Lec 15
hematological tests

Normal bleeding time = 1-5 minutes

Bleeding time is prolonged in: Platelet deficiency – Vessel wall defe

Bleeding time is normal in: Hemophilia

Precautions:

1. The skin site for the test should be scrubbed well with alcohol to increa the blood flow
2. The skin should be dry & the puncture should be 3-4mm deep to give f flowing blood
3. Don't squeeze on the puncture site
4. If bleeding continues for more than 10-12 min., you must stop the test press a sterile gauze on the wound

Clotting time

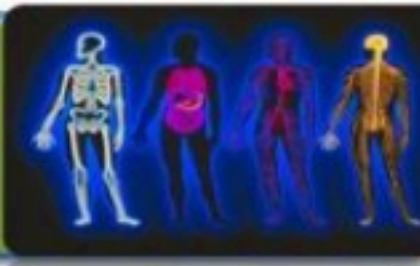
Procedure:

1. Draw 5ml venous blood by a new clean syringe …. This is the Zero time & then transfer the blood to a clean, dry test tube
2. Hold the test tube in a water bath at 37°C, take it out at 30 sec. intervals & it ….. The end point is when the tube can be tilted without spilling the blood

Normal clotting time: 5-10 minutes

Clotting time is prolonged in: Deficiency in one or more of the clotting factors (Such as In case of hemophilia) – Liver diseases – Vitamin K deficiency

Clot retraction time (CRT)



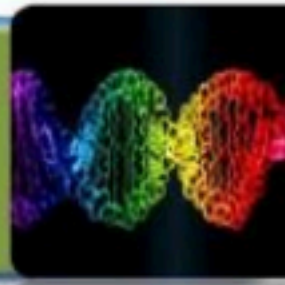
Procedure:

1. Transfer the test tube containing clotted blood to an incubator at 30°C.
2. Normally, the clot starts to shrink in about 30 min. …. Becomes half its size 2-3 hours

N.B.

- Clot retraction depends on the release of many factors from the platelets, & so CRT depends on the platelet count
- **CRT is prolonged in case of: Low platelet count**

Clot lysis time



Clot lysis: Is a process by which a clot becomes fluid

- ✓ Normally, the clot lysis in about 72 hours
- ✓ More or less than this time is considered abnormal

Prothrombin time (PT)

EXTRINSIC PATHWAY

Damage to tissues

[leaks]

Tissue Factor III (TF_3)

Ca^{2+} **Clotting Factor VII (CF_7)**

[combine to form]

Factor VII-Tissue Factor Complex ($TF\text{-}F_7$)

[activates]

INTRINSIC PATHWA

Damage to endotheliu

[activates]

Factor XII (F_{12})

Platelet Factor III (PF_3)

Clotting Factors VIII & IX (CF_8, CF_9)

Ca^{2+}

[combine to form]

Factor X-Activator Com

[activates]

Factor X

[forms]

Prothrombinase

(Prothrombin Activator)

Prothrombin → Thrombin

Fibrinogen → Fibrin

PT

For clotting to occur,

- ✓ **Prothrombin** is converted into **thrombin** by **(Prothrombin activator)**
- ✓ And then **thrombin** will convert **fibrinogen** into **fibrin** (Clot)

The time required for clotting to occur is called: Prothrombin time (PT)

Normal PT = 12 – 15 sec.
Normal Level of prothrombin = 30-40 mg/dl
Bleeding may occur if prothrombin level falls below 20% of normal

PT test is used to control anticoagulant dose

PT

PT is prolonged in case of: deficiency in certain clotting factors – Hig
dose of oral anticoagulant – Vitamin K deficiency

ANTICOAGULANT USED IN HAEMATOLOGY

Introduction to blood coagulation

Blood coagulation is the process of blood clotting which involves a number of substances which react enzymatically. There are over twelve clotting factors which react together to form a clot. These factors have been given numerals from factors I to XII but factor VI does not exist.

The following factors below are commonly used by their names including: I-Fibrinogen II- Prothrombin III- Thromboplastin or tissue factor IV Calcium (Ca++) For coagulation to occur, all factors must be present. If any factor is absent, then blood coagulation will not take place .If any of the factors is present insufficient quantities, then the process is slowed down. There are two systems of blood coagulation:

I. ***Intrinsic system*** Involves factors XII, XI, IX, VIII, X,V and platelet factor in the presence of calcium ions; when activated from an active principle *(Prothrombin activator or Activated factor X) which act on Prothrombin (factor II) to thrombin (IIa), that converts fibrinogen (I to fibrin Ia),* a network of fibres i.e. Clot.

2. ***Extrinsic system*** *This involves a tissue extract(tissue factor) that acts on factors VII to VIIa, then VIIa acts on X in the presence of Ca2+ and V to form an active principle, active thromboplastin (Xa). The active principle then converts prothrombin to thrombin which converts fibrinogen to fibrin.*

There are three stages of blood coagulation:

1. The formation of the active principle (active

thromboplastin) 2. Conversion of prothrornbin to thrombin

3. Conversion fibrinogen to fibrin.

Coagulation ends up with formation of fibrin. Removal of fibrin is termed fibrinolysis. Coagulation and fibrinolysis are always in equilibrium for normal life and this is termed haemostasis. Factors XIII makes the fibrin clot stable, if coagulation exceeds fibrinolysis, intra- vascular coagulation takes place and blocks veins and this can be lethal if not treated. If fibrinolysis exceeds coagulation, excessive bleeding will occur as in hernopophilia and may also lead to death.

ANTICOAGULANTS

These are substances which prevent blood clotting either in vivo or in vitro. This phenomenon is achieved by a coagulation factor, thus preventing blood coagulation. Usually the anticoagulants prevent one stage of clotting factors but some anticoagulants affect many factors. In hematology we have different types of anticoagulants used depending on what investigations are being carried out. This is because some anticoagulants are good for some tests.

1. SEQUESTRINE or Ethylene Di-amine tetra acetic acid (EDTA)

has different types including:

- K2 EDTA (1650 g/l) this is very soluble
- Na2 EDTA (108g/l) less soluble
- Li2 EDTA (160 g/l) f

airly soluble All the three are used for different purposes. Ka2 EDTA and Na2 EDTA are recommended in hematological investigation while Li2 EDTA is good for Clinical chemistry for determination of electrolyte (Ca++ Na + , and K+). The recommended amount of sequestrine is usually1.5 + O.25mg/ml of blood.

MODE OF ACTION: EDTA acts by removing Ca ++ from blood by converting it from the ionized to non Ionized form i.e. a chelating agent.

EDTA is a chelating agent. EDTA has a residual effect, that is, not all of it is used up and its mode of action continues so long as it is blood. The removal of Ca ++ ions affects the clotting system, thus the term sequestrine or Chelating agent.

Sequestrine (EDTA) is the recommended anticoagulation for routine hematological investigations for the following tests: · Haemoglobin determination · Full blood counts –platelet, red cell count and WBC counts · Blood film study · Haematocrit (PCV). The size of the cell is not usually affected. · Erythrocyte sedimentation rate (ESR). · EDTA is not poisonous and can be used in transfusion medicine.

EDTA is not recommended for Coagulation studies because of its chelation properties as it keeps removing Ca ions even if more calcium is added. In osmotic fragility tests (OFTs) where red cells are presented to different salt concentration, it is not an anticoagulant of choice.

Effects of excess EDTA

EDTA has shrinkage and degenerative changes on both RBCs and Leucocytes. When EDTA is used in excess (above 2mg/ml of blood) it causes crenation leading to decreased PCV and MCHC, There is false thrombocytosis, because platelets swell and disintegrate causing artificially high platelet count.

2. HEPARIN

Heparin can be extracted from lung tissue and is also present platelets and basophils. It is not a poisonous anticoagulant and can be used in blood transfusion. But mostly it is used for treatment of coagulation diseases such as deep venous

thrombosis (DVT). *Disadvantages of heparin are that it is expensive and has short half-life and it is only used where other anticoagulation are not available. It also tends to cause leucocyte clumping and blood films stained with rowmanowsky stains give a faint blue coloration in the background.*

MODE OF ACTION It neutralizes or inactivates thrombin so that it is not available for formation of fibrin from fibrinogen. It used at a concentration of 15-20 l.U. /ml of blood (0.1-0.2 mg/ml). It does not affect Red cell size. It doesn't alter salt concentration.

USES: Good for red cell count and hemoglobin estimation. Good for osmotic fragility test and PCV. It is unsuitable for platelet and leucocyte counts because it clumps these cells 3.

OXALATES

These affect coagulation by converting Calcium ions into calcium oxalate crystals i.e calcium is precipitated out of blood. These crystals may be engulfed by leucocytes and are insoluble. The three common oxalates **used are: • Potassium oxalate • Ammonium oxalate • Sodium oxalate Potassium oxalate causes the shrinkage of red cell (crenation) Ammonium oxalate causes the swelling of red cells (haemolysis)**

The Heller and Paul mixture is formed to minimize crenation and haemolysis by talking 4 parts of potassium oxalate and 6 parts of ammonium oxalate to anticoagulate 5mls of blood. The oxalates are poisonous and can not be used for blood transfusion purposes. They are used in concentration of 2mg/ml of blood. They can be used in coagulation studies, Haematocrit, haemoglobin estimation, red blood count and red cell indices.

4. TRISODIUM CITRATE

This is non-toxic anticoagulant usually applied in blood

transfusion as well as in hematology for evaluation of coagulation disorders.

MODE OF ACTION :- It acts by converting Ca+ + ions into non ionized form hence preventing coagulation. Calcium is not precipitated. It lowers the blood PH from 7.4 to between 7.3 -7.1 which is the best pH for storage of blood at 4 0 C for not less than 21 days. TYPES i) Trisodium citrate (Na3C6H5O7 .2H20) ii) Disodium citrate (2Na2C6H5O7 .11H2O)

USES

- ❖It is the anticoagulant of choice in blood transfusion Acid citrate dextrose and CPD
- ❖Used in coagulation studies
- ❖Used for erythrocytes sedimentation rate. For coagulation studies use 1 part of 32 g/Lcitrate to 9 parts of blood. For ESR use 1 part of 32 g/L citrate to 4parts of blood (blood in EDTA can be added to citrate in similar manner. The blood citrate is unsuitable for blood count because the solution has a dilution effect on cells. It causes platelet clumping it is unsuitable platelet count. Effect of anticoagulant on cells When blood is allowed to stand in the lab, degenerative changes occur. Most of them are due to presence of Anticoagulants, being exaggerated by presence of sodium or potassium oxalate. Films can be made within 1 hr of collection but sometimes up to 3hrs of collection is permissive 8-12 hrs changes are striking. 1. Effect on Neutrophils; · Nucleus stains homogeneously but is hypersegmented. · Cytoplasm margin may appear

less defined and small vacuoles are seen.

2. Mononuclear cells · Irregular lobulation which may result into disintegration. · Small vacuoles may also appear in cytoplasm

3. Lymphocytes · Nucleus may undergo budding and gives rise to lobes and may stain homogeneous. · Vacuoles appear in the cytoplasm.

4. Red cells · These are the least affected by standing up to 6 hrs at room temperature · Longer standing may lead to crenation and shrinkage. But these changes are reduced when kept at 40 C. · Blood film made before blood is added to anticoagulant is recommended · However platelet clumps make less easy to assess platelet numbers.

Apheresis

Apheresis; is a procedure in which whole blood is removed from the body and passed through an apparatus that separates out one (or more) particular blood constituents.

 It then returns the remainder of the constituents to the individual's circulation. Through the use of sophisticated automation, an apheresis procedure can be performed on either a **blood donor** or a **patient**.

According to the principles on which device depends, apheresis provides two purposes

1. **Donor apheresis**; can be performed on a donor to collect a **specific blood component,** (plasma, plt. , WBC, RBC, HSC). Apheresis is also used to harvest **stem cells** from the peripheral blood of donors and patients, avoiding the need for extraction from the bone marrow.

2. **Therapeutic apheresis**; can be performed on a patient to remove **disease-causing or unwanted cellular or plasma** constituents from a patient.

 Apheresis technology continues to evolve, and the procedure is now common place in blood donor centers and many hospitals and acute care settings.

