TRANSFUSION MEDICINE

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Objectives

1. Hemopoiesis.
2. Anemia, Types and Related Disorders.
3. Granulopoiesis and White Blood Cell Disorders.
4. Hematological Malignancies.
5. Hemostasis.
6. **Transfusion Medicine.**

BLOOD TRANSFUSION

Transfer of blood or blood components from a donor to a recipient.

The aim of blood transfusion is to transfer blood components safely

- **Principles:**
- Blood donation should always be **voluntary**.
- **Never** give transfusion unnecessarily.
- Blood transfusion should follow components **policy**.
- **Donor** must be fit and healthy.
- It should **not harm** the donor.
- It should **not transmit** any disease to the recipient.

Blood Donation

- Should be voluntary and safety measure for the blood donor is a must, including;
- Age 17–70 years (maximum 60 at first donation)
- Weight above 50 kg
- Haemoglobin >13 g/dL for men, >12 g/dL for women
- Maximum donation; 2-3 times/year for females and 3-4/year for males.
- Pregnant and lactating women excluded because of high iron requirements; donation deferred for 9 months post pregnancy.
- History of major surgery (defer 6 months).

❑ Exclusion of those with:

- Cardiovascular disease and significant respiratory disorders
- Epilepsy and other CNS disorders
- Gastrointestinal disorders with impaired absorption
- Insulin-dependent diabetes (IDDM) and Chronic renal disease.
- History of blood diseases such as leukemia, lymphoma, thalassemia major, sickle cell anemia, polycythemia and abnormal bleeding tendency should be deferred from donation.
- Delayed faint would be dangerous; Exclude; donor returning to occupations such as driving bus, plane or train, heavy machine or crane operator .. etc.
- Defer for 6 months after major surgery, body piercing or tattoo, after acupuncture
- Defer for 2 months after vaccinations, e.g. measles, mumps

❖ *Tests of the Blood Unit*

- Blood group, Rh status
- Human immunodeficiency virus (HIV) 1 and 2
- Hepatitis B virus (HBV) and C virus (HCV).
- Syphilis test, although *Treponema palladium* not survive in storage blood and not cause infection, but Ab may confuse diagnostic purposes later on.
- Human T-cell leukemia viruses (HTLV)
- Cytomegalovirus (CMV) - for immunosuppressed recipients
- Atypical antibodies.

❖Anticoagulants;

1- ACD (Acid citrate dextrose); used only in automated plasmapheresis.

2- CPD (Citrate phosphate dextrose).

3- CPD-A (Plus Adenine); mostly used now, high shelf life of blood.

Blood donation is taken by an aseptic technique into plastic bags designed to hold 450 ml ± 45 ml of blood, mixed with 63 ml of anticoagulant (ratio 1:7).

The citrate anticoagulants to the blood by combining with the blood calcium

❖ Storage Changes of Blood:

1- Depletion of **ATP**: progressive during storage of red cells, leading to changes in red cell shape (discs to spheres, loss of membrane lipid and increased rigidity).

2- Reduction in red cell **2,3-DPG**: lead to left shift of O2 dissociation curve Thus, less oxygen is given up to the tissues, until its level restored to normal approximately 24 hours after transfusion.

3- **Electrolyte** changes: as a result of equilibration of Na and K levels across the cell membrane once active transport has been halted by the cooling of blood to 4°C. There is rapid restoration of electrolyte levels after transfusion.

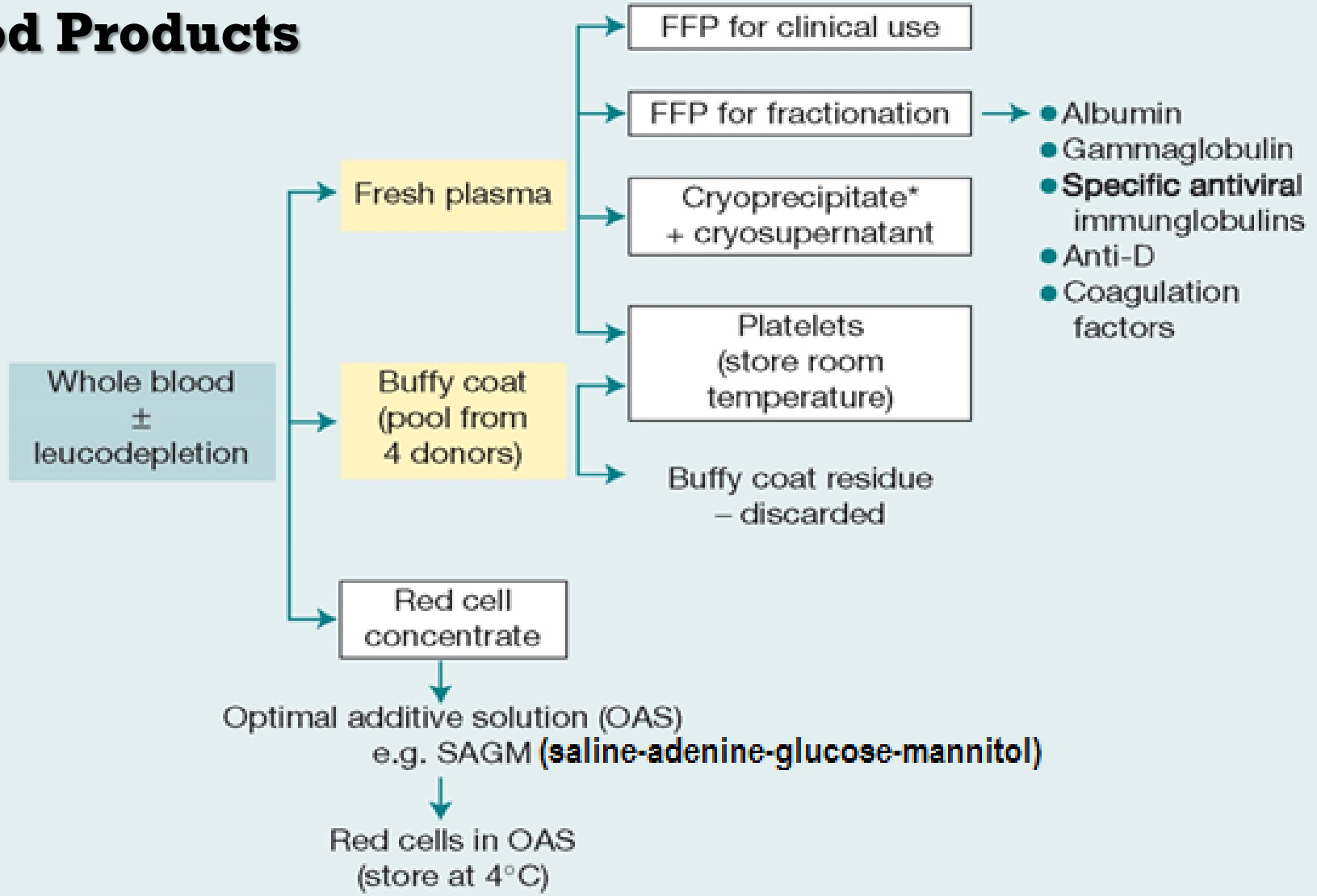
4- The **pH** of blood: decreases rapidly with storage, but most recipients can handle the acid load during transfusion without ill effect.

After transfusion of stored red cells, a proportion are removed from the circulation within the first 24 hours. The remainders appear to survive normally.

With increased length of storage, a greater proportion of red cells are removed within the first 24 hours.

The destruction at 24 hours of more than 30% of the total number of cells transfused is considered unacceptable.

Blood Products



Red Cell Concentrate

Red blood cells are indicated for the patient with increasing oxygen demands.

