thus necessiting use of dialyzer alongwith.



HP-Hemoperfusion , HD – Hemodialysis for renal failure
Ref - http://www.oriontamajaya.com/wp-content/uploads/2016/09/MG-SERIES-HEMOPERFUSION-CARTRIDGE-TREATMENT-GUIDELINES1.pdf

3. In blood fractionation, what is the "buffy coat", where do we find it in the test tube, and what is it composed of?



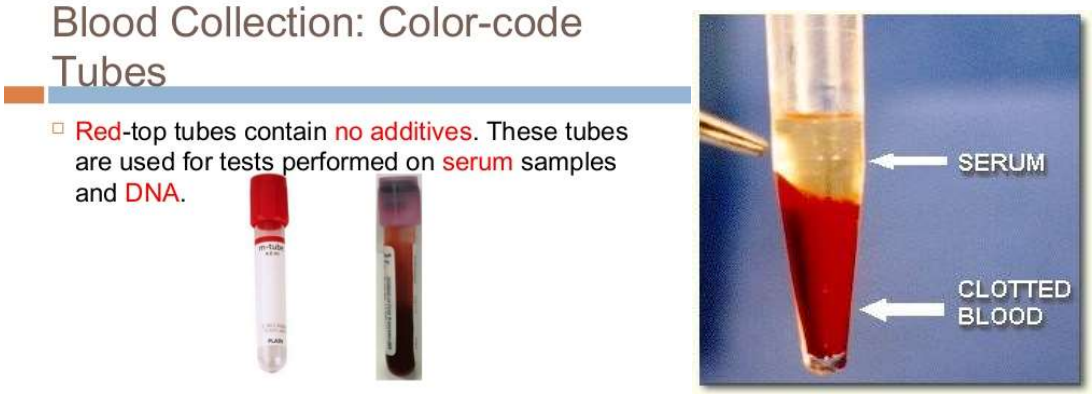
Blood components after centrifugation.

The buffy coat is the thin layer of translucent cells suspended between the red blood cells and plasma of blood consisting of platelets & leukocytes(WBCs) in the blood.

4. In collecting a blood sample from a patient:

A. If nothing is added to the receiving test tube (i.e. The collection sample), what happens to the blood sample and what is commonly derived from such a sample for lab testing?

Answer: If nothing is added to the receiving test tube, the blood shall begin clotting due to presence of clotting factors in it. The clotting will cause blood cells to be coagulated and leave straw colored liquid containing fluids with hormones,glucose,electrolytes, antibodies,antigens and nutients devoid of clotting factors.



Ref- Slide 20 https://www.slideshare.net/100002840600351/sample-collection-preservation-and-its-estimation-71483199

B. EDTA and heparin are common agents used in blood sample collection. What is the difference in using these two agents from the standpoint of what end sample we end up with for testing and what kinds of lab tests are commonly done for each type of sample.

Answer : EDTA (Ethylenediaminetetraacetic acid) is a Chelating agent for calcium ions and is an anti-coagulant. Used when whole blood is required for analysis.
Used for – Complete Blood Counts(CBC) , blood films

Heparin – Heparin(sodium heparin, lithium heparin or ammonium heparin) activates anti-thrombins and blocks coagulation .

Used for – plasma determinations in chemistry tests

5. If we want to collect the white blood cells from a patient using a centrifuge technique, what do we do…please describe the steps, where the WBC's end up and how we collect them from the sample.

Answer :