Types of Apheresis Procedures and Their Application

PROCEDURE	COMPONENT REMOVED	APPLICATION	
		DONAR	PATIENT
Plasmapheresis	Plasma	√	√
Plateletpheresis	Platelets	√	√
Leukapheresis	WBC	granulocytes are the primary component collected	Removal of granulocytes and/or lymphocytes
Erythrocytapheresis	RBC	√	√
HPC apheresis	Hematopoietic progenitor cells (HPC)	peripheral blood stem cells (PBSC)	√

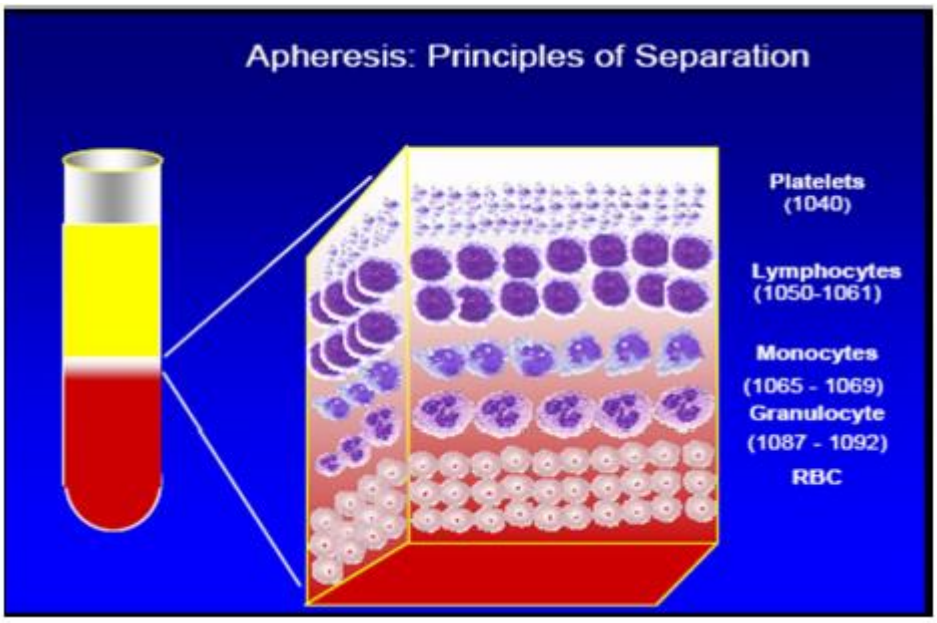
- **Plasmapheresis**: The process of removing plasma by apheresis device.
- **Plateletpheresis**: The process of removing platelets by apheresis device.
- **Erythrocytapheresis**: The process of removing RBC by apheresis device.
- **Leukapheresis**: The process of removing WBC by apheresis device.
- Apheresis technology utilizes this same principle for separating blood components.
- Blood is removed from an individual (usually with a large-bore needle), mixed with an anticoagulant, and transported directly to the separation device (typically a machine with a centrifuge bowl or belt).

 There it is separated into specific components.

 Once the components have been separated, any component can be withdrawn. The remaining portions of the blood are then mixed and returned to the donor or patient.

Methodology

 Apheresis is performed using automated technology, and separation is typically performed by **centrifugation**, less commonly by **membrane filtration**. Apheresis instruments in use today have a computerized control panel, allowing the operator to select the desired component to be collected or removed.



 After collecting the blood from patient or donor, the blood is anticoagulated, processed, the specific component collected, others reinfused back to circulation.

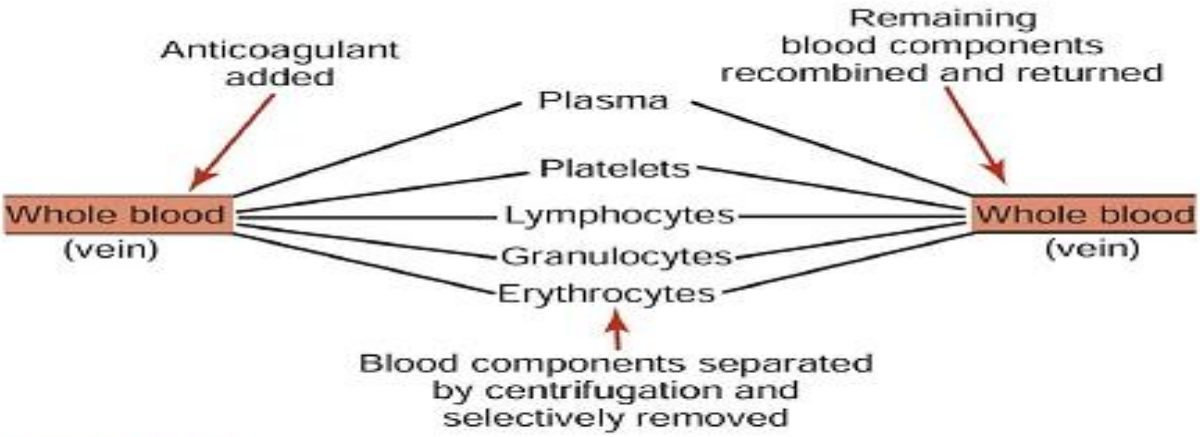


Figure 14–2. Principles of apheresis.

 Two Methods of Centrifugation in common use:

 Intermittent Flow Centrifugation; requires only one venipuncture, in which the blood is drawn and reinfused through the same needle. Once the desired component is separated, the remaining components are reinfused to the donor, and one cycle is complete.

Apheresis procedures performed on patients usually require many cycles to reach an acceptable therapeutic endpoint.

 Continuous Flow Centrifugation; procedures withdraw, process, and return the blood to the individual simultaneously. Two venipuncture sites are necessary. The process of phlebotomy, separation, and reinfusion is uninterrupted.

Membrane filtration technology uses **membranes with specific pore sizes**, allowing plasma to pass through the membrane while the cellular portion passes over it.

The most common anticoagulant used in apheresis is **acid citrate dextrose.**

Therapeutic apheresis

Therapeutic apheresis, like donor apheresis, involves the removal of a specific blood component, with return of the remaining blood constituents to the patient.

However, with TA, since the component being removed is considered pathological (or contributing to the patient's underlying disease state), significantly larger volumes of blood must be processed in order to remove as much of the offending agent as possible.

Therapeutic apheresis has become an accepted and standard therapy for many hematologic, neurological, renal, metabolic, autoimmune, and rheumatic diseases, among others.

The TA procedure is classified according to the blood component removed:

Cytapheresis procedure may be used to selectively remove RBCs, WBCs, or platelets.

Plasmapheresis procedure is used to remove plasma when the pathological substance is found in the circulation. Plasmapheresis considered as the most useful of other types of TA.

Factors Removed by Therapeutic Plasmapheresis

1. Immune complexes (e.g., systemic lupus erythematosus)
2. Alloantibodies (e.g., antibody-mediated transplant rejection)
3. Autoantibodies (e.g., Guillain-Barré syndrome, Goodpasture's syndrome)
4. Immunoglobulins causing hyperviscosity
5. Protein-bound toxins or drugs
6. Lipoproteins (e.g., familial hypercholesterolemia, hypertriglyceridemia)

Adverse Effects of Apheresis

- Citrate toxicity

• Vascular access complications (hematoma, sepsis, phlebitis, neuropathy)

• Hypovolemia

• Allergic reactions

• Hemolysis

• Air embolus

• Depletion of clotting factors

• Circulatory and respiratory distress

• Transfusion-transmitted diseases

• Lymphocyte loss

• Depletion of proteins and immunoglobulins

Blood Groups

The term **human blood group systems** is defined by the International Society of Blood Transfusion (ISBT) as systems in the human species where cell-surface antigens—in particular, those on blood cells—are "controlled at a single gene locus or by two or more very closely linked homologous genes with little or no observable recombination between them", and include the common ABO and Rh (Rhesus) antigen systems, as well as many others; **43** human systems are identified as of June 2021.

The term "blood group" refers to the entire blood group system comprising red blood cell (RBC) antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. "Blood type" refers to a specific pattern of reaction to testing antisera within a given system.

At present, 43 blood group systems representing over 300 antigens are listed by the International Society of Blood Transfusion . The genes of these blood group systems are **autosomal**, except XG and XK which are **X-borne**, and MIC2 which is present on both X and Y chromosomes. The antigens can be :
1- **integral proteins** where polymorphisms lie in the variation of amino acid sequence (e.g., rhesus [Rh], Kell)

2- **glycoproteins or glycolipids** (e.g., ABO). Some of the important groups are mentioned here [Table 1].

Table 1: Blood group systems				
Name	Symbol	Number of antigens	Gene name	Chromosome
ABO	ABO	4	ABO	9
MNS	MNS	43	GYPA, GYPB, GYPE	4
P	P1	1	P1	22
Rhesus	Rh	49	RhD, RhCE	1
Lutheran	LU	20	LU	19
Kell	KEL	25	KEL	7
Lewis	LE	6	FUT3	19
Duffy	FY	6	FY	1
Kidd	Jk	3	SLC14A1	18

ABO Blood Group System

The oldest known blood group system is the ABO or ABH system, which discovered at 1901. It still remains the most important of all blood groups in transfusion practice.

The **ABO system** is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals have **antibodies** in their serum to **antigens** that are absent from their RBCs. This occurs without any exposure to RBCs through transfusion or pregnancy.

The ABO system consist of the basic **antigen H** and 2major groups of antigens; these are **(A) antigen** and **(B) antigen**. The basic material of these antigens is a glycoprotein or glycolipid backbone to which sugars are attached, the terminal sugars specify the antigen.

H-antigen

H‑antigen is the **precursor** to the ABO blood group antigens. It is present in all RBCs irrespective of the ABO system. Persons with the rare Bombay phenotype are homozygous for the H gene (HH), do not express H‑antigen on their RBCs. As H‑antigen acts as precursor, its absence means the absence of antigen A and B. However, the individuals produce iso-antibodies to H‑antigen as well as to antigens A and B.

BLOOD GROUP ANTIBODIES:

Antibody production generally initiated within **first 4-6 months** of life and peaks when an individual is between 5 and 10 years of age and declines later in life**.**

1. *Naturally-occurring antibodies (Abs):*

These antibodies occur in the plasma of subjects who:

- Lack the corresponding antigen.
- Have not been transfused.
- Have not been pregnant.

These antibodies are:

- Usually **IgM**
- React optimally at cold temperature (4°C)
- Although reactive at 37°C, are called **cold antibodies**
- The most important are anti-A and anti-B
- Although anti-A (from blood group B individuals) and anti-B (from blood group A individuals) contains predominantly IgM antibody,

there may be small quantities of IgG present. Naturally occurring antibodies to A and/or B antigens are found in the

- plasma of subjects whose red cells lack the corresponding antigen.

2. Immune antibodies:

Develop in response to:

- Blood Transfusion
- Transplacental passage during pregnancy of fetal red cells possessing Ags that the mother lacks.

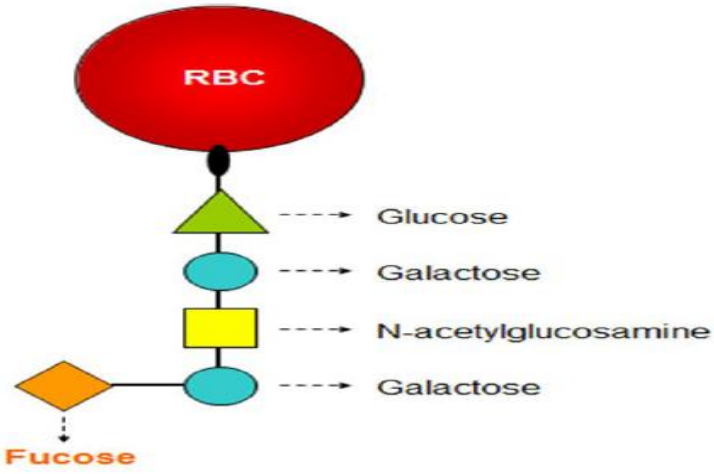
These antibodies are:

- Commonly **IgG**.
- Immune Abs react optimally at 37°C (**warm Abs**).
- Although some IgM Abs may also develop usually in the early phase of an immune response.
- Only IgG Abs are capable of transplacental passage from mother to
- fetus.
- The most important immune Ab is the Rh Ab (anti-D).

BLOOD GROUP ANTIGENS

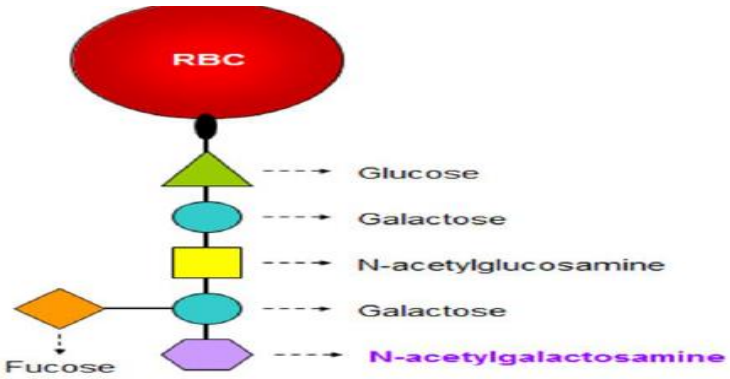
- ABO system consists of three allelic genes: **A**, **B** and **O**.
- The A and B genes control the synthesis of specific transferase enzymes responsible for the addition of single carbohydrate residues on H substance.
- The **O gene** is amorph and does not transform the H substance.