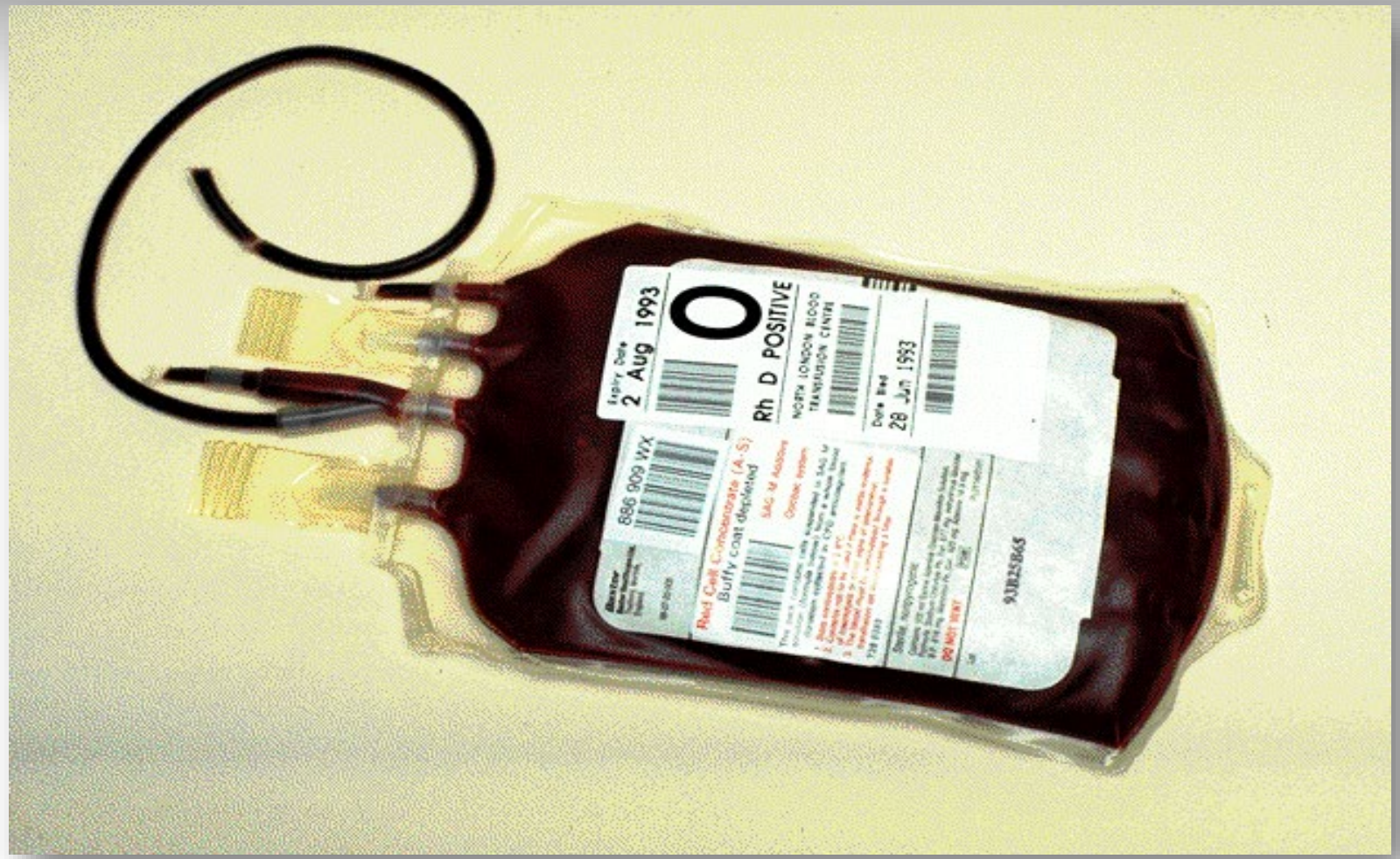
Normally, a rise in hemoglobin concentration of about 1.0 gm/dL is expected with every unit of red cells transfused into an adult.

Generally, for a patient with a hemoglobin of;

1- >10.0 g/dL, it is likely inappropriate to give the RBC concentrate.

2- 7.0-10.0 g/dL, it is likely to be appropriate to give the RBC concentrate if there are signs or symptoms of impaired O2 delivery.

3- <7.0 g/dL, it is likely to be appropriate to give the RBC concentrate.



Red cell concentrate

Blood Components

1- Platelet Concentrates:

- Blood donations should be kept at room temperature after collection and platelets separated as soon as possible.

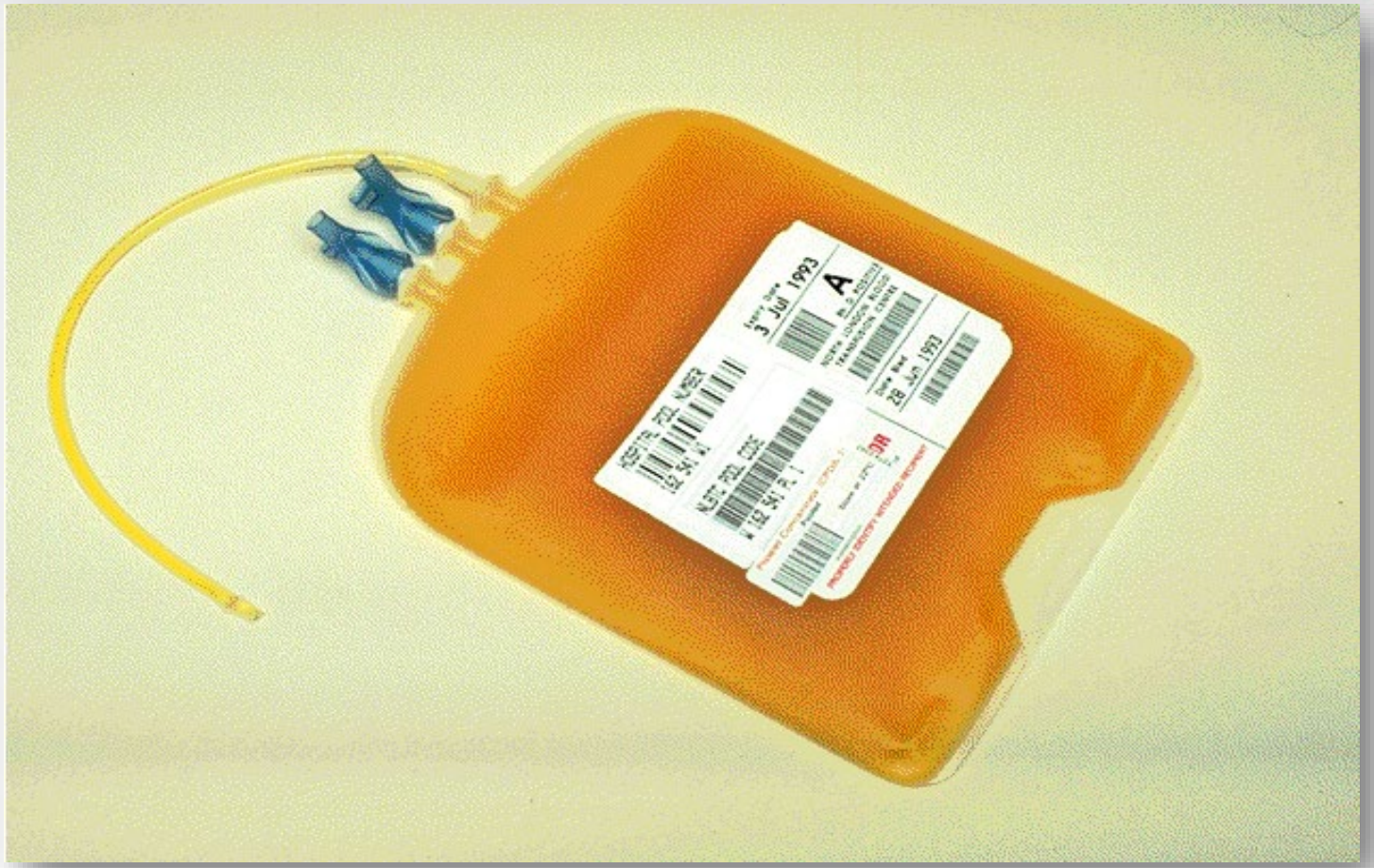
- Each single-donor platelet concentrate should contain a minimum of 5.5 $x10^{10}$ resuspended in 50 ml of plasma. 5-6 single-unit concentrates may be obtained from one donor by an aphaeresis procedure lasting approximately 90 minutes

- Post-transfusion survival is poor in platelets stored in 4°C as well as hemostatic effect is short-lived

- Storage at 20-22°C is therefore preferred and given for actively bleeding patients.

- Platelets prepared in conventional blood packs have a shelf-life 5 days, now extended with new plastics which allow the diffusion of oxygen.

Platelet concentrate;
Each single-donor contain a minimum of 5.5 x10^{10} resuspended in 50 ml of plasma and 5-6 single-unit may be obtained from one donor by an aphaeresis procedure lasting approximately 90 min.