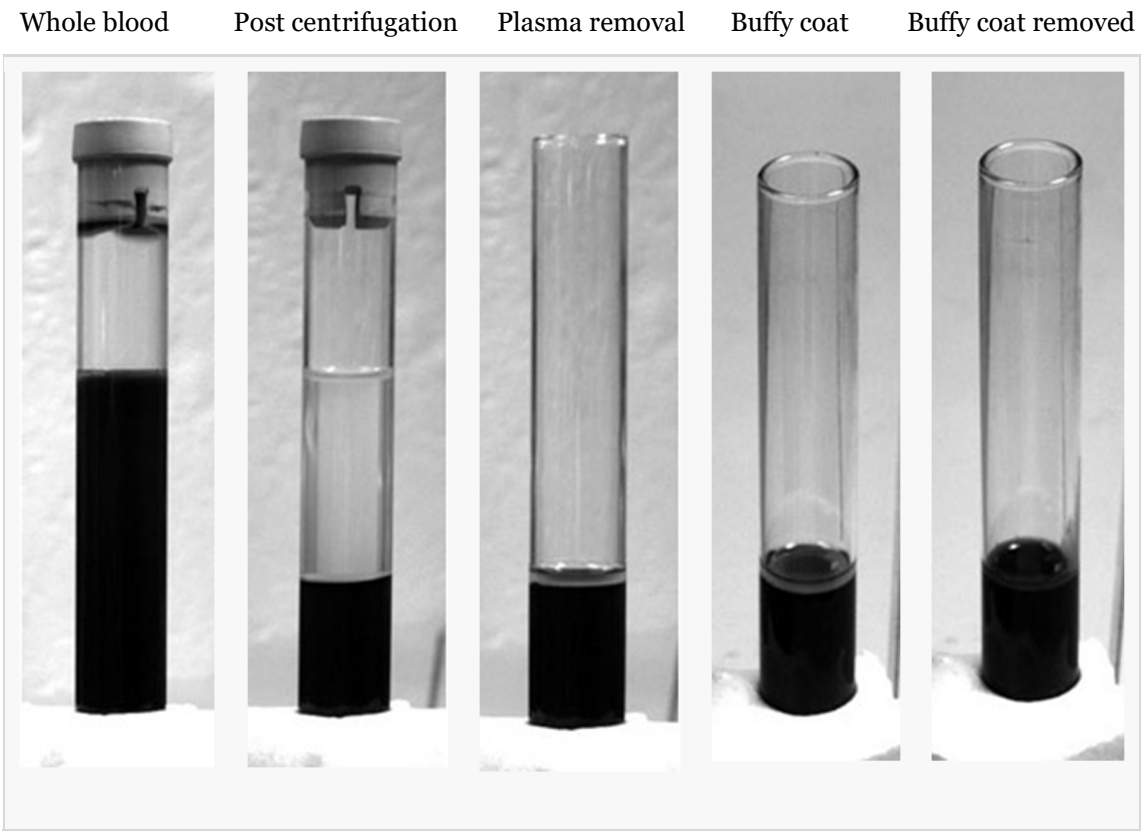
1. **Collect 2.5-10 ml blood samples**
   Collect 2.5-10 ml blood samples according to standard procedures in tubes containing EDTA
2. **Centrifuge samples at ~1500-2000 X g for 10-15 min at room temp**
   Fractionate the whole blood by centrifuging at 1500-2000 X g for 10-15 min at room temperature.
3. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs called Buffy Coat

4. Remove the plasma (upper layer) with a transfer pipet, being careful not to disturb the WBCs

5. Do not disturb the WBC layer, also called the buffy coat, which forms a thin film between the upper plasma layer and the lower layer of packed RBCs

6. **WBC Recovery**
   Carefully aspirate the exposed WBC layer in a volume of about 0.5 ml or less.
7. Alternately use Cytology brush to recover WBCs

Below Fig 1 demonstrates the process.



Ref - https://www.thermofisher.com/in/en/home/references/protocols/nucleic-acid-purification-and-analysis/rna-protocol/blood-fractionation-protocol-for-collection-of-white-blood-cells.html

Figure 1. Appearance of Blood Samples during Recovery of WBCs

1. Whole blood in the collection tube
2. Blood after centrifugation
3. WBCs and RBCs after plasma removal
4. Top view of the WBCs (buffy coat)
5. Top view of sample after WBC removal

WBCs can be stored at this point at ambient temperature (up to about 30°C) for up to about 5 days. Storage for longer than 5 days should be at -20°C.

6. Please describe the original Cohn fractionation technique and a modern use of that procedure.

Answer :

Cohn Fractionation Process –

It consists of use of ethanol varying concentration, pH, ionic concentrations & protein concentration to separate blood proteins due to their differential solubility in blood plasma.

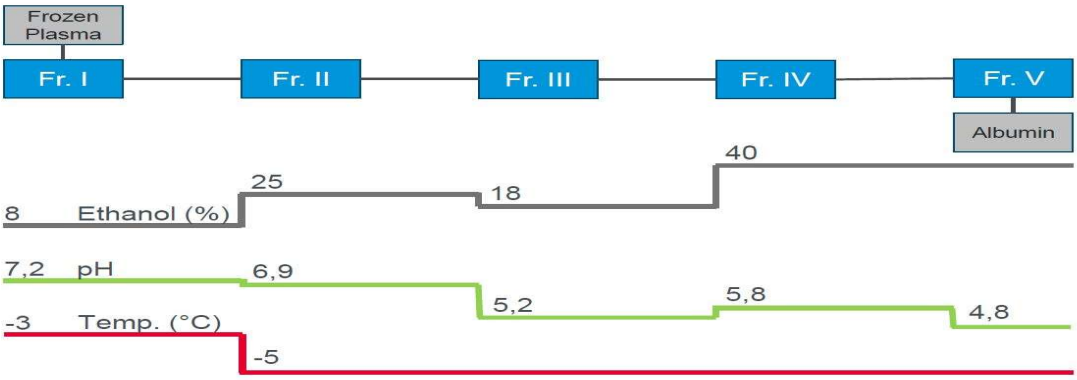
It uses Cold ethanol for the process and is also known as cold ethanol fractionation process.