- Although there are six possible genotypes (AA, AO, BB, BO, OO, AB) the absence of a specific anti-O prevents the serological recognition of more than four phenotypes.
- H antigen is a **short oligosaccharides** act as a foundation upon which A and B antigens are built, act as a precursor by adding fucose sugar



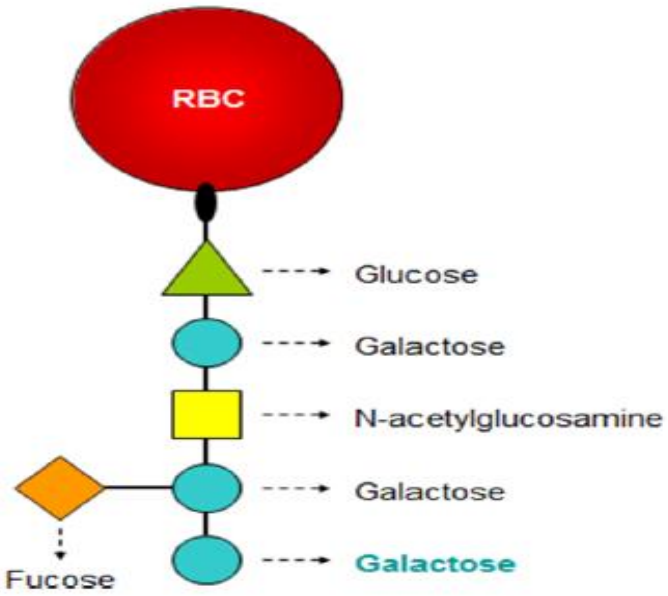
Formation of H antigen

- A and B genes code for enzymes that add an immunodominant sugar to the H antigen
- The A gene codes for an enzyme (**N-acetylgalactosaminyltransferase**) that adds N- acetylgalactosamine sugar to the terminal sugar of the H antigen to become A group



Formation of A antigen

- The B gene codes for an enzyme (D-galactosyltransferase) that adds D-galactose sugar to the terminal sugar of the H antigen to become **B group**



Formation of B antigen

Phenotype	Genotype	Antigens	Naturally-occurring Abs
O	OO	H	Anti-A , anti-B, anti-A,B
A	AA or AO	A	Anti-B (anti-H is very rare)
B	BB or BO	B	Anti-A
AB	AB	AB	None

ABO subgroups

- The ABO phenotypes can be divided into categories termed subgroups. These subgroup occurred due to qualitative and quantitative differences.

- Quantitative difference due to ↓ Number of antigen sites, ↓ Amount of transferase enzyme and↓ Amount of branching structure.
- Qualitative difference due to differences in the precursor oligosaccharide chains and subtle differences in transferase enzymes.

For example , Blood group A can be divided into a number of sub groups (A, A1, A2, A3, A4, A5) of which A1 and A2 are the most relevant clinically A2 is weaker than A1.

Lec.3

Rh Blood Group System

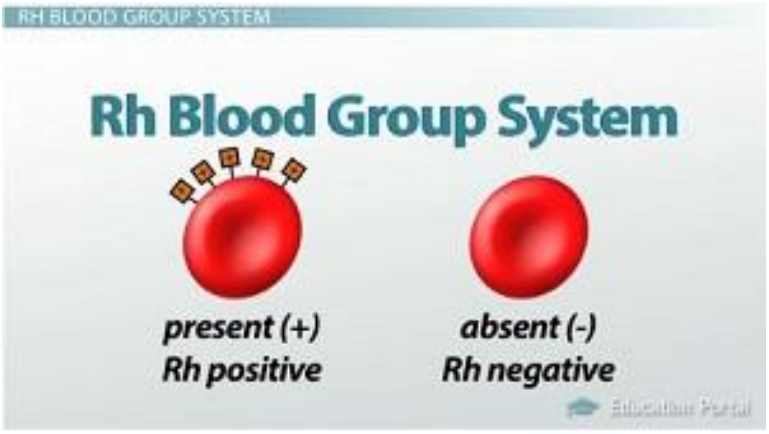
Rh blood group system is a system for classifying blood groups according to the presence or absence of the Rh antigen on the cell membranes of RBC, it is also important when transfusing blood. The designation Rh is derived from the use of the blood of rhesus monkeys in the basic test for determining the presence of the Rh antigen in human blood.

The major difference between the O-A-B system and the Rh system is the following: In the O-A-B system, the plasma agglutinins responsible for causing transfusion reactions develop spontaneously, whereas in the Rh system, spontaneous agglutinins almost never occur. Instead, the person must first be massively exposed to an Rh antigen, such as by transfusion of blood containing the Rh antigen, before enough agglutinins to cause a significant transfusion reaction will develop.

Rh Antigens—"Rh-Positive" and "Rh-Negative" People.

There are 6 common types of Rh antigens, each of which is called an *Rh factor*. These types are designated C, D, E, c, d, and e. A person who has a C antigen does not have the c antigen, but the person missing the C antigen always has the c antigen. The same is true for the D-d and E-e antigens. Also, because of the manner of inheritance of these factors, each person has one of each of the three pairs of antigens.

The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens. Anyone who has this type of antigen is said to be Rh positive, whereas a person who does not have type D antigen is said to be Rh negative. However, it must be noted that even in Rh-negative people, some of the other Rh antigens can still cause transfusion reactions, although the reactions are usually much milder.



About 85% of all white people are Rh positive and 15%, Rh negative. In American blacks, the percentage of Rh-positives is about 95%, whereas in African blacks, it is virtually 100%.

Rh Immune Response

Formation of Anti-Rh Antibody (Agglutinins)

When RBCs containing Rh factor are injected into a person whose blood does not contain the Rh factor—an Rh-negative person—anti-Rh agglutinins develop slowly, reaching maximum concentration of agglutinins about 2-4 months later.

This immune response occurs to a much greater extent in some people than in others. With multiple exposures to the Rh factor, an Rh-negative person eventually becomes strongly "sensitized" to Rh factor.

Rh antibodies stimulated as a result of transfusion or pregnancy, they are immune. Associated with Haemolytic Transfusion Reaction (HTR) and Haemolytic Disease of the Fetus Newborn (HDFN).

Characteristics of Rh Transfusion Reactions

1. If an Rh-negative person has ***never before been exposed*** to Rh-positive blood, transfusion of Rh-positive blood into that person will likely cause no immediate reaction.

   However, anti-Rh antibodies can develop in sufficient quantities during the next 2-4 weeks to cause agglutination of the transfused cells that are still circulating in the blood. These cells are then hemolyzed by the tissue macrophage system. Thus, a delayed transfusion reaction occurs, although it is usually mild.
2. ***Upon subsequent transfusion*** of Rh-positive blood into the same person, who is now already immunized against the Rh factor, the transfusion reaction is greatly enhanced and can be immediate and as severe as a transfusion reaction caused by mismatched type A or B blood.

What happen during pregnancy for the Rh-positive offspring of Rh-incompatible parents?

During pregnancy, there are a similar danger exists for the Rh-positive offspring of Rh-incompatible parents, when the mother is Rh-negative and the father is Rh-positive.

1. The first child of such parents is usually in no danger, but the mother has acquired anti-Rh antibodies by virtue of incompatible blood transfusion.
2. During labour a small amount of the fetus's blood may enter the mother's blood stream.
3. The mother will then produce anti-Rh antibodies, which will attack any Rh-incompatible fetus in subsequent pregnancies.
4. This process produces erythroblastosis fetalis, or hemolytic disease of the newborn, which can be fatal to the fetus or to the infant shortly after birth.

Erythroblastosis Fetalis ("Hemolytic Disease of the Newborn")

Erythroblastosis fetalis is a disease of the fetus and newborn child characterized by agglutination and phagocytosis of the fetus's RBCs. In most instances of erythroblastosis fetalis, ***the mother is Rh negative and the father is Rh positive.***

The baby has inherited the Rh-positive antigen from the father, and the mother develops anti-Rh agglutinins from exposure to the fetus's Rh

antigen. In turn, the mother's agglutinins diffuse through the placenta into the fetus and cause RBC agglutination.

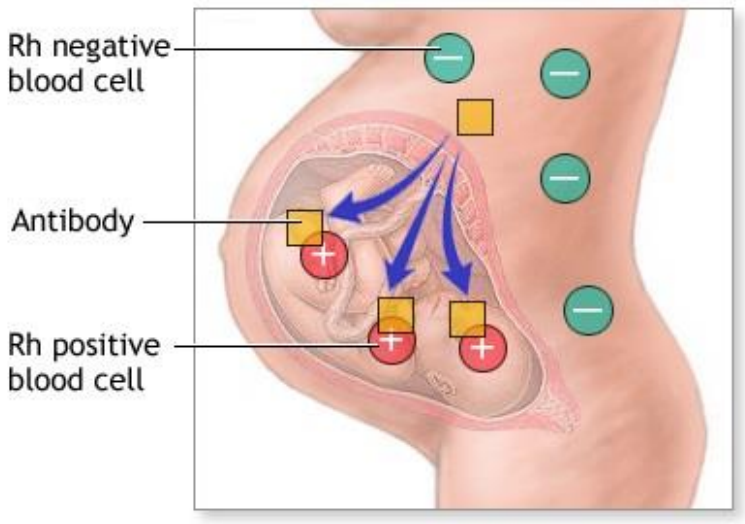
Incidence of the Disease

1. An Rh-negative mother having her first Rh-positive child usually does not develop sufficient anti-Rh agglutinins to cause any harm.
2. However, about 3% of second Rh-positive babies exhibit some signs of erythroblastosis fetalis
3. About 10% of third babies exhibit the disease
4. The incidence rises progressively with subsequent pregnancies.

Effect of the Mother's Antibodies on the Fetus

After anti-Rh antibodies have formed in the mother, they diffuse slowly through the placental membrane into the fetus's blood, as shown in figure below. There they cause agglutination of the fetus's blood.

The agglutinated RBCs subsequently hemolyze, releasing hemoglobin into the blood. The fetus's macrophages then convert the hemoglobin into bilirubin, which causes the baby's skin to become yellow (jaundiced). The antibodies can also attack and damage other cells of the body.



Clinical Picture of Erythroblastosis

1. The jaundiced, erythroblastotic newborn baby is usually anemic at birth, and the anti-Rh agglutinins from the mother usually circulate in the infant's blood for another 1-2 months after birth, destroying more and more RBCs.
2. The hematopoietic tissues of the infant attempt to replace the hemolyzed RBCs.
3. The liver and spleen become greatly enlarged and produce RBCs in the same manner that they normally do during the middle of gestation.
4. Because of the rapid production of RBCs including many nucleated blastic forms are passed from the baby's bone marrow into the circulatory system, and it is because of the presence of these nucleated blastic RBCs that the disease is called ***erythroblastosis fetalis***.

5. Although the severe anemia of erythroblastosis fetalis is usually the cause of death, many children who barely survive the anemia exhibit permanent mental impairment or damage to motor areas of the brain because of precipitation of bilirubin in the neuronal cells, causing destruction of many, a condition called ***kernicterus***.

Treatment of Neonates with Erythroblastosis Fetalis.

One treatment for erythroblastosis fetalis is to replace the neonate's blood with Rh-negative blood. About 400 milliliters of Rh-negative blood are infused over a period of 1.5 or more hours while the neonate's own Rh-positive blood is being removed.

This procedure may be repeated several times during the first few weeks of life, mainly to keep the bilirubin level low and thereby prevent kernicterus.

Prevention of Erythroblastosis Fetalis

The disease can be avoided by vaccinating the mother with Rh immunoglobulin after delivery of her firstborn if there is Rh-incompatibility.

The Rh vaccine destroys any fetal blood cells in the mother's body before the mother's immune system can develop antibodies.

The administered anti-D antibody also attaches to D-antigen sites on Rh-positive fetal RBCs that may cross the placenta and enter the circulation of the expectant mother, thereby interfering with the immune response to the D antigen.