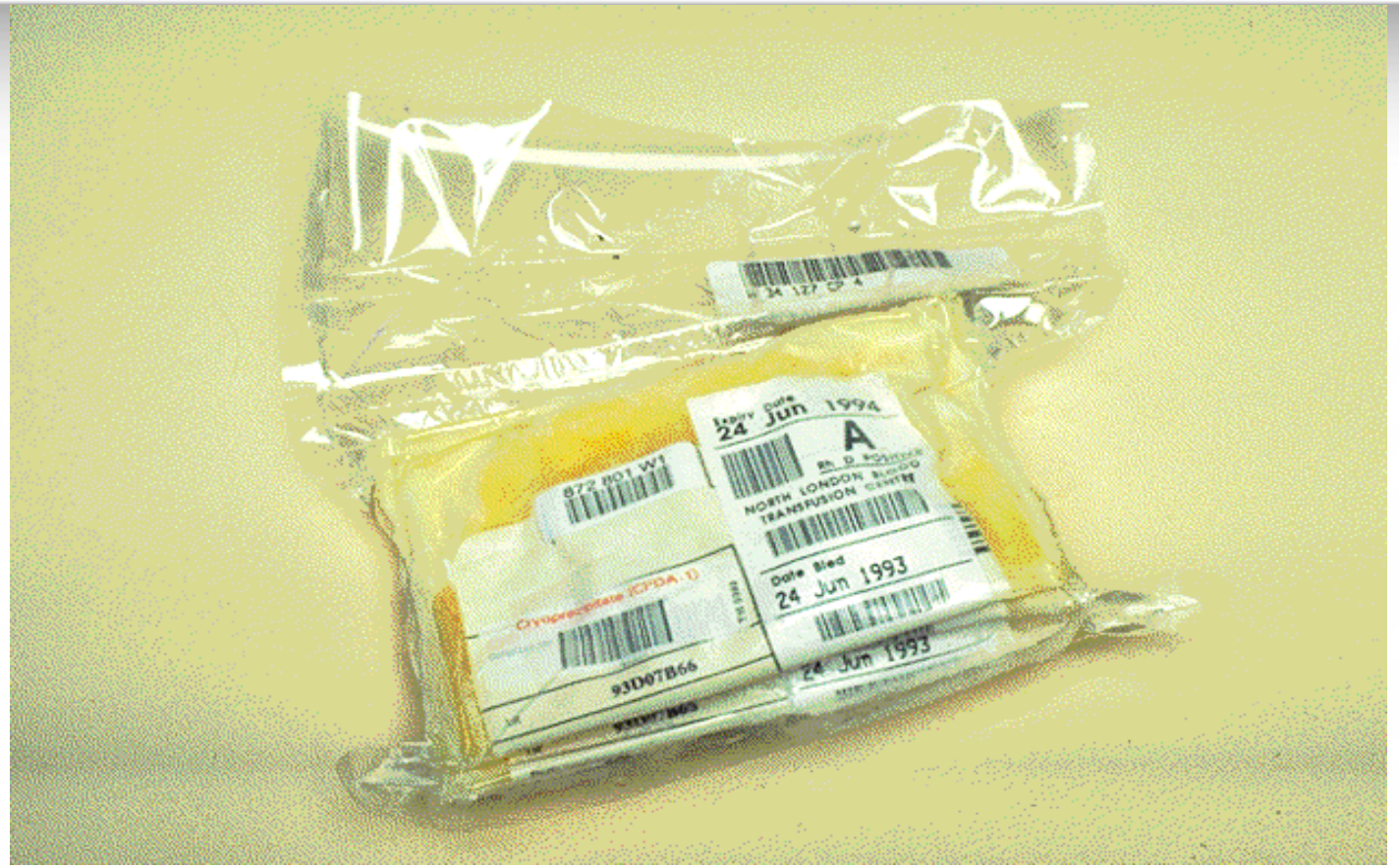
2- Granulocyte concentrates:

Extremely labile, must be separated from whole blood immediately after collection and transfused within hours of preparation and heavily contaminated with red cells.

Granulocyte concentrates prepared by aphaeresis are the satisfactory means of achieving a therapeutic dose for adult neutropenic patients

3- Cryoprecipitate:

Prepared from blood within 6 hours of collection. Plasma is separated, frozen, and allowed to thaw (classically at 4°C. overnight). The factor VIII;c and fibrinogen are left as a precipitate, which is then refrozen with 15 ml of plasma, and stored at -30°C or below for up to 12 months. Each unit should contain: FVIII:c, fibrinogen, together with fibronectin, von Willebrand's Factor and FXIII



Cryoprecipitate; approximately 15 ml of plasma contains: a minimum of 80 iu of factor VIII:c, approximately 150 mg of fibrinogen, together with fibronectin, von Willebrand's Factor and FXIII

4- Fresh frozen plasma (FFP):

This plasma has been separated from red cells within 6 hours of blood collection, and immediately frozen. A longer delay leads to reduction of the labile factors V and VIII:c. Fresh frozen plasma contains all coagulation factors, and should be stored at -30°C or below for up to 12 months.

5- Single-donor plasma; refers to plasma separated from whole blood at any time after 18 hours following donation.

6- Cryoprecipitate poor plasma (cryosupernatant): used for the remaining plasma after the removal of cryoprecipitate.

Both 5 and 6 components lack the labile coagulation factors but may be used interchangeably with FFP in most instances. They should be stored refrigerated for 5 days, or frozen at -30°C or below for up to 12 months.

Fresh frozen plasma; plasma has been separated from red cells within 6 hours of blood collection, and immediately frozen, contains all coagulation factors, and should be stored at -30°C or below up to 12m.

Red Cell Antigens and Blood Group Antibodies;

- About 400 RBC group antigens are described
- ABO and Rh groups are the most important groups clinically
- Importance of the blood group antigen in the blood transfusion is that persons who lack antigen may produce Abs reacting with that Ag which may lead transfusion reaction. i.e; recipient who transfused with a different blood group (Ag), leads to destruction of the transfused red cells by the recipient Ab.

Blood Group Antibodies (Abs);

- **Naturally** occurring Abs;
- Occur in plasma of subjects lack the corresponding Ag who not transfusion or been pregnant.
- It is of IgM of cold type which react optimally at 4°C, but also at 37°C.
- **Immune** Abs;
- Develop in response to Ags of red cell transfusion or transplacental during pregnancy
- It is of IgG, although some IgM may occur, usually of warm type which react optimally at 37°C. IgG is the only Ab can pass through placenta.

ABO System

- The ABO gene is located on chromosome 9
- Has three alleles, A, B and O, leading to 6 genotypes OO, AA, AO, AA, BB, BO and AB
- Only 4 phenotypes can be recognized serologically, because of the absence of anti-O
- The individual who lack the specific Ag develops natural Ab against corresponding to that Ag
- This blood group system is considered the most important, because of the high risk of a severe hemolytic transfusion reaction if ABO incompatible blood is transfused

Phenotype	Genotype	Antigens	Naturally occurring antibodies
O	OO	O	Anti-A Anti-B and Ani-A,B
A	AA or AO	A	Anti-B
B	BB or BO	B	Anti-A
AB	AB	AB	None

Rh System

- Previously known as Rhesus.
- Rh genes are located on chromosome 1
- One of the most complex blood groups known in humans and constitute of about 49 Ags are known and D, C, E, c, and e, are the most significant
- Primary importance, being the main cause of hemolytic disease of the newborn (HDN). It is unlike ABO system, as its Abs of immune type and rarely have naturally occurring ones
- Anti-D is most Ab of this system responsible for the clinical problem and usually Rh D+ and Rh D is mostly used as identification of Rh^{+} and Rh^{-} for the clinical purposes.

Dangerous 'Universal' Donors

Group O red cells can be given to A, B or AB recipients.

Group O donors were formerly, inappropriately called **'universal donors'**. Group O donors have anti-A, -B and -A,B in their plasma, which will react with the recipient's A or B cells.

Normally, if group A, B or AB recipients are transfused with a relatively small number of group O units of whole blood, the anti-A or -B that is transfused will be **diluted** out and neutralized by the plasma of the adult recipient.

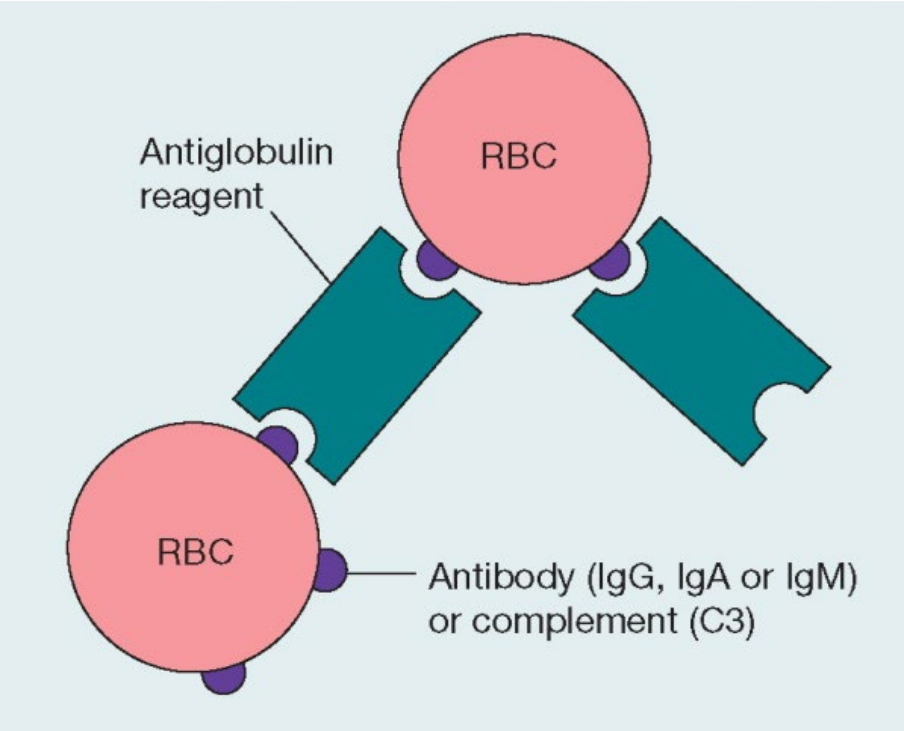
However, if the transfused units **contain potent immune hemolytic antibodies**, this neutralization and dilution effect may be insufficient and the antibodies may cause a marked destruction of the A or B red cells of the recipient, leading to a severe acute hemolytic transfusion reaction **(HTR)**.