Steps –

a. Room temperature for start of process
b. Fraction I precipitates at 8% ethanol, pH 7.2 , -3° C , 5.1% protein ; 25% ethanol ,pH 6.9 and 3% protein .
c. Fraction II & III are removed ; Fraction II (range 8-25% , pH 6.9 , -5° C), Fraction III ((range 25-18% , pH 5.2 , -5° C)
d. Fraction IV contains unwanted proteins, removed by raising pH from 5.2 to 5.8 , raising pH from 18-40%.
e. Fraction V contains albumin by reducing pH to 4.8 and ethanol concentration at 40% with protein concentration of 1% of original plasma

Points :

a. Yield is upto 80% and purity is 85-90% .
b. For albumin purification , extraction with water and ethanol concentration adjustment to 10% , pH of 4.5 at -3° C is maintained.
c. Process reprecipitation is done to improve yield, with raising ethanol concentration back to 40%, pH 5.2 and at -5° C



Ref - The Cohn plasma fractionation process: separation is achieved by adjusting the pH value, temperature and ethanol concentration. Esposito, Vincenzo et al. https://www.gea.com/en/news/insights/2017/taking-blood-plasma-fractionation-further.jsp

Modern Uses :

Cohn's process is used in modern times with conjugation to provide Fractions for further purification/elution in the Chromatography process where several different adsorbents are used at varying concentrations and biological affinity to elute the required proteins.

This process is used with chromatographic and other separation processes to purify many labile and biologically important compounds in medicine and pharma industry.

Important proteins and clotting factors produced from blood plasma using Cohn's is albumin, Immunoglobin G (IgG) , Coagulation Factor VIII, Factor IX etc.

Eg : Albumin separation is done starting with intermediate Factor V of Cohn's process and subjecting it to DEAE Sephadex or Sepharose Ion Exchange resin columns for chromatography.

7. What is the difference between "blood grouping" procedure and "tissue typing " procedure.

Answer:

Sr No	Blood Grouping	Tissue Typing
1	Blood grouping is done by the ABO system where, antigens are specifically grouped by antigen groups of A,B or no antigens present on cell surface of RBCs.	Tissue typing traditionally was done by cross matching lymphocyte of recipient cell with specific antisera but is discontinued for non specificity and replaced with DNA Typing of HLAs of donor & recipient for typing
2	Recipient with similar or no antigens on surface of the RBCs as the donor will accept the donation whereas, presence of unknown antigens to the recipient will trigger agglutination reaction (due to antibodies being developed to the 'foreign' antigen) and lead to medical complications	Recipients in case of organ transplants need to look at HLA Typing of the donor. A HLA sensitized recipient to donor antigens will lead to foreign antigen recognition and immune rejection and the transplant shall fail and further lead to immune complications in the recipient.
3	Blood grouping considers only RBC antigens to avoid agglutination reactions .	Tissue typing considers HLA types present on immune cells as marker for immune rejection.

8. Please describe how a patient is coaxed to produce more circulating stem cells, and include in your answer a full description of the drug Neupogen (its formulation, description, dosage etc…maybe locate the Package Insert for this)

Answer:
A patient can have more circulating stem cells by administration of cytokines (blood cell growth factors) with or without myelosuppressive chemotherapy in a process call 'Stem Cell Mobilization'.

A drug called Neupogen, a man-made granulocyte colony-stimulating factor (G-CSF) is used for this purpose via stimulation of neutrophils required for body's immunity against infection. Its molecule is specifically **recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF)**

Neupogen specifications:

Formulation:

Active ingredient: filgrastim
Inactive ingredients: acetate, polysorbate 80, sodium, sorbitol, and (WFI) water for Injection

Dosage – partial or complete pre filled syringe per administration .
Clinical prescription Dosage range from 4-69 mcg/kg IV or 4-17.25 mcg/kg SC

Vial

Injection: 300 mcg/mL in a single-dose vial
Injection: 480 mcg/1.6 mL in a single-dose vial

Prefilled Syringe

Injection: 300 mcg/0.5 mL in a single-dose prefilled syringe
Injection: 480 mcg/0.8 mL in a single-dose prefilled syringe (3

Description - sterile, clear, colorless, preservative-free liquid

Administration – Parenteral (Sc or IV)

Clinical Prescribed Dosage as per Insert –

- Patients with cancer receiving myelosuppressive chemotherapy or induction and/or consolidation chemotherapy for AML o

Recommended starting dose is 5 mcg/kg/day subcutaneous injection, short intravenous infusion (15 to 30 minutes), or continuous intravenous infusion.