ABO vs. Rh

Trait	ABO	Rh
Antigen Composition	Glycolipid, glycosphingolipid or glycoprotein	Glycoprotein
Ag Location on Cell Membrane	Outer surface	Transmembrane
Ag Location in Body	Red cells, platelets, lymphocytes, endothelial and epithelial cells, and in secretions	Red cells only
Effect of Enzymes	Enhanced	Enhanced
Antibody Class	IgM (some IgG)	IgG
Natural or Immune Ab	Natural	Immune
Clinically Significant?	Yes	Yes

Red cell and platelet storage lesions

storage lesions Series of biochemical and biomechanical changes in **red cells** or **platelets** during ex vivo preservation that reduce their survival and function

Erythrocytes are prone to modifications due to :

1. High oxygen environment (prone to oxidative stress and hemoglobin auto-oxidation)

2. Absence of nucleus and other organelles – no repair mechanism

The storage effects on RBC :

First : Metabolic effects

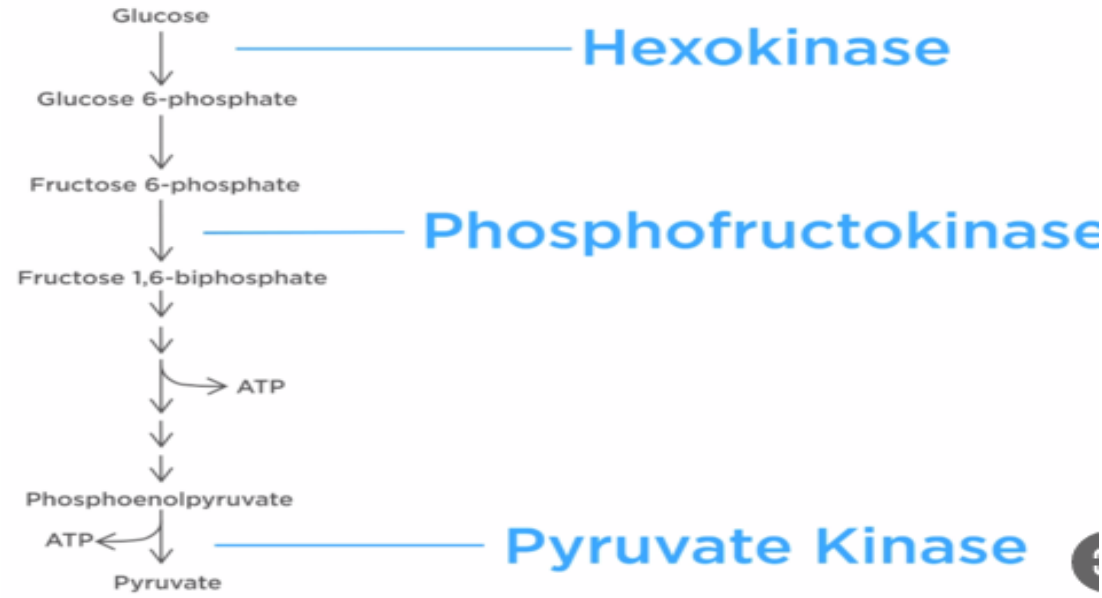
1. Lack of mitochondria > Energy production only by glycolysis .

One molecule of glucose produce :

- two molecules of lactate
- two molecules of adenosine triphosphate (ATP)
- two protons -> increase the acidity of the storage solution over time

2. Acidosis leads to :

- inhibition of phosphofructokinase and hexokinase
- slower glycolysis
- reduced ATP production



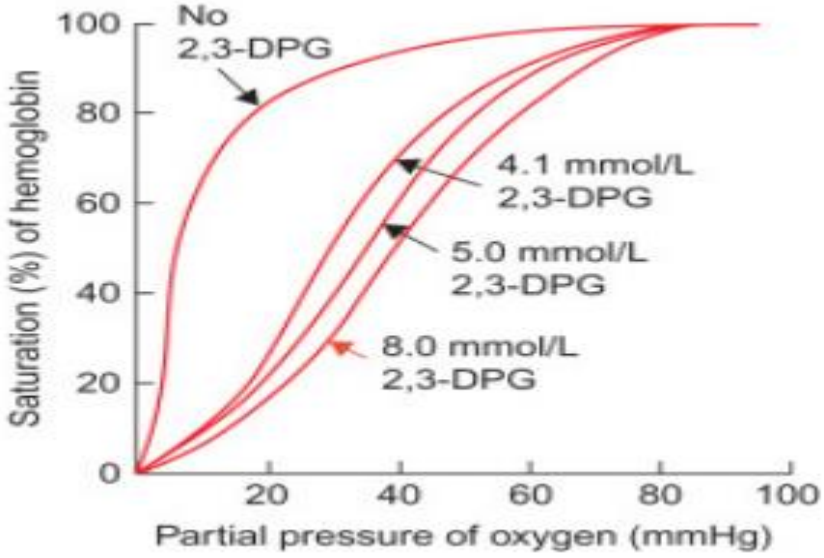
3. Decreased glutathione reductase levels -> reduce the ability of the RBC membrane to avoid oxidative damage.

4. The decrease in pH -> breakdown of 2,3- diphosphoglycerate (promotes hemoglobin transition from a **high-oxygen-affinity state** to a **low-oxygen-affinity state**. The 2,3-BPG binds to the central compartment of the hemoglobin , changing its conformation and shifting the oxygen dissociation curve to the right.)

- low level of 2,3-DPG leads to a left shifted oxygen disassociation curve
- increase hemoglobin O2 saturation and affinity

5. 2,3-DPG restored in vivo after transfusion :

- one hour ------ 30%
- 24 hrs. ------- 50%
- 3 days -------- full restoration



6. Storage of red cells at 4 ± 2°C :

- helps maintain red cell functionality and viability by reducing the red cell metabolic rate.
- At 4°C, the metabolic rate is ten times lower than at 25°C
- glucose or dextrose are added to storage mediums to allow red cells to continue glycolysis.

Second : Biomechanical effects

- Normal shape of the RBC is a **biconcave** disc.

- Maximum surface area > efficient gas exchange and flexibility to travel through the capillaries.

The red cell membrane consists of :

- lipid bilayer that is interspersed with proteins .
- The lipid bilayer includes phospholipids, cholesterol and fatty acids .

Phosphatidylserine is an important component :

- under normal circumstances is present entirely on the inner layer.
- When expressed on the outer layer (in senescent red cells) -> highly thrombogenic & leads to the removal of red cells by macrophage.

Auto-oxidation of hemoglobin within the red cell leads to precipitation of structurally distorted forms of **methemoglobin** .

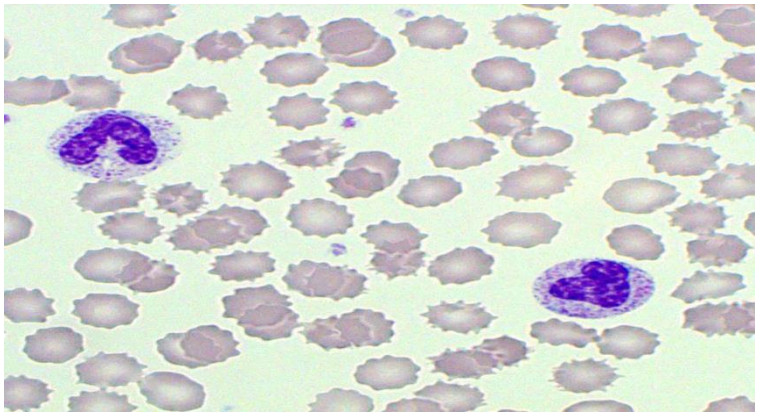
- Methemoglobin is a form of hemoglobin that has been oxidized, changing its heme iron configuration from the **ferrous (Fe^{2+})** to the **ferric (Fe^{3+})** state. Unlike normal hemoglobin, methemoglobin does not bind oxygen and as a result cannot deliver oxygen to the tissues

Membrane changes occur in parallel with metabolic changes :

- RBC shape maintenance -> dependent on ATP
- Shape changes due to ATP depletion

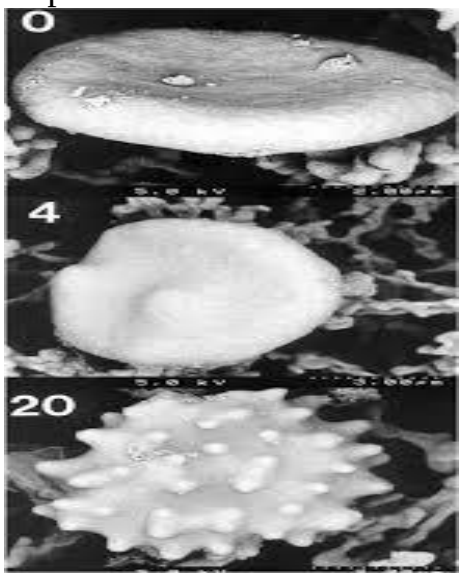
Echinocytes (burr cells)

- Greek – sea urchin
- Disk shaped cells with projections on the membrane
- reversible



Sphero-echinocytes

- With further depletion of total ATP and ADP pool
- spherical red cells with thorny projections
- decreased surface to volume ratio and deformability -> reduce RBC post-transfusion survival
- Irreversible change



RBC Microvesiculation occurs from the tips of echinocytic spines

RBC microvesiculation ->decreased surface to volume ratio and increased cell rigidity

- reduction in RBC deformability
- occlusion in capillary beds or intravascular rupture of RBCs.

Sialic acid content decreases with storage:

- reduction of the electrostatic repulsive forces that protect RBCs from aggregation
- the amount of membrane Sialic acid is a major factor in distinguishing young from aged RBC
- blood viscosity and potentially block flow in small vessels
- impairment of tissue perfusion.

Third : Oxidative effects

- For hemoglobin to be able to reversibly bind oxygen (oxyhemoglobin ↔ deoxyhemoglobin) within the red cell, its component heme-irons must be maintained in their reduced, or ferrous **(Fe^{2+})**, form.

- Under normal circumstances, a small amount of oxyhemoglobin undergoes spontaneous oxidation, generating **methemoglobin** (which has oxidized or **ferric (Fe^{3+})** iron and cannot bind oxygen) and reactive oxygen species .

- Methemoglobin

  - Inherently unstable
  - haemin (also known as ferric or oxidized haeme)

- Free haemin and iron, in conjunction with reactive oxygen species, can generate highly hazardous hydroxyl radicals that can cause oxidative injury to membrane lipids and proteins.

- Under normal circumstances, red cells are protected against this oxidative injury

  - the rate of spontaneous oxidation of hemoglobin is slow
  - NADH-dependent cytochrome-b5 reductase (CYTb5) reduces methaemoglobin back into oxyhemoglobin
  - cytosolic antioxidants (primarily reduced glutathione or GSH) and membrane anti-oxidants (primarily ascorbic acid or vitamin C) neutralize the generated reactive oxygen species.

- Under aerobic storage conditions -> RBCs are constantly exposed to a pro-oxidative environment.

- Superoxide dismutase and methaemoglobin reductase repair any oxidative damage via the hexose-monophosphate shunt.

- Glutathione stores decline during storage

  - likelihood of hydroxyl radical formation via the Fenton reaction increases
  - Fenton’s reaction – ferrous and ferric ions react with peroxide to form hydroxyl radicles , capable of degrading a number of organic and inorganic substances.

$Fe^{2+} + H_2O_2$ ----> Fe^{3+} + **.**OH + OH-

$Fe^{3+} + H_2O_2$ ----> Fe^{2+} + **.**OOH + H+

- Oxidative insult to proteins and lipids

  - protein oxidation and lipid peroxidation
  - formation of lysophospholipid, may contribute to transfusion-related acute lung injury

- RBC function and viability deteriorate as the oxidative injury persists over storage.

Platelets storage lesion

Platelets are cellular fragments derived from the cytoplasm of megakaryocytes

Do not contain a nucleus

Have mitochondria and various cytoplasmic granules.

Do not possess either a Golgi body or rough endoplasmic reticulum

Platelets are released and circulate approximately 9 to 12 days as small, disk-shaped cells

Biochemical changes

In the resting state

- 15% ATP - by glycolysis
- 85% by TCA cycle – with O_2 consumption

In the activated state

- 50% ATP by glycolysis - increase lactate production.

Decreased pO_2 in the plastic platelet container

- Increasing the rate of glycolysis to compensate for the decrease in ATP regeneration from the oxidative (TCA) metabolism
- This increases glucose consumption and causes an increase in lactic acid
- This results in a fall in pH - <6.4 after 5-7 days of storage at 22°C.

Lactic acid is buffered by bicarbonate - When the bicarbonate buffers are depleted during PC storage , pH rapidly falls to less than 6.2

Activation

- Platelets get activated following exposure to :
  - foreign surfaces – plastic bag
  - low pH – metabolic alteration
  - shear stress - during component separation
- Upon activation, the platelets lose their discoid morphology and become more spherical with multiple pseudopods.
- Conformational changes in **Glycoprotein** IIb/IIIa complex exposes binding sites for adhesive proteins (fibrinogen, vWF) resulting in platelet aggregates.
- Activation Platelet activation causes :
  1. **Release of granular contents**
     - Function -> recruitment of leucocytes and platelets
     - promote, immunity against infection
     - contribute to wound healing
     - presence of these contents in storage medium -> various transfusion reactions
  2. Expression of sequestered membrane proteins (CD62, CD63) & phospholipids
     - Negatively charged phospholipids providing a surface for the prothrombinase complex (X-Va) thereby contributing to procoagulant activity
- Agitation while storage cause **platelet lysis** and **calpain** (protease) activation
- Platelet lysis

- discharge cytosolic lactate dehydrogenase (LDH) and granular contents
- accumulation in the storage solution

- Activation of calpain
  - degradation of cytoskeletal proteins like actin
  - generate platelet microvesicles.
- Microvesicle formation leads to decrease in mean platelet volume (MPV) and also contributes to procoagulant activity

Blood component

A single blood donation can provide transfusion therapy to multiple patients in the form of RBCs, platelets, fresh frozen plasma, and cryoprecipitate. Other products such as derivatives of plasma (e.g., immune serum globulin) also benefit patients in various diseases or conditions.