For this reason, the practice of transfusing group O blood to non-O recipients should be strongly discouraged.

Blood Grouping;

The principle of the ABO and Rh blood grouping system based on agglutination reaction.

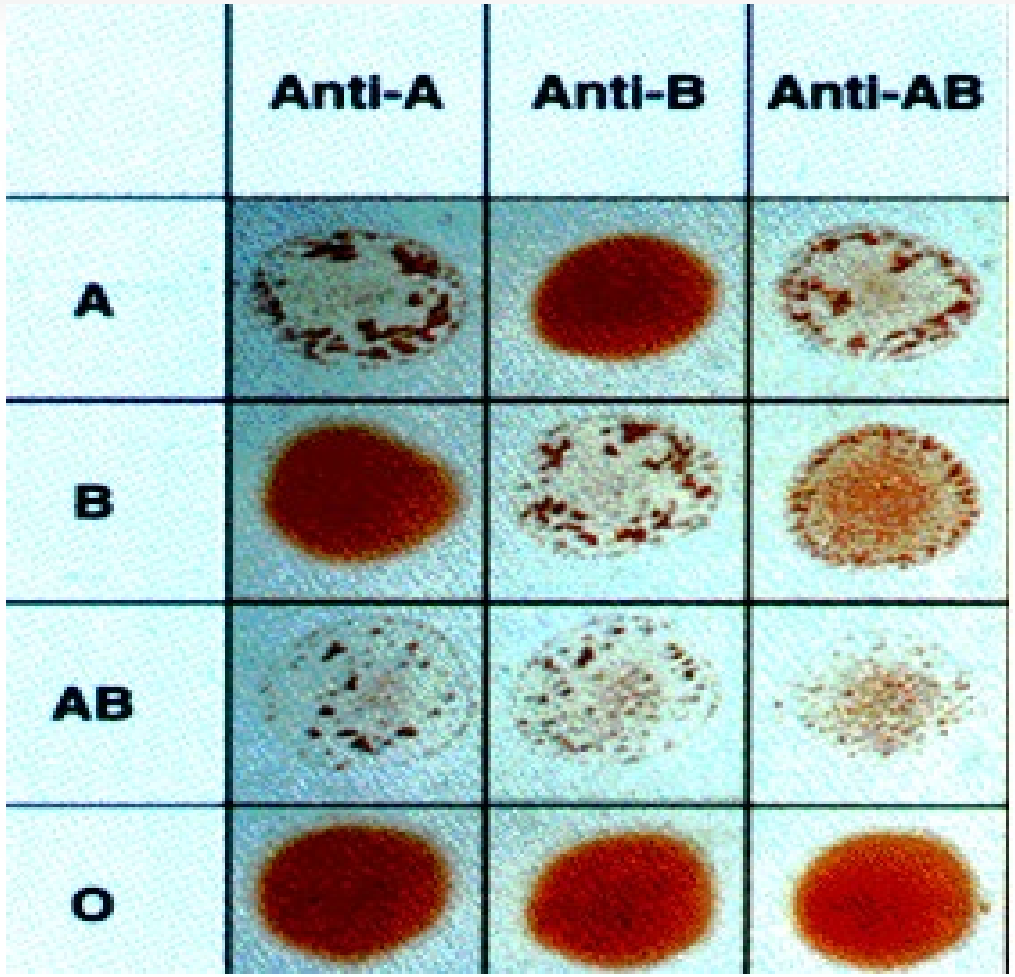
- When red blood cells carrying one or both, the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping.



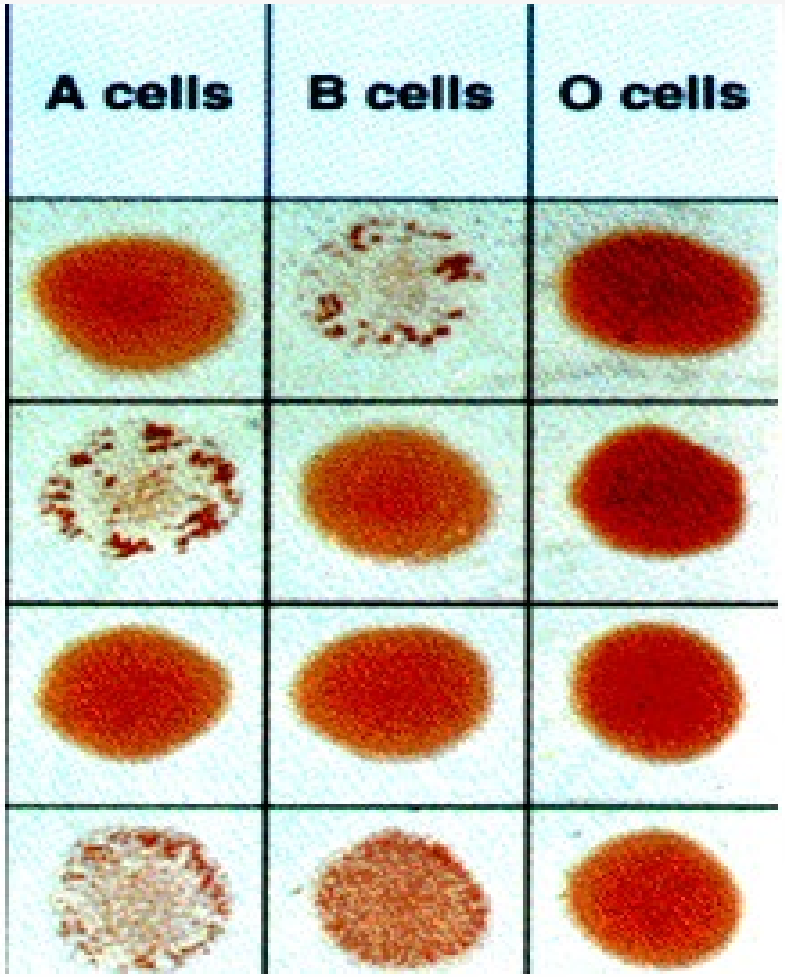
The Abs sera used for the main ABO and Rh grouping are;
Anti-A, Anti-B and Anti-D (for Rh system)

Antigens on the Surface of RBC	Antibodies in the Serum	ABO Blood Group	Genotype
A	Anti-B	A	AA or AO
B	Anti-A	B	BB or BO
A and B	Neither Anti-A nor Anti-B	AB	AB
Neither A nor B	Anti-A, Anti-B and Anti-A,B	O	OO

Cells



Serum



Cross-matching;

- ABO and Rh grouping
- Alloantibodies (Abs in the serum) for the recipient need to be determined, by indirect antiglobulin (Coombs') test.
- Cross-matched with the appropriate blood;

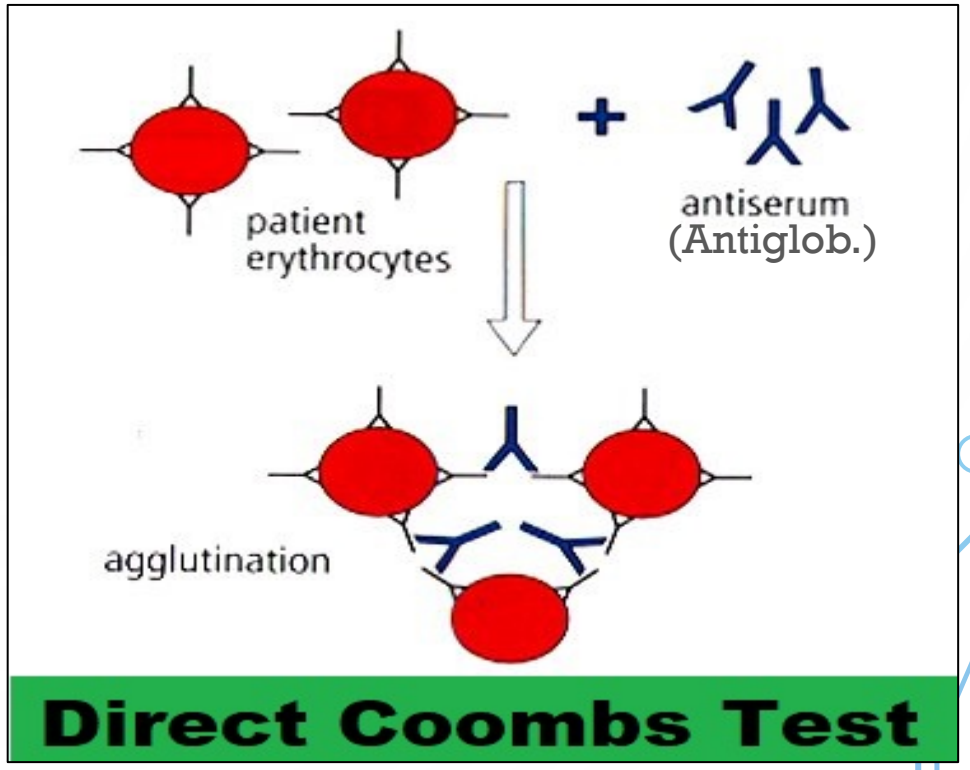
Donor cells tested against recipient serum and agglutination detected visually or microscopically after mixing and incubation at the appropriate temperature (37°C).