- Patients with cancer undergoing bone marrow transplantation o

10 mcg/kg/day given as an intravenous infusion no longer than 24 hours.

- Patients undergoing autologous peripheral blood progenitor cell collection and therapy o

10 mcg/kg/day subcutaneous injection. Administer for at least 4 days before first leukapheresis procedure and continue until last leukapheresis

- Patients with congenital neutropenia

Recommended starting dose is 6 mcg/kg subcutaneous injection twice daily

- Patients with cyclic or idiopathic neutropenia o

Recommended starting dose is 5 mcg/kg subcutaneous injection daily

- Patients acutely exposed to myelosuppressive doses of radiation o

10 mcg/kg/day subcutaneous injection

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9. Please describe the process of stem cell harvesting as presented in your Syllabus Reference #12 Autologous Stem Cell Collection

Stem cell harvesting

1. **Bone Marrow Stem cell Harvesting**

Bone marrow is retrieved from the BM site under anaesthesia in a operative procedure which lasts approximately 2 hours . It retrieves upto 1 litre of bone marrow containing stem cells

2. **Peripheral Blood Stem Cell harvesting**

Post myelosuppressive chemotherapy or in many cases without suppression, cytokines are administered to the patient to increase circulating stem cell counts in peripheral blood(PB). Cytokines Neupogen® [GM-CSF] , Leukine® are used for this process known as ‘stem cell mobilization’

Patients are evaluated daily by monitoring CD34+ cells and process of apheresis is used for stem cell collection, where blood is collected directly in apheresis machine which filters out purely stem cells and returns blood to patient. This process is continued for many days to collect requisite amount of stem cells from PB.

3. **Stem Cell Processing**

Stem Cells contain stem cells, immune cells and other blood cells combined. Purification is carried out by

a. Purging – Magnetic removal of cancer cells using monoclonal antibodies (mABs) containing heavy metal nickel which make the cancer cells attached to antibodies heavy and settle at the bottom. Thus allowing separation.

b. CD34 selection- CD 34 being a marker of haemopoietic stem cells, is used to enrich the fraction of cells with stem cells. Using magnetic cell sorting using CD34 selecting antibody.

4. **Ex Vivo Expansion**

Expanding the extracted stem cells in vitro is a very important and non optimized stem till date for increasing amount of stem cells. In vitro and culture of these expanded stem cells ex vivo could help in transplantation of large amounts of functional stem cells to the recipient and be future target of therapy in stem cell transplantation.

10. If we use tissue typing (HLA typing) to generate a "match" for a kidney transplant, please answer these
questions:

A. What does a "match" mean in terms of HLA tissue typing?

Match means similarity of major HLA antigens present on the donor to that of the recipient. Minimum of 6 HLAs out of 8 must match for transplantation and more for a closer and less immune responsive match.

B. If we have a match, why does the kidney recipient need lifetime immunosuppressive drugs?

Immunosuppresion is required as matching is done against HLA-A,HLA-B and HLA-DR loci. As HLAs are extremely polymorphic, it is impossible to have full HLA matching (major & minor HLAs) except in case of donation by identical twin. The HLA-identical donor has major HLAs matched but in most cases. Some minor HLAs are unmatched and in many cases will cause immune reaction without immune therapy.

Hence immunosuppression lifelong is required to protect against these immune reactions caused by unmatched minor HLAs (or major HLA in case of partial HLA matched)

C. If we have a match, why do 25%-50% kidney transplants fail?

Due to lack of full HLA match, most transplants have a partial or HLA identical match when transplanted. Immunosuppressive therapy s require lifelong for the renal transplant to continue functioning.

In this period, if the therapy doesn't function as required, immune reaction against the donated organ will appear leading to rejection and failure of transplant.

25-50% rejection rate is due to chronic immune damage to kidneys despite immunosuppressive therapy due to the actions of innate immunity among other reasons.

11. What is the best, or at least most common marker for stem cells in peripheral blood, and how is such a test carried out?

The most common stem cell marker in peripheral blood is CD34 antigen.

CD34 measurement is carried out using FACS (Flow Assisted Cell Sorting) in a Flow Cytometer.

End of exam–