Blood components could be **cellular** or **non-cellular** (plasma-derived)

Cellular blood component

Whole Blood

 Constituents; Whole blood contains RBCs and plasma, with a hematocrit level of approximately 38%.

 Benefit; Whole blood transfusions provide both oxygen carrying capacity and volume expansion.

 The platelets, white cells, and labile clotting factors do not survive in stored whole blood, so whole blood is rarely used for transfusion today with the exception of autologous units in some transfusion facilities.

 Most whole blood donations are made into components, red cells, platelets, plasma, cryoprecipitate or some combination of these components.

 Storage; If the donation remains as whole blood, it must be

stored at 1°C to 6°C, and the shelf-life is dependent on the

preservative used.

 Volume; 450 ml blood + 63ml anticoagulant solution.

Packed Red Blood Cells

 RBCs are prepared from whole blood by centrifugation or sedimentation.

 In addition, they may be obtained directly by apheresis.

 RBCs may be prepared at any time during the normal storage time.

 The amount of plasma removed from the whole blood unit will vary depending on the anticoagulant-preservative solution used. If CPDA-1 is used, 200 to 250 mL of plasma can be removed, leaving the RBC product with a hematocrit of 65% to 80%.

 If additive solutions (AS) are employed, an additional 50 mL of plasma can be removed, because 150 mL of adenine saline is added back to the cells, achieving the desired hematocrit level of less than 80%.

 Typically, RBCs with AS added will have a **hematocrit** of 55% to 65%.

 Volume; RBCs typically have a final red cell volume of 160 to 275 mL or 50 to 80 g of hemoglobin suspended in the residual plasma or additive solution.

 Benefits; RBC transfusions are indicted in patients who require an increase in RBC mass and oxygen-carrying capacity.

 They are particularly useful when the patient is also at risk of circulatory overload; for example, patients with anemia in addition to cardiac failure.

RBC Aliquots

 Benefits; Aliquoted red cells are the product most often transfused during the neonatal period or in infants younger than 4 months of age.

 Indications for transfusion include :

- ✓ anemia caused by spontaneous fetomaternal or fetoplacental hemorrhage,
- ✓ twin-twin transfusion,
- ✓ obstetric accidents,
- ✓ and internal hemorrhage.

 Volume; small volumes of RBCs (10 to 25 mL); several aliquots may be prepared from a single-donor unit.

 Storage; The aliquoted blood has an expiration time of 24 hours and should be stored at 1°C to 6°C until issued.

 Initial testing for neonates includes **ABO**, **Rh**, and **antibody screen for unexpected antibodies**, which can be performed on serum or plasma from the infant or mother.

 If the initial screen for RBC antibodies is negative, it is not necessary to cross-match donor RBCs for the initial or subsequent transfusions.

 The anticoagulant most often used for neonate transfusions is CPDA-1. A transfusion of 10 mL/kg in a unit with a hematocrit level of 80% should raise the hemoglobin by 3 g/dL.

RBCs Irradiated

Patients who are immuno-compromised or who are receiving a bone marrow or stem cell transplant, fetuses undergoing an intrauterine transfusion, and recipients of blood from relatives must receive irradiated blood.

Platelet Concentrates

 Platelet concentrates can be produced during the routine conversion of **whole blood** into concentrated RBCs or by **apheresis**. Most blood centers are producing apheresis platelets because of the high yield and minimal RBC contamination.

 Platelets have widespread use for a variety of patients who are thrombocytopenic (platelets less than 50,000/μL)

 Platelet concentrates prepared from whole blood are generally referred to as **random-donor platelets** to distinguish them from **single-donor platelets** produced by apheresis.

 Random-donor platelet concentrates should contain at least **5.5×10^{10}** platelets, contain approximately 40 to 70 mL plasma, stored at 20°C to 24°C with continuous agitation, and have a shelf-life of 5 days.

 Apheresis or single-donor platelets contain at least **30×10^{10}** platelets (therapeutic equivalent of four to six random donor platelets), are stored at 22°C to 24°C with continuous agitation, contain approximately 300 mL of plasma, and have a shelf-life of 5 days.

Platelet Aliquots

 As with RBCs, platelet transfusions for neonates require only small volumes, and several aliquots may be prepared from a single unit with the use of a sterile bag (Pedi-Pak).

 Transfusion of platelet concentrates is indicated for neonates whose counts fall below 50,000/μL and who are experiencing bleeding.

Leukoreduced components (RBCs & platelets)

 Leukoreduced red cells is a product in which the absolute WBC count in the unit is reduced to less than 0.0005×10^9 /L. special filters procure at least a 99.9% (a 2- to 4-log) removal of leukocytes either prestorage of component or during receiving of component.

- ✓ Leukoreduced components have been useful in trying to avoid the following reactions associated with products containing leukocytes:
- ✓ febrile non-hemolytic transfusion reactions;
- ✓ transfusion-related acute lung injury;
- ✓ and transmission of Epstein-Barr virus, CMV, and human T-cell lymphotrophic virus.

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BLOOD TRANSFUSION

Blood transfusion is generally the process of receiving blood or blood products into one's circulation intravenously. Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets.

Blood Bank

A **blood bank** is a place where blood collected from donor, typed separated to components, testing is performed (to reduce the risk of transfusion related adverse events) and preserved for later use in blood transfusion. However, it sometimes refers to a collection center, and indeed some hospitals also perform collection.

History

During the first blood transfusions were made directly from donor to receiver before coagulation, it was discovered that by adding anticoagulant and refrigerating the blood it was possible to store it for some days, thus opening the way for the development of blood banks.

John Braxton Hicks was the first to experiment with chemical methods to prevent the coagulation of blood at St Mary's Hospital, London in the late 19th century. His attempts, using phosphate of soda, but, were failed.

The first non-direct transfusion was performed on 1914 by the Belgian doctor Albert Hustin, although this was a diluted solution of blood. The

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Argentine doctor Luis Agote used a much less diluted solution in the same year. Both used sodium citrate as an anticoagulant.

The First World War acted as a catalyst for the rapid development of blood banks and transfusion techniques. In October 1915, Robertson performed his first wartime transfusion with a syringe to a patient suffering from multiple shrapnel wounds.

Robertson, a medical researcher and U.S. Army officer was instrumental in establishing the first blood banks

Collecting and Processing

There are certain standards are set for the collection and processing of each blood product. "Whole blood" is the proper name for one defined product, specifically inseparated venous blood with an approved preservative added.

Autologous donations are sometimes transfused without further modification, however whole blood is typically separated (via centrifugation) into its components, with red blood cells (RBC) in solution being the most commonly used product.

Units of WBC and RBC are both kept refrigerated at **1** to **6 °C**, with maximum permitted storage periods (shelf lives) of **35** and **42** days respectively. RBC units can also be frozen when buffered with glycerol, but this is an expensive and time consuming process, and is rarely done. Frozen red cells are given an expiration date of up to ten years and are stored at **−65 °C**.

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The less dense blood plasma is made into a variety of frozen components, and is labeled differently based on when it was frozen and what the purpose use of the product is. If the plasma is frozen immediately and is intended for transfusion, it is typically labeled as *fresh frozen plasma*. If it is intended to be made into other products, it is typically labeled as *recovered plasma* or *plasma for fractionation*.

The layer between the red cells and the plasma is referred to as *the buffy coat* and is sometimes removed to make platelets for transfusion. Platelets are typically pooled before transfusion and have a shelf life of **5** to **7** days. Thy are stored at room temperature **22 °C** and must be rocked/agitated. Since they are stored at room temperature in nutritive solutions, they are at relatively high risk for growing bacteria.

Some blood banks also collect products by ***apheresis***. The most common component collected is plasma via plasmapheresis.

Storage and Management:

Routine blood storage is **42** days or **6** weeks for stored packed **red blood cells** ("StRBC" or "pRBC") and involves refrigeration but usually not freezing.

Transfusions of **platelets** are comparatively far less numerous, but they present unique storage/management issues. Platelets may only be stored for **7** days, due largely to their greater potential for contamination, which is in turn due largely to a higher storage temperature.

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RBC storage lesion

Insufficient transfusion efficacy can result from red blood cell (RBC) blood product units damaged by so called **storage lesion**, a set of biochemical and biomechanical changes which occur during storage.

With red cells, this can decrease viability and ability for tissue oxygenation. Although some of the biochemical changes are reversible after the blood is transfused, the biomechanical changes are less so, and renewal products are not yet able to adequately reverse this phenomenon.

In general, there are not yet any *in vitro* tests to assess quality deterioration or preservation for specific units of RBC blood product prior to their transfusion, though there is exploration of potentially relevant tests based on RBC membrane properties such as erythrocyte deformability and erythrocyte fragility (mechanical).

Platelet storage lesion

Platelet storage lesion is a very different phenomenon from RBC storage lesion, due largely to the different functions of the products and purposes of the particular transfusions, along with different processing issues and inventory management considerations.

Human Blood groups

The term **human blood group systems** is defined by thInternational Society of Blood Transfusion (ISBT) as systems in the human species where cell-surface antigens—in particular, those on blood cells—are "controlled at a single gene locus or by two or more very closely linked homologous genes with little or no observable recombination between them", and include the

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common ABO and Rh (Rhesus) antigen systems, as well as many others; **43** human systems are identified as of June 2021.

The term "blood group" refers to the entire blood group system comprising red blood cell (RBC) antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. "Blood type" refers to a specific pattern of reaction to testing antisera within a given system.

At present, 43 blood group systems representing over 300 antigens are listedby the International Society of Blood Transfusion . The genes of these blood group systems are autosomal, except XG and XK which are X‑borne, andMIC2 which is present on both X and Y chromosomes. The antigens can be :

1- integral proteins where polymorphisms lie in the variation of amino acid sequence (e.g., rhesus [Rh], Kell)

2- glycoproteins or glycolipids (e.g., ABO). Some of the important groups are mentioned here [Table 1].

Table 1: Blood group systems

Name	Symbol	Number of antigens	Gene name	Chromosome
ABO	ABO	4	ABO	9
MNS	MNS	43	GYPA, GYPB, GYPE	4
P	P1	1	P1	22
Rhesus	Rh	49	RhD, RhCE	1
Lutheran	LU	20	LU	19
Kell	KEL	25	KEL	7
Lewis	LE	6	FUT3	19
Duffy	FY	6	FY	1
Kidd	Jk	3	SLC14A1	18

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The oldest known blood group system is the ABO or ABH system, which discovered at 1901. It still remains the most important of all blood groups in transfusion practice.

The **ABO system** is the most important of all blood groups in transfusion practice. It is the only blood group system in which individuals have **antibodies** in their serum to **antigens** that are absent from their RBCs. This occurs without any exposure to RBCs through transfusion or pregnancy.

The ABO system consist of the basic **antigen H** and 2major groups of antigens; these are **(A) antigen** and **(B) antigen**. The basic material of these antigens is a glycoprotein or glycolipid backbone to which sugars are attached, the terminal sugars specify the antigen.

H-antigen is the **precursor** to the ABO blood group antigens. It is present in all RBCs irrespective of the ABO system. Persons with the rare Bombay phenotype are homozygous for the H gene (HH), do not express H-antigenon their RBCs.

As H-antigen acts as precursor, its absence means the absence of antigen A and B. However, the individuals produce isoantibodies to H-antigen as well as to antigens A and B.

Blood Donation & Selection of Donation

A *blood donation* occurs when a person voluntarily has blood drawn and used for transfusions and/or made into biopharmaceutical medications by a process called *fractionation* (separation of whole-blood components). Donation may be of *whole blood* (WB), or of specific components directly (the latter called *apheresis*).

Types of Donation:

Blood donations are divided into groups *based on who will receive the collected blood:-*

1- *An 'allogeneic'* (also called *'homologous'*) *donation* is when a donor gives blood for storage at a blood bank for transfusion to an unknown recipient.
2- *A 'directed' donation* is when a person, often a family member, donates blood for transfusion to a specific individual. Most blood transfusion authorities believe that blood from family and friends (directed donations) is no safer than blood from regular volunteer donors.
3- *A 'replacement donor' donation* is a hybrid of the two and is common in developing countries such as Ghana. In this case, a friend or family member of the recipient donates blood to replace the stored blood used in a transfusion, ensuring a consistent supply.
4- When a person has blood stored that will be transfused back to the donor at a later date, usually after surgery, that is called *an 'autologous' donation*.