Antiglobulin test (Coombs' Test);

Using antihuman globulin (AHG) sera which is produced by the injection of animals with human Ig, which then purified.

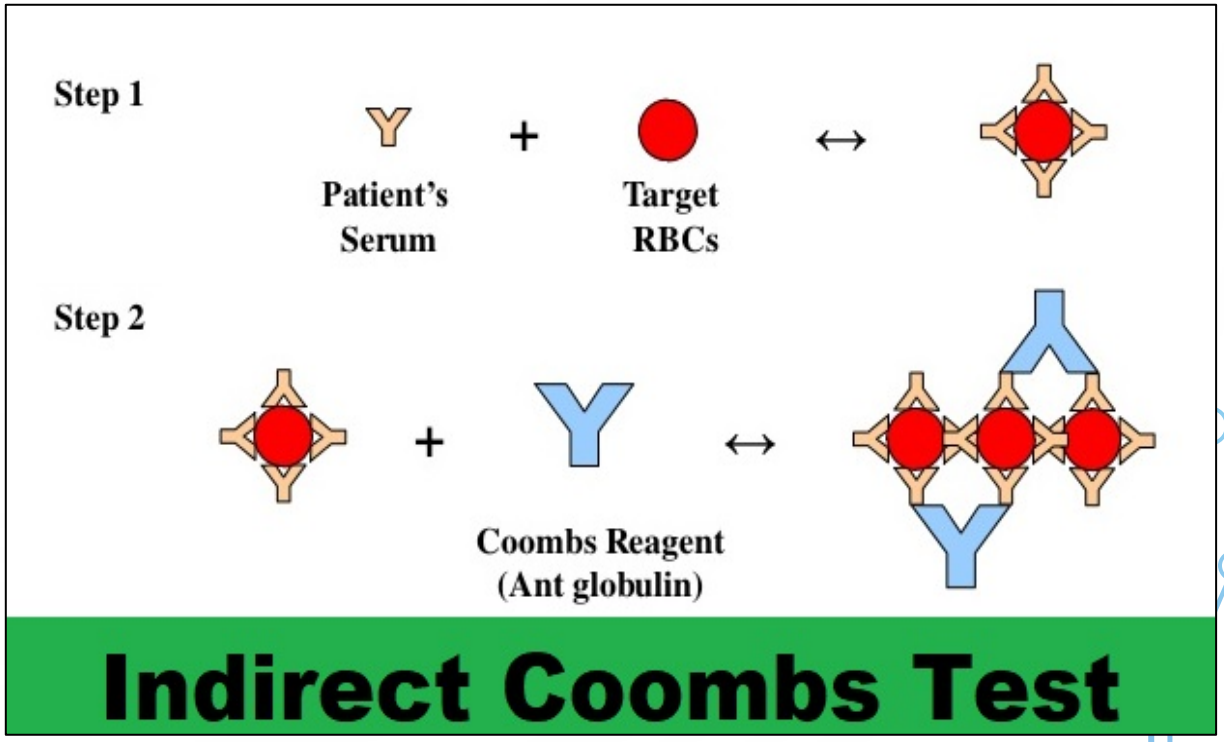
- **Direct**, to detect the Ab or complement on the *in vivo* sensitized RBC surface after washing of the cells to make easier for detection of Ag-Ab complex on the cells

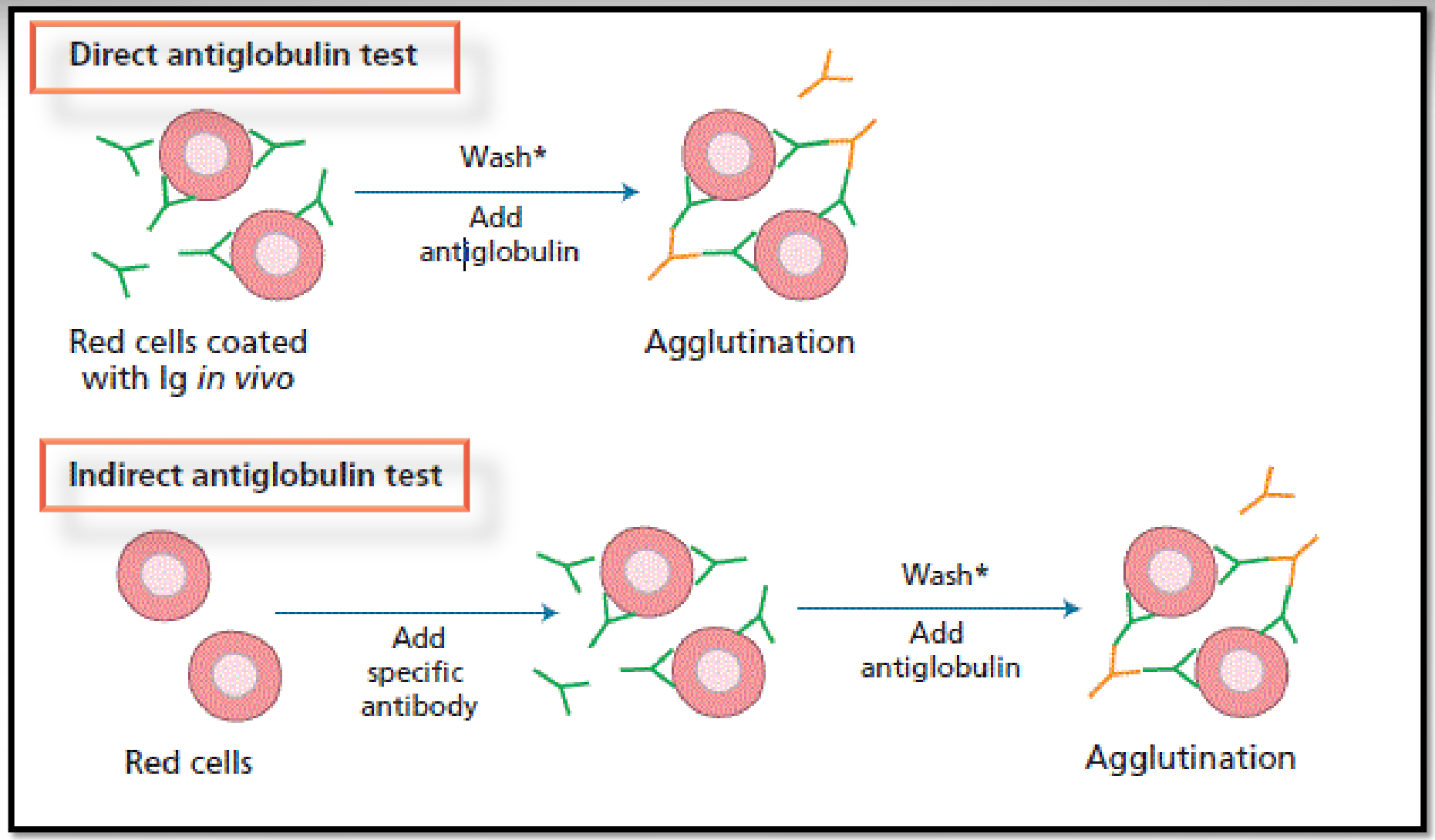
Positive in cases of hemolytic disease of newborn, autoimmune hemolytic anemia and transfusion reaction.



❖ **Indirect,** to detect Ab in the patient's serum by the *in vitro* sensitized RBC surface by the patient's serum, and it's of 2 steps;

- The first step performed to make the Ag-Ab complex between the patient's serum and known cells
- Second step after washing by adding the AHG
- Used for the Ab screening for transfusion and in pregnant with blood group (mainly Rh, because of IgG type which can pass placenta) in noncompatible husbands




Direct antiglobulin test
Wash*
Add
antiglobulin
Red cells coated
with Ig *in vivo*
Agglutination
Indirect antiglobulin test
Add
specific
antibody
Wash*
Add
antiglobulin
Red cells
Agglutination

Complications of Blood Transfusion

1- Immunological complications

2- Non-immunological complications

Complications;

- Hazards of infections; mainly Hepatitis B, C, and D, HIV, HTCV I and II (Human T-cell Leukemia Virus) and CMV (Cytomegalovirus).
- Hemolytic transfusion reaction; immediate or delayed reaction. The immediate usually life-threatening reaction of IV hemolysis may occur within minutes or 1-2 hours associated with DIC, and acute renal failure.
- Other type of immune reactions may develop whether due to HLA Ags (graft-versus host disease, GVHD) or plasma proteins.
- Circulatory overload especially in patients with cardiac failure.
- Iron overload in frequently transfused patients (eg; thalassemias)

1- Immunological Complications

1- Hemolytic transfusion reaction:

Most of the cases are due to clerical or administrative error .