Blood that is used to make medications can be made from allogeneic donations or from donations exclusively used for manufacturing.

Recipient safety

Donors are screened for health risks that could make the donation unsafe for the recipient.

Autologous donors are not always screened for recipient safety problems since the donor is the only person who will receive the blood.

Donors are also asked about *medications* such as **dutasteride**, since they can be dangerous to a pregnant woman receiving the blood.

Donors are *examined for signs and symptoms of diseases that can be transmitted in a blood transfusion*, such as HIV, malaria, and viral hepatitis.

Donor safety

The donor is also examined and asked specific questions about their medical history to make sure that donating blood is not hazardous to their health. The *donor's hematocrit or hemoglobin* level is tested to make sure that the loss of blood will not make them anemic. *Pulse, blood pressure, and body temperature* are also evaluated. Elderly donors are sometimes also deferred on age alone because of health concerns.

The safety of donating blood during pregnancy has not been studied thoroughly, and pregnant women are usually deferred.

Blood testing

The donor's blood type must be determined if the blood will be used for transfusions.

1. The collecting agency usually identifies whether the blood is type A, B, AB, or O.
2. *The donor's Rh (D) type.*
3. *Also* will screen for *antibodies to less common antigens*. More testing is usually done before a transfusion.

   Group O is often referred as the "universal donor" but this only refers to *red cell transfusions*. For *plasma transfusions* the system is reversed and AB is the universal donor type.
4. Most blood is tested for diseases, including some sexually transmitted diseases (STDs).

The tests used are high-sensitivity screening tests and no actual diagnosis is made. Some of the test results are later found to be false positives using more specific testing.

False negatives are rare, but donors are discouraged from using blood donation for the purpose of anonymous STD screening because a false negative could mean a contaminated unit. The blood is usually discarded if these tests are positive, but there are some exceptions, such as autologous donations. The donor is generally notified of the test result.

Donated blood is tested by many methods, but *the basic tests* recommended by the World Health Organization are these four:

1. Hepatitis B Surface Antigen
2. Antibody to Hepatitis C
3. Antibody to HIV, usually subtypes 1 and 2
4. Serologic test for Syphilis

Site preparation and drawing blood

1. The blood is drawn from a large arm vein close to the skin, usually the median cubital vein on the inside of the elbow.
2. The skin over the blood vessel is cleaned with an antiseptic such as iodine or chlorhexidine to prevent skin bacteria from contaminating the collected blood and also to prevent infections where the needle pierced the donor's skin.
3. A large needle (16 to 17 gauge) is used to minimize shearing forces that may physically damage red blood cells as they flow through the needle.
4. A tourniquet is sometimes wrapped around the upper arm to increase the pressure of the blood in the arm veins and speed up the process.
5. The donor may also be prompted to hold an object and squeeze it repeatedly to increase the blood flow through the vein.

Apheresis

Apheresis is a blood donation method where the blood is passed through an apparatus that separates out one particular constituent and returns the remainder to the donor. Usually the component returned is the red blood cells, the portion of the blood that takes the longest to replace.

Using this method an individual can donate plasma or platelets much more frequently than they can safely donate whole blood. These can be combined, with a donor giving both plasma and platelets in the same donation.

Platelets can also be separated from whole blood, but they must be pooled from multiple donations. From three to ten units of whole blood are required for a therapeutic dose. Plateletpheresis provides at least one full dose from each donation.

Plasmapheresis is frequently used to collect source plasma that is used for manufacturing into medications much like the plasma from whole blood. Plasma collected at the same time as plateletpheresis is sometimes called *concurrent plasma*.

Apheresis is also used to collect more red blood cells than usual in a single donation (commonly known as "double reds") and to collect white blood cells for transfusion.

Recovery and time between donations

Donors are usually kept at the donation site for 10–15 minutes after donating since most adverse reactions take place during or immediately after the donation. Blood centers typically provide light refreshments or a lunch allowance to help the donor recover.

The needle site is covered with a bandage and the donor is directed to keep the bandage on for several hours.

In hot climates, donors are advised to avoid dehydration (strenuous games, alcohol) until a few hours after donation.

Donated plasma is replaced after 2–3 days. Red blood cells are replaced by bone marrow into the circulatory system at a slower rate, on average 36 days in healthy adult males. In one study, the range was 20 to 59 days for recovery. These replacement rates are the basis of how frequently a donor can donate blood.

Plasmapheresis and plateletpheresis donors can donate much more frequently because they do not loss significant amounts of red cells. The exact rate of how often a donor can donate differs from country to country.

Iron supplementation decreases the rates of donor deferral due to low hemoglobin, both at the first donation visit and at subsequent donations. Iron-supplemented donors have higher hemoglobin and iron stores.

Complications

Donors are screened for health problems that would put them at risk for serious complications from donating.

- First-time donors, teenagers, and women are at a higher risk of a reaction. One study showed that 2% of donors had an adverse reaction to donation. Most of these reactions are minor.
- Hypovolemic reactions can occur because of a rapid change in blood pressure. Fainting is generally the worst problem encountered.
- The process has similar risks to other forms of phlebotomy. Bruising of the arm from the needle insertion is the most common concern.
- A number of less common complications of blood donation are known to occur. These include arterial puncture, delayed bleeding, nerve irritation, nerve injury, tendon injury, thrombophlebitis, and allergic reactions.
- Donors sometimes have adverse reactions to the sodium citrate used in apheresis collection procedures to keep the blood from clotting. Since the anticoagulant is returned to the donor along with blood components that are not being collected, it can bind the calcium in the donor's blood and cause hypocalcemia. These reactions tend to cause tingling in the lips, but may cause convulsions, seizure,

hypertension, or more serious problems. Donors are sometimes given calcium supplements during the donation to prevent these side effects.

- The final risk to blood donors is from equipment that has not been properly sterilized. In most cases, the equipment that comes in direct contact with blood is discarded after use. Re-used equipment was a significant problem in transfer of diseases.

Donor Health Benefits

1- In patients prone to iron overload, blood donation prevents the accumulation of toxic quantities.

2- Donating blood may reduce the risk of heart disease for men, but the link has not been firmly established and may be from selection bias because donors are screened for health problems.

3- Repeated blood donation is effective in reducing blood pressure, blood glucose, HbA1c, low-density lipoprotein/high-density lipoprotein ratio, and heart rate in patients with metabolic syndrome.

Lec. 4

Complications of blood transfusion

Blood transfusions are associated with several complications, many of which can be grouped as **immunological** or **infectious**. There is also increasing focus on complications **arising directly or indirectly from potential quality degradation during storage.**

While some complication risks **depend on patient status** or **specific transfusion quantity** involved, a major risk of complications **simply increases in direct proportion to the frequency and volume of transfusion.**

"For recipient"

1. Immunological complications

A\ Acute hemolytic reactions

* It occur with transfusion of red blood cells, and occurs in about 0.016% of transfusions, with about 0.003% being fatal.

* This is due to destruction of donor erythrocytes by preformed recipient antibodies.

* Most often this occurs due to clerical errors or improper typing and cross matching.

* Symptoms include fever, chills, chest pain, back pain, hemorrhage, increased heart rate, shortness of breath, and rapid drop in blood pressure. Kidney injury may occur due to the effects of the hemolytic reaction (pigment nephropathy).

* When suspected, transfusion should be stopped immediately, and blood sent for tests to evaluate for presence of hemolysis. * Treatment is needed.

B\ Delayed hemolytic reactions

* It occur more frequently (about 0.025% of transfusions) and are due to the same mechanism as in acute hemolytic reactions.

* The consequences are generally mild and a great proportion of patients may not have symptoms, however, evidence of hemolysis and falling hemoglobin levels may still occur.

* Treatment is generally not needed, but due to the presence of recipient antibodies, future compatibility may be affected.

C\ Febrile non-hemolytic reactions

* They are due to recipient antibodies to donor white blood cells, and occurs in about 7% of transfusions.

* This may occur after 1 to 6 hours after receiving the transfusions.

* Fever is generally short lived and is treated with antipyretics, and transfusions may be finished as long as an acute hemolytic reaction is excluded.

* This is a reason for the now-widespread use of leukoreduction - the filtration of donor white cells from red cell product units.

D\ Allergic reactions

* It may occur when the recipient has performed antibodies to certain chemicals in the donor blood.

* Symptoms include urticaria, pruritus, and may proceed to anaphylactic shock.

* Treatment is the same as for any other type **I** hypersensitivity reactions.

E\ Post transfusion purpura

* It is a rare complication that **occurs after transfusion containing platelets that express a surface protein HPA-1a**.

* Recipients who lack this protein develop sensitization to this protein from prior transfusions, and develop thrombocytopenia about 7–10 days after subsequent transfusions.

* Treatment is with intravenous immunoglobulin, and recipients should only receive future transfusions with HPA-1a negative cells.

F\ Transfusion-associated acute lung injury (TRALI)

* TRALI is a syndrome of acute respiratory distress, often associated with fever, non-cardiogenic pulmonary edema, and hypotension.

* Symptoms can range from mild to life-threatening, but most patients recover fully within 96 hours,

* The mortality rate from this condition is less than 10%.

* Although the cause of TRALI is not clear, it has been consistently **associated with anti-HLA antibodies**.

* Because these types of antibodies are commonly formed during pregnancy, several transfusion organizations have decided to use only plasma from men for transfusion.

Non-immunological Complications

1. **Transfusion inefficacy**, while not itself a "complication," can lead to various complications due in part to the need to repeat transfusions; inefficacy can be especially serious for critical-care patients requiring rapid restoration of oxygen delivery.

2. **Transfusion-associated volume overload** is a common complication simply due to the fact that blood products have a certain amount of volume.

3. **Hypothermia**: Core body temperature can go down as low as 32 °C and can produce physiologic disturbances if the transfused blood was cold. Prevention should be done with warming the blood to ambient temperature prior to transfusions.

4. **Sever hemorrhage**: Transfusions with large amounts of red blood cells, whether due to severe hemorrhaging and/or transfusion inefficacy, can lead to a tendency for bleeding. The mechanism is thought to be due to spread intravascular coagulation, along with dilution of recipient platelets and coagulation factors.

5. **Metabolic alkalosis** can occur with massive blood transfusions due to the breakdown of citrate stored in blood into bicarbonate.

6. **Hypocalcemia** can also occur with massive blood transfusions due to the complex of citrate with serum calcium, thus lowering the ionized plasma calcium concentration. This is usually prevented by rapid hepatic metabolism unless the patient is hypothermic. Calcium is an important co-factor, especially in coagulation, and has a key role in mediating the contractility of myocardial, skeletal and smooth muscles. Hypocalcaemia results in hypotension, small pulse pressure, flat ST-segments and prolonged QT intervals on the ECG.

7. **Hyperkalaemia**: The potassium concentration of blood increases during storage. After transfusion, the RBC membrane Na^+–K^+ ATPase pumping mechanism is re-established and cellular potassium reuptake occurs rapidly. Hyperkalaemia rarely occurs during massive transfusions unless the patient is also hypothermic and acidotic.

Infectious Complications

Complications of blood transfusion are rare but can be life-threatening.

Blood products are contaminated with bacteria. This can result in life-threatening infection, also known as transfusion-transmitted bacterial infection.

Sources of contaminants include the donor's blood, donor's skin, phlebotomist's skin, and from containers.

Contaminating organisms vary greatly, and include skin flora, gut flora, or environmental organisms.

From the most important transmissible infections:

1- HIV transmission

2- Hepatitis C virus transmission

3- Other rare transmissible infections include hepatitis B, syphilis, Chagas disease, cytomegalovirus infections, and HTLV.

"For the donor"

The biggest risk is probably that of vasovagal syncope. The best defense, as a donor, is being well-hydrated and remaining at the donation center for 10-15 minutes after finishing the donation, to make sure he or she is feeling well. Other risks to donors included:

a) Bruise at the needle site
b) Sore arm
c) Hematoma at needle site
d) Sensory changes in the arm used for donation (eg, burning pain, numbness, tingling)
e) Fatigue
f) Nausea and vomiting

Lec.8

Hemolytic anemia

- **Anemia** is the result of premature destruction of red cells exceeding the erythropoietic capacity of the bone marrow.

- **Hemolytic anemia** is a form of anemia due to hemolysis, the abnormal breakdown of red blood cells (RBCs), either in the blood vessels (**intravascular hemolysis**) or elsewhere in the human body (**extravascular**, but usually in the spleen) before their normal life span of 120 days.

Causes

- The bone marrow is mostly responsible for making new red cells.

- Hemolytic anemia occurs when the bone marrow isn't making enough red cells to replace the ones that are being destroyed.