This reaction is caused by premature destruction, almost always of donor cells by antibodies present in the recipient serum. It can be immediate or delayed.

a- Immediate :

- This is the most dangerous type and usually caused by ABO incompatibility.

- IgM and complement activation leading to **intravascular lysis** with production of the anaphylatoxins C3a & C5a which are liberated during complement activation causing smooth muscle contraction, platelets aggregation, increased capillary permeability, release of vasoactive amines and hydrolases from mast cells and granulocytes.

Clinical Features:

- Occur within less of one hour from the start of transfusion with a mortality rate in such ABO-incompatible cases is 5–10%.
- Heat in the vein.
- Throbbing headache and flushing of the face.
- Chest tightness, nausea and lumber pain.
- Hypotension and tachycardia.
- DIC, hemoglobinuria (passing red urine), acute renal failure, collapse and death in severe cases.

Stop transfusion immediately, keep the IV line and maintain the circulating blood volume, restore the blood pressure and urinary flow.

b- Delayed Transfusion reaction:

Usually 7-10 days after transfusion and is caused by antibodies, which are present in low liter and not detected at time of cross matching. So this reaction is neither predictable nor preventable.

The antibodies are caused by sensitization due to previous pregnancy or transfusion.

Usually **presented** with; fever, jaundice and lowering of Hb.

2. Febrile Reaction;

- Most common immunological reaction, due to WBC and platelets Ags.
- Seen in patients having multiple blood transfusion or pregnancy.
- Caused by Abs to HLA Ags ,WBC (usually) and platelets specific Ags.
- The onset of the reaction is delayed (30-90) min after start of transfusion.
- The main symptom is fever.

Management: by slow the transfusion, antipyretic and No need to terminate the transfusion.

3- Post-transfusion Purpura; (reaction to platelets Ag)

- Seen in women with history of multiple pregnancies or in those with history of multiple transfusion.
- Caused by Abs to platelets Ag (PI).
- The reaction occurs 7-10 days after transfusion.
- The main feature is purpura due to thrombocytopenia (caused by destruction of the platelets by the Abs).
- It is usually self limiting.

4. Reaction due to plasma protein antibodies:

Majority are due to Anti IgA antibodies, mainly presented as urticaria and treated by antihistamine. Rarely more severe anaphylactic reaction occur

5- Sensitization to red cells antigens:

Because the ABO and RhD antigens are the only Ags matched between donor & recipient, there is a possibility of sensitization to other red cells Ags.

In clinical practice this sensitization could lead to: hemolytic disease of the newborn if the recipient is a female, difficulties in compatibility testing in further transfusion.

2- Non-Immunological Complications

1. Reaction due to bacterial pyrogens or bacteria:

Although its rare complication, it has very high mortality rate characterized by sudden onset of high fever, shock and bleeding due to DIC. Blood may be contaminated by cold-growing organisms (pseudomonas or colon- aerogenes group).

The infusion of large number of Gram-negative microorganisms results in a serious reaction i.e., endotoxic shock.

2. Circulatory overload:

This increase in blood volume may be dangerous in the elderly with a compromised cardiovascular function, pregnancy and in those with severe anemia.

3. Thrombophlebitis; indwelling venous cannula.

4. Air embolism; Only large volumes of air, and not the entry of a few bubbles, result in a clinically significant air embolism.

5. Hemosiderosis; Each unit of blood contain approximately 200 mg of iron(2ml=1mg). After 50 units in adults, and lesser amount in children. This is a major problem in thalassemia major and other severe chronic refractory anemias, and this could be prevented by giving chelating agent.

6. Transmission of infections:

- **Viruses;** Hepatitis (B and C), Retroviruses (HIV and HTL), Herpesviruses (CMV and EBV)
- **Bacteria;** Endogenous or Exogenous
- **Parasites;** Malaria (all Plasmodium falciparum), Chagas' disease by Trypanosoma cruzi

7. Complications of Massive Transfusion.

These tend to occur in cases of replacement the total blood volume within 24 hr (For adult about 10 units /24 hr).

a. Dilution of platelets: As blood stored more than 48 hr has no functional platelets. Transfusion of 8-10 units of blood to an adult will lead to thrombocytopenia.

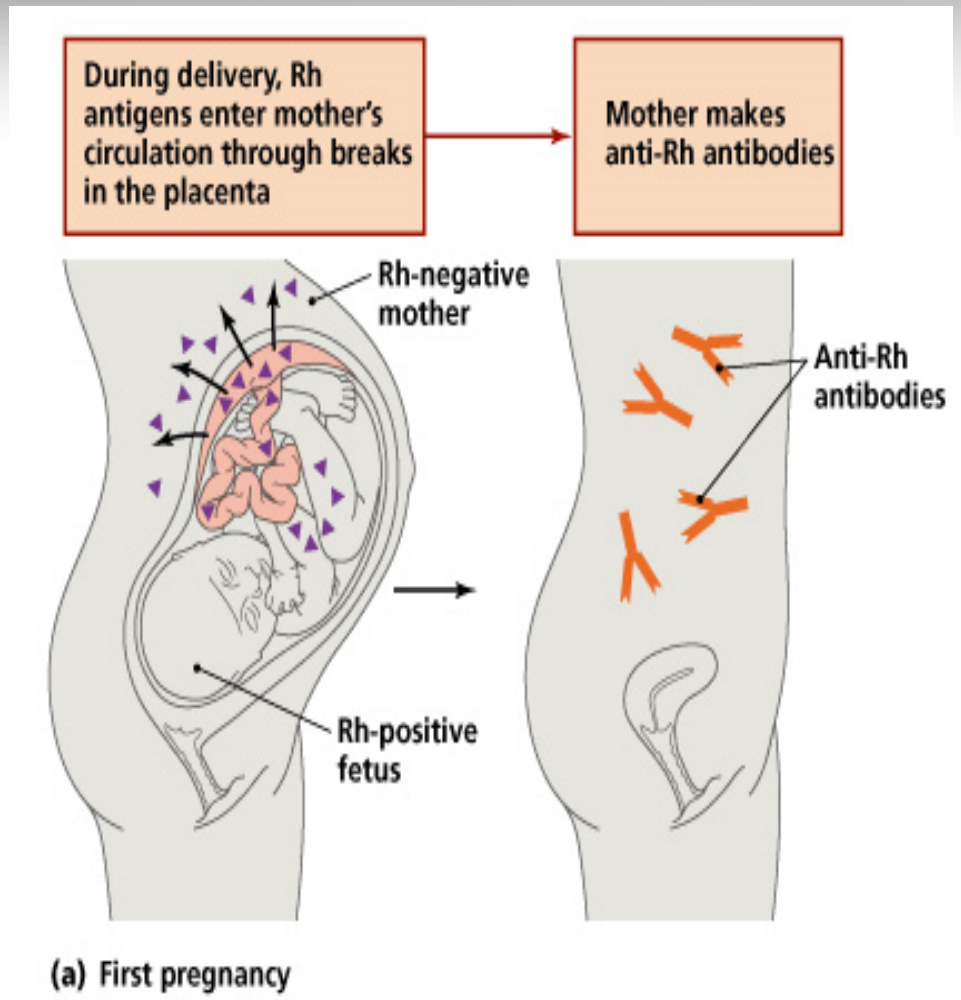
b. Dilution of coagulation factors: This occurs if blood stored more than 14 days is given. Blood stored less than 14 days has adequate level of most of the coagulation factors except FV and FVIII as they are the most labile factors.