- There are several possible causes of hemolytic anemia. Red blood cells may be destroyed due to:

1. An autoimmune problem in which the immune system mistakenly sees own red blood cells as foreign substances and destroys them.

2. Genetic defects within the red cells (such as sickle cell anemia, thalassemia and glucose-6-phosphate dehydrogenase G6PD deficiency)

3. Exposure to certain chemicals, drugs, and toxins

4. Infections

5. Blood clots in small blood vessels

6. Transfusion of blood from a donor with a blood type that does not compatible.

Signs and symptoms:

- The patient may not have symptoms if the anemia is mild. If the problem develops slowly, the first symptoms may be:

1. Feeling weak or tired more often than usual, or with exercise
2. Headaches
3. Problems concentrating or thinking

Signs and symptoms

- If the anemia gets worse, symptoms may include:

4. Lightheadedness when you stand up
5. Pale skin
6. Shortness of breath
7. Sore tongue
8. Enlarged spleen

Pathophysiology

There are two mechanisms of hemolysis.

1. **Intravascular hemolysis** is the destruction of red blood cells in the circulation with the release of cell contents into the plasma.

Mechanical trauma from a damaged endothelium, complement fixation and activation on the RBC surface, and infectious agents may cause direct membrane degradation and cell destruction.

Pathophysiology

2. **Extravascular hemolysis** is the removal and destruction of red blood cells with membrane alterations by the macrophages of the spleen and liver.

Circulating blood is filtered continuously through thin walled splenic cords into the splenic sinusoids (with fenestrated basement membranes), macrophage with long dendritic processes.

A normal 8-micron red blood cell can deform itself and pass through the 3-micron openings in the splenic cords. Red blood cells with structural alterations of the membrane surface (including RBC coated by antibodies) are ***unable to cross this network and are phagocytosed and destroyed by macrophages.***

Classification

- They may be classified according to the means of hemolysis, being either ***intrinsic*** in cases where the cause is related to the red blood cell (RBC) itself, or ***extrinsic*** in cases where factors external to the RBC destruction.

1- Hereditary hemolytic anemia (Intrinsic causes)

In inherited hemolytic anemias, the genes that control how red blood cells are made are faulty. This class can be due to :

1. Defects of red blood cell membrane (as in **hereditary spherocytosis** and **hereditary elliptocytosis**)
2. Defects in hemoglobin production (as in **thalassemia**, **sickle cell anemia** and **congenital dyserythropoietic anemia**).
3. Defective red cell metabolism (as in **glucose-6-phosphate dehydrogenase deficiency** and **pyruvate kinase deficiency**).

2- Acquired hemolytic anemia (Extrinsic causes)

This class may be caused by immune-mediated causes, drugs and other various causes:

1. ***Immune-mediated causes*** could include ***transient factors*** as in ***Mycoplasma pneumoniae infection*** (**cold agglutinin disease**) or ***permanent factors*** as in ***autoimmune diseases*** like **autoimmune hemolytic anemia.**
2. ***Paroxysmal nocturnal hemoglobinuria (PNH)*** is a rare, acquired, potentially life-threatening disease of the blood characterized by complement-induced intravascular hemolytic anemia.
3. ***The causes of hypersplenism***
4. ***Burns***
5. ***Lead poisoning***

Laboratory finding

I- Increased RBC destruction

- Decreased RBC life span
- Increased haem(heme) catabolism
  - Increased serum unconjugated bilirubin
  - Increased urobilinogen excretion
- Absence or decrease of serum haptoglobin
- > 1 g /dl /week fall in blood Hb level
- Reduced glycosylated Hb
- Signs of intravascular hemolysis
  - Hemoglobinemia
  - Hemoglobinuria
  - Hemosiderinuria
  - Methemalbuminemia
  - Reduced serum hemopexin level

II-Increased bone marrow activity and RBC production

- **Blood**
  - Reticulocytosis
  - Macrocytosis
  - Polychromatophilia
  - Erythroblastosis
  - Leukocytosis and thrombocytosis
- **Bone marrow**
  - Erythroid hyperplasia
- ***Ferrokinetic***
  - *Increased plasma iron turnover*
  - *Increased RBC iron turnover*
- ***Biochemical***
  - *Increased RBC creatine*
  - *Increased activity of RBC enzymes eg: hexokinase, etc*

Mechanism of Hemolytic anemia

- Hemolytic anemia involves the following:

1. Abnormal and accelerated destruction of red cells and, in some anemias, their precursors
2. Increased breakdown of hemoglobin, which may result in:
   a) increased bilirubin level with jaundice
   b) increased fecal and urinary urobilinogen
   c) Hemoglobinemia, methemalbuminemia, hemoglobinuria and hemosiderinuria.

3. Bone marrow compensatory reaction:

   a) Erythroid hyperplasia with accelerated production of red cells, reflected by reticulocytosis, and slight macrocytosis in peripheral blood

   b) Expansion of bone marrow in infants and children with severe chronic hemolysis - changes in bone configuration visible on X-ray

4. The balance between red cell destruction and bone marrow compensation determines the severity of anemias.

In a healthy person

1. A red blood cell survives ***90 to 120 days*** in the circulation, so about ***1%*** of human red blood cells break down each day.
2. The ***spleen*** (part of the reticulo-endothelial system) is the main organ that removes old and damaged RBCs from the circulation.
3. ***The breakdown and removal of RBCs from the circulation is matched by the production of new RBCs in the bone marrow***.

✓ In conditions where the rate of RBC breakdown is increased, the body initially compensates by ***producing more RBCs***; however, breakdown of RBCs can ***exceed*** the rate that the body can make RBCs, and so anemia can develop.

✓ ***Bilirubin,*** a breakdown product of hemoglobin, can accumulate in the blood, causing jaundice.

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Homeostasis and bleeding disorders

Hemostasis

Hemostasis (haemostasis) is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel (the opposite of hemostasis is hemorrhage). It is the first stage of wound healing. This involves coagulation, blood changing from a liquid to a gel.

Intact blood vessels are central to moderating blood's tendency to form clots. The endothelial cells of intact vessels prevent blood clotting with a heparin-like molecule and thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin.

When endothelial injury occurs, the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von Willebrand factor which initiate the maintenance of hemostasis after injury.

Hemostasis has three major steps:

1) Vasoconstriction
2) Temporary blockage of a break by a platelet plug
3) Blood coagulation, or formation of a fibrin clot.

Coagulation

Coagulation (**clotting**) is the process by which blood changes from a liquid to a gel, forming a clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair.

The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin.

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Coagulation (Bleeding) Disorders

Coagulation disorders **(bleeding disorder, Coagulopathy)** is a condition in which the blood's ability to coagulate (form clots) is impaired. This condition can cause a tendency toward prolonged or excessive bleeding (bleeding diathesis or bleeding disorder), which may occur spontaneously or following an injury or medical and dental procedures.

The most commonly known coagulation disorder is ***hemophilia***, a condition in which patients bleed for long periods of time before clotting. There are other coagulation disorders with a variety of causes.

Types of Coagulation disorders:

Coagulation disorders arise from different causes and produce different complications. Some common coagulation disorders are:

1. ***Hemophilia A (Factor VIII deficiency):*** an inherited coagulation disorders. This genetic disorder is carried by females but most often affects males.

2. ***Christmas disease (Hemophilia B or Factor IX deficiency***): is less common than hemophilia A with similar in symptoms.

3. ***Disseminated intravascular coagulation disorder (consumption coagulopathy):*** occurs as a result of other diseases. This type of coagulation disorder occur because of malfunction of clotting factors that cause to clot in small vessels throughout the body. This action leads to a lack of clotting factors and platelet at a site of injury that requires clotting.

4. ***Thrombocytopenia*** It may be acquired or congenital. It represents a defective or decreased production of platelets.

5. Von Willebrand's disease is a hereditary disorder with ***prolonged bleeding time*** due to a clotting factors (Von Willebrand's factor) deficiency and impaired platelet function.

6. Hypoprothrombinemia: This disorder is a deficiency in prothrombin, or factor II, a glycoprotein formed and stored in the liver. Prothrombin, under the right conditions, is converted to thrombin, which activates fibrin and begins the process of coagulation.

7. Hemophilia C (Factor XI deficiency) is the second most common bleeding disorder among women.

8. Factor VII is also called ***serum prothrombin conversion accelerator (SPCA) deficiency.***

Normal blood clotting involves blood components called platelets and as many as 20 different plasma proteins. These are known as blood clotting or coagulation factors. These factors interact with other chemicals to form a substance called fibrin that stops bleeding. Problems can occur when certain factors are low or missing.

Some bleeding disorders are present at birth and are passed through families (inherited). Others develop from:

- Illnesses such as vitamin K deficiency or severe liver disease
- Treatments such as the use of drugs to stop blood clots (anticoagulants) or the long-term use of antibiotics

Bleeding disorders can also result from a problem with the number or function of the platelets that promote blood clotting. These disorders can also be either inherited or develop later (acquired).

Signs and symptoms:

Symptoms may include any of the following:

- Bruising easily
- Heavy bleeding may cause damage to joints, muscles, or internal organs.
- Heavy menstrual bleeding
- Nose bleeds that do not stop easily
- Excessive bleeding with surgical procedures
- Umbilical cord bleeding after birth

Mechanism:

The normal clotting process depends on the interplay of various proteins in the blood. Coagulopathy may be caused by reduced levels or absence of blood-clotting proteins, known as clotting factors or coagulation factors.

Genetic disorders, such as hemophilia and Von Willebrand's disease, can result from a reduction in clotting factors.

Anticoagulants such as warfarin will also prevent clots from forming properly. Coagulopathy may also occur as a result of dysfunction or reduced levels of platelets.

Diagnosis:

Tests that may be done include:

- Complete blood count (CBC)
- Bleeding time
- Partial thromboplastin time (PTT)
- Platelet aggregation test
- Prothrombin time (PT) test

Treatment:

Treatment depends on the type of disorder. It may include:

- Clotting factor replacement
- Fresh frozen plasma transfusion
- Platelet transfusion
- Other treatments

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Platelet disorder

Platelets are cell fragments that function in the clotting system. *Thrombopoietin* (THPO) is a ***glycoprotein hormone*** produced by the liver and kidney, helps control the number of circulating platelets by stimulating the bone marrow to produce megakaryocytes, which in turn shed platelets from their cytoplasm.

Platelets circulate for **7-10** days. About one third are always transiently sequestered in the spleen.

The platelet count is normally **140,000-440,000/µL**. However, the count can vary slightly ***according to menstrual cycle phase***, ***decrease*** during near-term pregnancy (gestational thrombocytopenia), and ***increase*** in response to inflammatory cytokines.

Platelets are eventually destroyed by apoptosis, a process independent of the spleen.

Platelet disorders

Platelet disorders are include:

- ***Essential Thrombocythemia & Reactive Thrombocytosis***
- ***Thrombocytopenia***
- ***Platelet Dysfunction***

Any of these conditions, even those in which platelets are increased, ***may cause defective formation of hemostatic plugs and bleeding.***

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The risk of bleeding is inversely proportional to the platelet count and platelet function (Table 1). When platelet function is reduced (as a result of uremia or aspirin use) the risk of bleeding increases.

Table 1: Platelet Count and Bleeding Risk

Platelet Count	Risk of Bleeding*
≥ 50,000/µL	Minimal
20,000–50,000/µL	Minor bleeding after trauma
< 20,000/µL	Spontaneous bleeding
< 5000/µL	Severe, possibly life-threatening spontaneous bleeding

Thrombocythemia & Thrombocytosis

Essential thrombocythemia is a myeloproliferative disorder involving overproduction of platelets because of a clonal abnormality of a hematopoietic stem cell.

Reactive thrombocytosis is platelet overproduction in response to another disorder. There are many causes, including acute infection, chronic inflammatory disorders (eg, Rheumatoid Arthritis RA, inflammatory bowel disease, Tuberculosis TB, sarcoidosis), iron deficiency, and certain cancers.

Thrombocytopenia

Causes of thrombocytopenia can be ***classified by mechanism*** and include:

1- Decreased platelet production
2- Increased splenic sequestration of platelets with normal platelet survival (splenomegaly)

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3- Increased platelet destruction or consumption (both immunologic and non-immunologic causes)
4- Dilution of platelets
5- A combination of these mechanisms.

A large number of drugs may cause thrombocytopenia, typically by triggering immunologic destruction.

Overall, ***the most common specific causes of thrombocytopenia*** include
1- Gestational thrombocytopenia
2- Drug-induced thrombocytopenia due to immune-mediated platelet destruction (heparin, trimethoprim)
3- Drug-induced thrombocytopenia due to dose-dependent bone marrow suppression (eg, chemotherapeutic agents)
4- Thrombocytopenia accompanying systemic infection
5- Immune thrombocytopenia

In patients with thrombocytopenia, ***the peripheral smear may suggest the cause*** (Table 2).