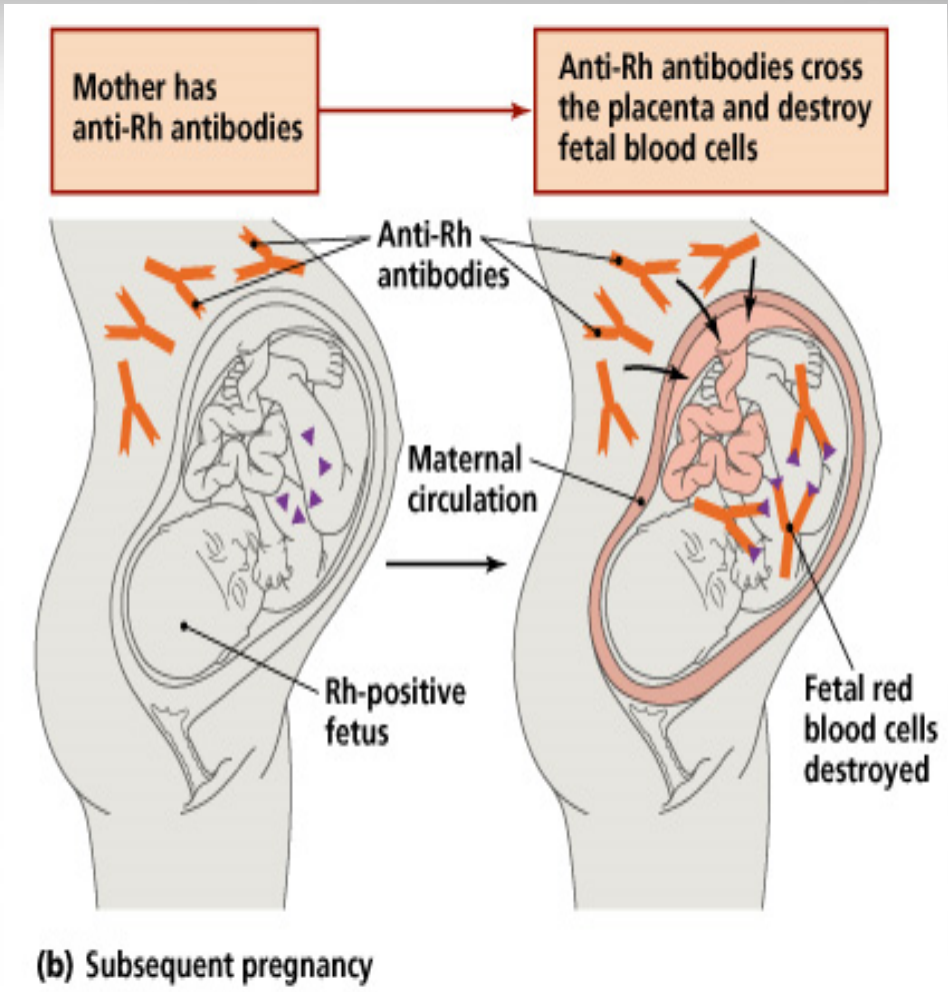
c. Metabolic changes.

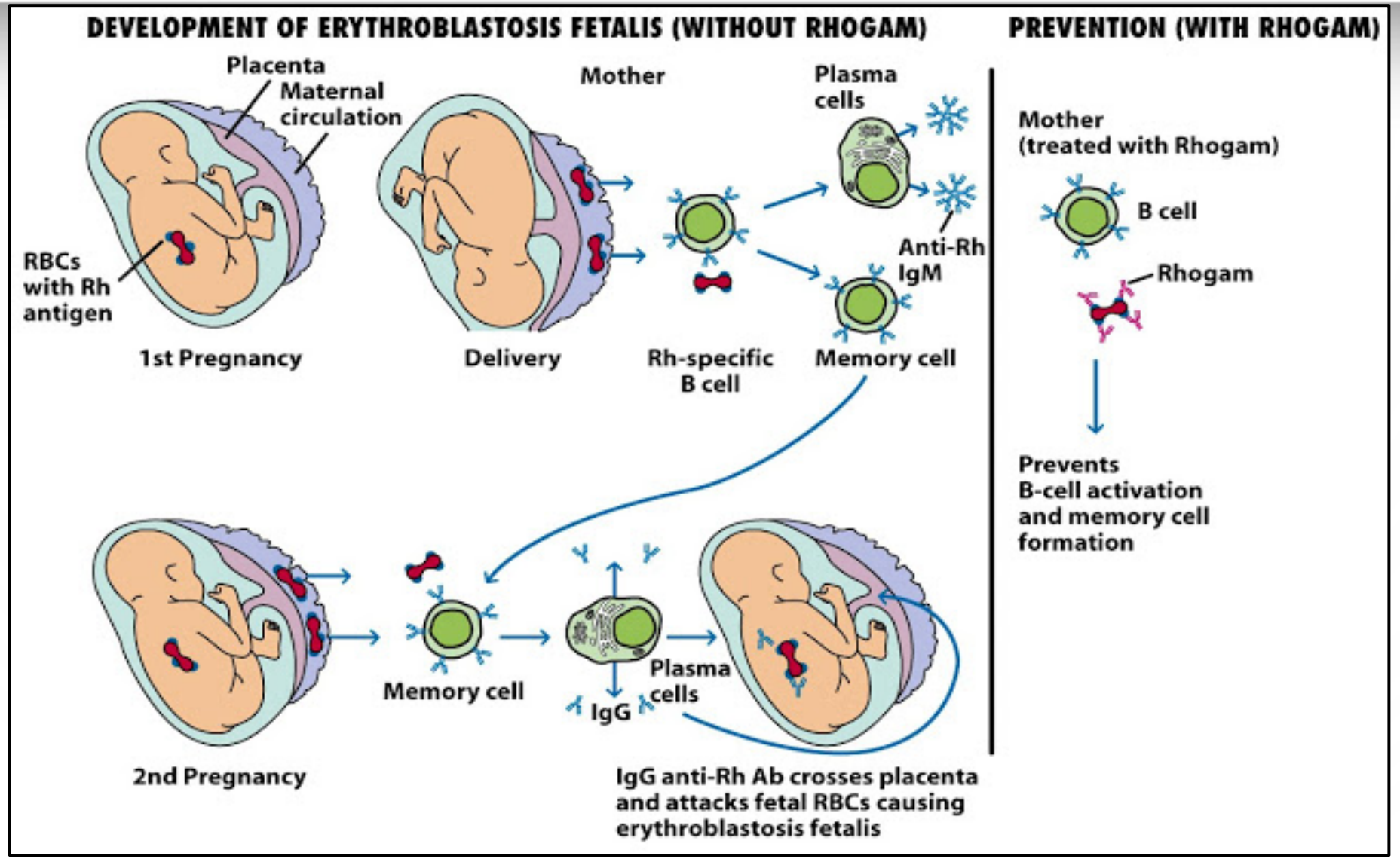
HEMOLYTIC DISEASE OF THE NEWBORN (HDN)

Immune reaction caused by the passage of Ab of IgG type formed in the maternal circulation by previous sensitization.

- Caused; due to the passage of the fetal incompatible red cells to the mother's circulation leading to the formation of Abs (i.e; sensitization)
- The Ab passing across the placenta into the circulation of the fetus where they react with fetal RBCs leading to their destruction
- The **sever** form mostly of Anti-D (RhD) antibody
- Wide range of other antibodies may be the cause
- ABO blood group system is the **most frequent** cause of HDN this is usually mild


During delivery, Rh antigens enter mother's circulation through breaks in the placenta
Mother makes anti-Rh antibodies
Rh-negative mother
Anti-Rh antibodies
Rh-positive fetus
(a) First pregnancy


Mother has anti-Rh antibodies
Anti-Rh antibodies cross the placenta and destroy fetal blood cells
Anti-Rh antibodies
Maternal circulation
Rh-positive fetus
Fetal red blood cells destroyed
(b) Subsequent pregnancy


DEVELOPMENT OF ERYTHROBLASTOSIS FETALIS (WITHOUT RHOGAM)
PREVENTION (WITH RHOGAM)
Placenta
Maternal circulation
RBCs with Rh antigen
1st Pregnancy
Mother
Delivery
Plasma cells
Anti-Rh IgM
Rh-specific B cell
Memory cell
Mother (treated with Rhogam)
B cell
Rhogam
Prevents B-cell activation and memory cell formation
2nd Pregnancy
Memory cell
Plasma cells
IgG
IgG anti-Rh Ab crosses placenta and attacks fetal RBCs causing erythroblastosis fetalis

CLINICAL FORMS;

Its usually occur in 2nd pregnancy of incompatible blood group, but may occur due to sensitization by incompatible transfusion.

I- Sever Disease; erythroblastosis fetalis (Hydrops fetalis)

- The fetal hematopoietic tissues (the liver, spleen and bone marrow) respond to hemolysis by increasing production of erythrocyte
- Increased erythrocyte production outside the bone marrow, extramedullary hematopoiesis result in their enlargement
- If increased erythropoiesis cannot compensate for erythrocyte destruction, a progressively severe anemia develops
- This severe anemia may cause the fetus to develop cardiac failure with generalized edema (hydrops fetalis), resulting in death in utero.

II- Moderate disease;

- Infants born with anemia, jaundice, hepatosplenomegaly, edema and heart failure shortly after birth
- The sever jaundice -if not treated properly- may lead to bile pigment deposition in the basal ganglia with development of *kernicterus*.