1- ***If the smear shows abnormalities other than thrombocytopenia***, such as nucleated RBCs or abnormal or immature WBCs, bone marrow aspiration is indicated. Bone marrow aspiration reveals the number and appearance of megakaryocytes and is the definitive test for many disorders causing bone marrow failure.

However, ***normal number and appearance of megakaryocytes does not always indicate normal platelet production***. For example, in patients with immune thrombocytopenia, platelet production may be decreased despite the normal appearance and increased number of megakaryocytes.

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2- ***If the bone marrow is normal but the spleen is enlarged***, increased splenic sequestration is the likely cause of thrombocytopenia.

3- ***If the bone marrow is normal and the spleen is not enlarged***, excess platelet destruction is the likely cause. Measurement of antiplatelet antibodies is not clinically useful.

Table 2: Peripheral Blood Findings in Thrombocytopenic Disorders

Findings	**Conditions**
Normal RBCs and WBCs	Drug-induced thrombocytopenia Gestational thrombocytopenia HIV-related thrombocytopenia Immune thrombocytopenia Posttransfusion purpura
RBC fragmentation (schistocytes)	Metastatic cancer DIC Preeclampsia with DIC Thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome
WBC abnormalities	Hypersegmented polymorphonuclear leukocytes in megaloblastic anemias Immature cells or increased mature lymphocytes in leukemia Markedly diminished granulocytes in aplastic anemia
Frequent giant platelets (approaching the size of RBCs)	Bernard-Soulier syndrome Disorders related to the myosin, heavy chain 9, non-muscle gene (*MYH9*) Other congenital thrombocytopenias
RBC abnormalities, nucleated RBCs, and immature granulocytes	Myelodysplasia
DIC = disseminated intravascular coagulation.	

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Platelet dysfunction

Platelet dysfunction may stem from an intrinsic platelet defect or from an extrinsic factor that alters the function of normal platelets. Dysfunction may be hereditary or acquired.

Hereditary disorders of platelet function consist of von Willebrand disease, the most common hereditary hemorrhagic disease, and hereditary intrinsic platelet disorders, which are much less common.

Acquired disorders of platelet function are commonly due to diseases (eg, renal failure) as well as to aspirin and other drugs.

Diagnosis

- Clinical presentation of petechiae and mucosal bleeding
- Complete blood count CBC with platelets, coagulation studies, peripheral blood smear
- Sometimes bone marrow aspiration
- Sometimes von Willebrand antigen and factor activity studies

Platelet disorders are suspected in patients with petechiae and mucosal bleeding.

Excessive platelets and thrombocytopenia are diagnosed based on the platelet count; coagulation studies are normal unless there is a simultaneous coagulopathy. In patients with a normal CBC, platelet count, INR, and PTT, platelet dysfunction is suspected.

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Suspected platelet dysfunction

In patients with platelet dysfunction, ***a drug cause*** is suspected if symptoms began only after the patient started taking a potentially causative drug (eg clopidogrel, ticagrelor). Platelet dysfunction caused by drugs may be severe, but specialized tests are rarely needed.

A hereditary cause is suspected if there is a lifelong history of easy bruising; bleeding after tooth extractions, surgery, childbirth, or circumcision; or heavy menstruation. In some patients, platelet aggregation tests may identify a defect in how the platelet responds to various platelet agonists (adenosine diphosphate [ADP], collagen, thrombin) and thereby demonstrate the type of platelet defect.

Platelet dysfunction caused by ***systemic disorders*** is typically mild and of minor clinical importance. In these patients, the causative systemic disorder is the clinical concern, and hematologic tests are unnecessary.

Treatment

- Stopping drugs that impair platelet function
- Rarely platelet transfusions

Blood components

Non cellular blood components

Fresh Frozen Plasma FFP

 Produced from **whole blood** or **apheresis** collections.

 FFP must be frozen within 6-8 hours of collection.

 FFP is stored at −18°C or colder for 1 year or at –65°C for 7 years, with FDA approval.

 FFP will contain the maximum levels of **both labile (FVIII & FV) and stable(other factors) clotting factors**, about 1international unit (IU) per mL.

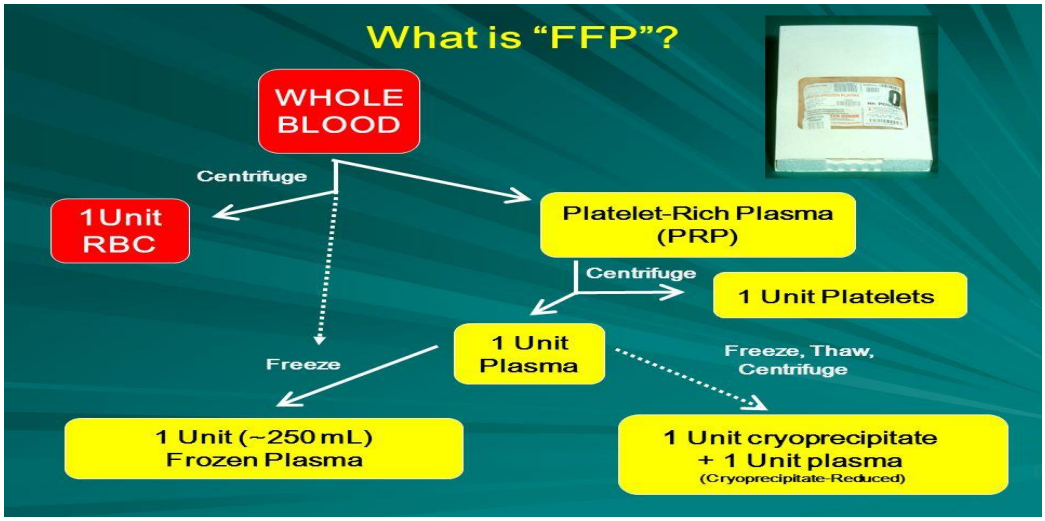
 FFP are thawed at temperatures between 30°C and 37°C or in an FDA-approved microwave device or water bath.

 Once thawing is complete, the product may be stored at 1°C to 6°C for up to 5 days.

 A single unit of FFP from whole blood collection, should contain 150 to 250 mL of plasma

 FFP prepared from apheresis collections may contain from 400 to 600 mL.

 The use of FFP is indicated in patients who are actively bleeding and have multiple clotting factor deficiencies.



Cryoprecipitate

According to the temperature of thawing, cryoprecipitate is divided into two types:

First: Cryoprecipitate-reduced plasma

 Cryoprecipitate-reduced plasma, also known as **cryosupernatant** or **cryoreduced-plasma**, is the remaining supernatant after the removal of cryoprecipitate from FFP, which is subsequently refrozen. This product is deficient in factor VIII (antihaemophilia factor), factor XIII (fibrin stabilizing factor), Von Willebrand factor (vWF) , fibrinogen (factor I), and fibronectin.

 Prepared from FFP after thawing at **30-37 °C** and centrifugation to prepare cryoprecipitate.

 The resulting cryo-poor plasma must be refrozen within 24 hours and stored at –18°C or colder for 1 year from the time of collection.

 This product contains **albumin**; **factors II (prothrombin) , V (labile factor), VII (stable) , IX (Christmas factor) , X (Stuart-power factor) , XI (plasma thromboplastin antecedent**) .

 This product is most often used for transfusion or plasma exchange in patients with **TTP** (thrombotic thrombocytopenic purpura).

Second: Cryoprecipitate Antihemophilic Factor

 Cryoprecipitate is the cold-precipitated concentration of factor VIII (the antihemophilic factor (AHF)), **FXIII** and von Willebrand factor (**VWF**).

 It is prepared from FFP thawed slowly between **1°C and 6°C**.

 The product contains most of the factor VIII and part of the fibrinogen from the original plasma.

 Cryoprecipitate has a shelf-life of 12 months in the frozen.

 Cryoprecipitate is indicated in the treatment of factor XIII deficiency, as a source of fibrinogen for hypofibrinogenemia, and as a secondary line of treatment for classic hemophilia (hemophilia A) and von Willebrand disease.

Plasma Derivatives

 Plasma derivatives are products manufactured from human plasma by chromatographic and other fractionation techniques. Some proteins can also be purified from serum. A number of plasma proteins (or their modified forms) are also produced using recombinant DNA technologies, in which the

protein is produced from an expression system into which a gene has been introduced and also monoclonal antibody purification.

 Source plasma is defined as plasma collected by plasmapheresis and intended for further manufacture into plasma derivatives.

 Recovered plasma is plasma recovered from whole blood donations.

 The plasma is frozen when sent to the manufacturer.

 The manufacturing process usually begins with a separation of the cryoprecipitate from the plasma.

 The cryoprecipitate is then used to produce factor VIII concentrate.

 The residual plasma is separated into various proteins by manipulating the pH, alcohol content, and temperature and viral inactivated by any of several methods, including heat, solvent detergent treatment, and nanofiltration.

 Most derivative plasma is also further tested for hepatitis A and parvovirus.

- **Some examples of derivatives of plasma**

 Activated Factor VII (Factor VIIa); indicated in patients with congenital factor VII deficiency. It has also been used in other situations such as trauma, massive transfusion, and liver transplantation, where bleeding has proved difficult to control and the patient's life is threatened.

 Factor VIII Concentrates (FVIII); product of choice in hemophilia A patient.

 Factor IX Concentrates; for hemophilia B patients**.**

 Factor XIII Concentrates; for Factor XIII deficiency

 Immune Serum Globulin; indicated for patients with immunodeficiency diseases (i.e., severe combined immunodeficiency) and for providing passive antibody prophylaxis against hepatitis and herpes.

Second: Phlebotomy

 Modern blood collection packs are designed to hold 450ml ± 45 ml of blood, mixed with 63 ml of citrate-phosphate-dextrose-adenine(CPDA) anticoagulant.

 Blood is to be collected only by trained personal working under the direction of a qualified physician.

 Blood collection must be by an aseptic method, using a sterile, closed system.

 Donation of whole blood should, ideally, not last more than 10 minutes.

 A completely efficient method of sealing the tube is obligatory. The organization should be such to minimize the possibility of errors in labeling blood containers and blood samples.

Care of the donor after phlebotomy:

After removing the needle from the vein, the phlebotomist should:

1. Apply firm pressure with sterile gauze over the point of entry of the needle into vein.

2. The donor has to remain reclining on bed or in donor chair for a few minutes under observation by staff.

3. Allow the donor to sit up under observation until his/her condition appears satisfactory.

4. The medical director may wish to include some or all of the following instructions:

a. Eat and drink something before leaving the donor site.

b. Do not leave until released by the staff.

c. Drink more fluids than usual in the next 4 hours.

d. Do not smoke for 30 minutes.

e. If there is bleeding from the phlebotomy site, raise arm and apply pressure to the site.

f. If fainting or dizziness occurs, either lie down or sit with the head between the knees.

g. Donors who work in certain occupations (e.g., construction workers, operators of machinery or persons working at heights) should be cautioned that faintness may occur if they return to work immediately after giving blood.

h. Remove bandage after a few hours.

5. Thank the donor and encourage repeat donation after the proper interval.

Third: Adverse Donor Reactions

Most donors tolerate giving blood very well but adverse reactions occur occasionally .

1. Mild Reactions

Reactions in this category include one or more of the following:

fainting, *nausea*, *vomiting*, hyperventilation, or *muscle spasm*.

The following instructions apply for the donor fainting:

1. Remove the tourniquet and withdraw needle.

2. Place cold compresses on the donor's forehead.

3. Raise the donor's legs above the level of the head.

4. Loosen tight clothing and secure airway.

5. Monitor vital signs

2. Moderate Reactions

A moderate reaction can include any of the reactions listed above in addition to loss of consciousness.

The following instructions apply:

1. Check vital signs frequently.

2. Administer oxygen.

3. Severe Reactions

A donor having convulsions defines a severe reaction.

The following should be followed by the donor room staff:

1. Call for help immediately; notify blood bank physician

2. Try to prevent the donor from injuring self or others.

3. Ensure an adequate airway.

Hematoma

A hematoma is a localized collection of blood under the skin, resulting in a bluish discoloration. It is caused by the needle going through the vein, with subsequent leakage of blood into the tissue. If a hematoma develops, the following instructions apply:

1. Remove the tourniquet and needle from donor's arm

2. Apply pressure with sterile gauze pads for 7 to 10 minutes, with the donor raising his or her arm above the heart

3. Apply ice to the area for 5 minutes

Polycythemia and therapeutic phlebotomy

Many medical conditions require blood donation as part of their management (e.g. hereditary hemochromatosis & secondary polycythemia) Blood collected from this individual may be unsuitable for transfusion because of the patient/donor's underlying medical condition.

By tradition, therapeutic phlebotomy services have been located in an area separate from volunteer blood donation and have required a medical prescription from an attending physician.