III- Mild disease; mild anemia with or without jaundice

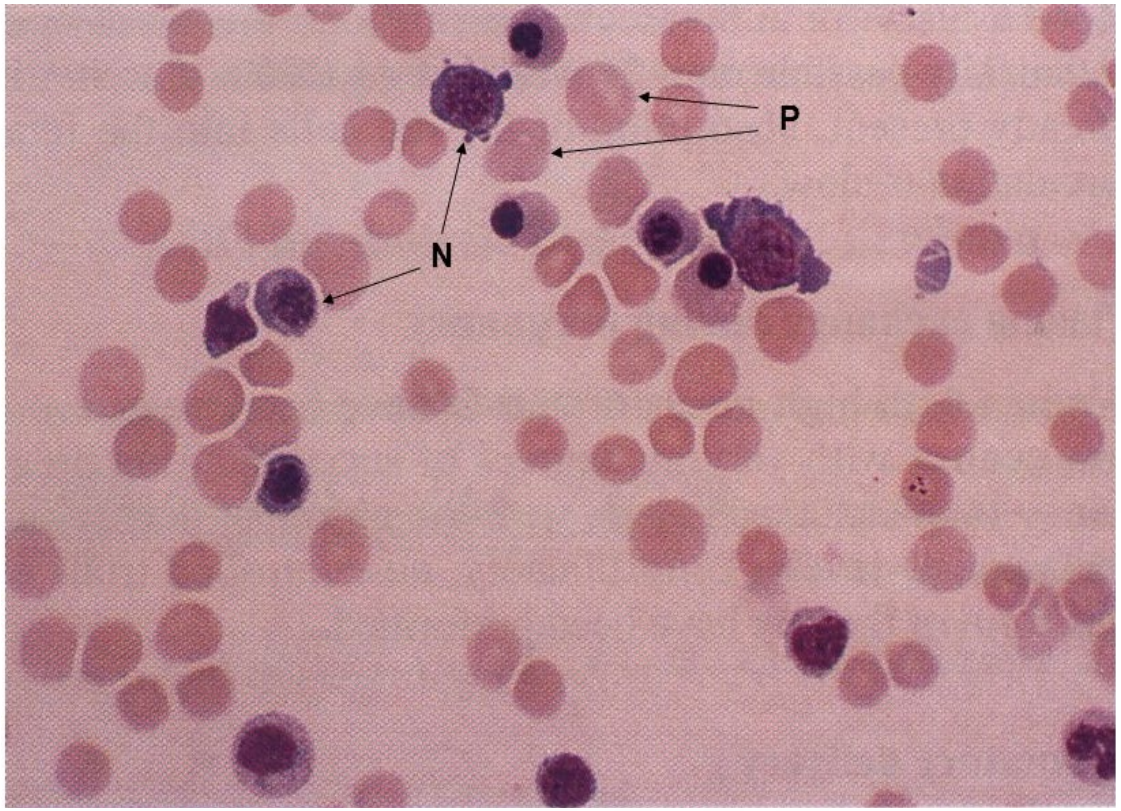
Laboratory Features;

❖Features of hemolysis;

- Low Hb level of normochromic
- High reticulocyte count
- High bilirubin level

❖Blood film;

- Contracted, crenated cells
- Polychromasia (P) (reticulocytes)
- Normoblastemia (N)



Treatment;

- Prevention; Anti-D injection for the Rh-negative pregnant women *with Rh^{+ve} husband. Usually given in the 3ed trimester or even short period after delivery*
- Prenatal and antenatal care; to prevent and follow sensitization
- Treatment of the infant (according to the severity);

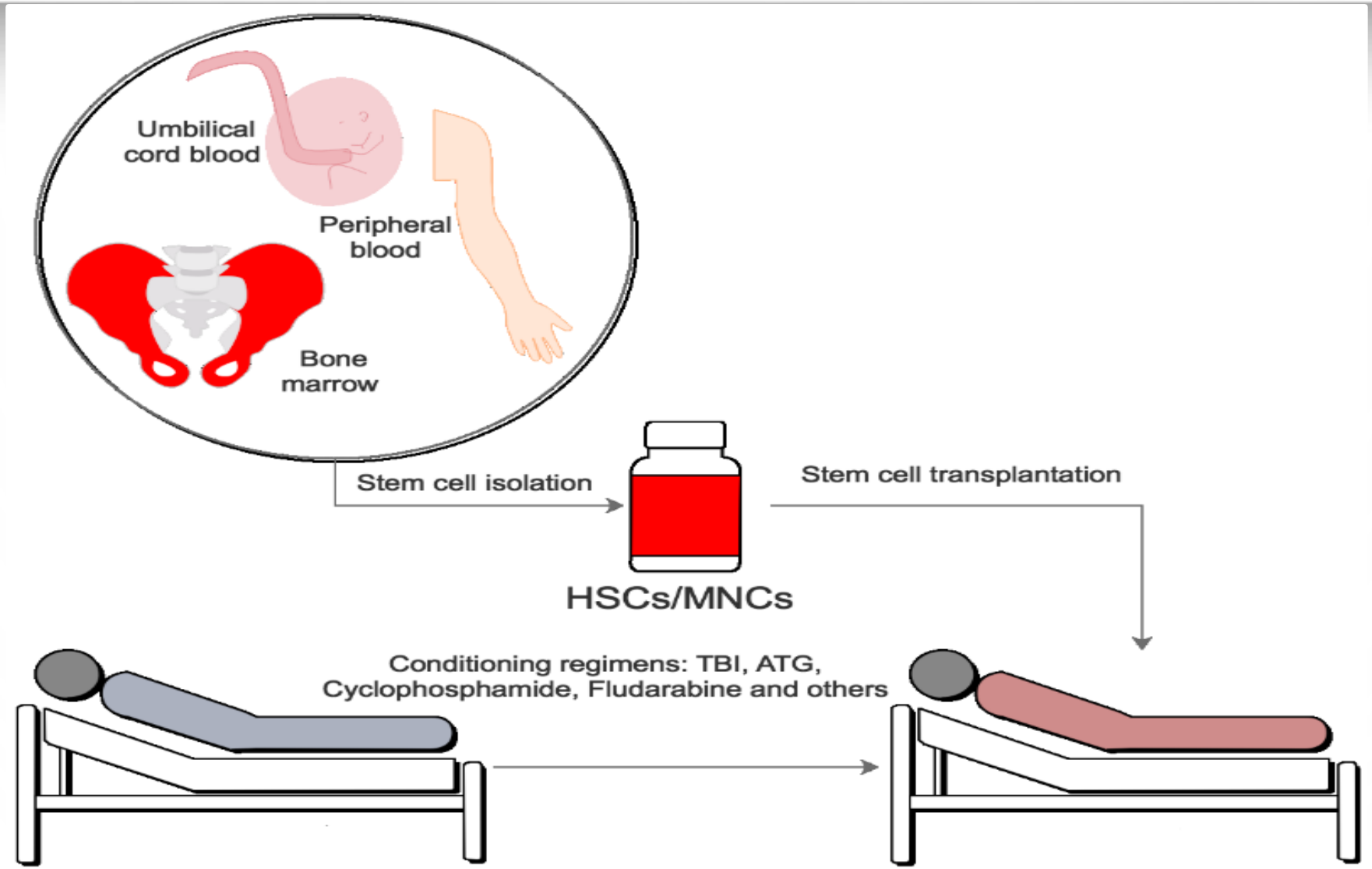
1. Phototherapy
2. Exchange transfusion

STEM CELL TRANSPLANTATION

Stem cell transplantation (SCT); is the infusion of healthy stem cells (SCs) into the body to stimulate new bone marrow growth

- The patient receives healthy blood-forming cells (SCs) to replace their own that have been destroyed by disease or by the radiation or high doses of anticancer drugs
- It is a transfusion of blood and immune cells rather than a surgical procedure.
- It can be harvested from bone marrow (bone marrow transplantation BMT), from peripheral blood (peripheral blood transplantation PBSCT) or Umbilical
- **Autologous**; of the same individual
- **Allogenic**; from another person

- Usually in a specialized well-equipped center with qualified team.
- Donor **selection** in allogenic SCT, in which must be HLA-matched and fulfils the other criteria for blood donation.
- The SCs need to be **harvested** from the selected site (peripheral blood, BM or umbilical cord) to adequate amount which is measured by its $CD34^+$ cells, then processed prior transplantation.
- **Conditioning** of the patient according to the disease treated; using chemotherapy and/or irradiation.
- **Post-transplantation** care; which due to bone marrow suppression (pan cytopenia) and possibility of the immune reactions (graft-versus host disease GVHD)



TBI; total body irradiation, ATG; Anti-Thymocyte globulin

Indications;

1. Hematological malignancies; leukemias, lymphomas, multiple myelomas (MM).
2. Aplastic anemia; including Fanconi's anemia.
3. Paroxysmal Nocturnal Hemoglobinuria (PNH), Myelodysplastic syndrome (MDS) and myelofibrosis (MF)
4. Inherited Hb disorders (hemoglobinopathies); thalassemia and sickle cell anemia.
5. Others; not yet proved.

Complications;

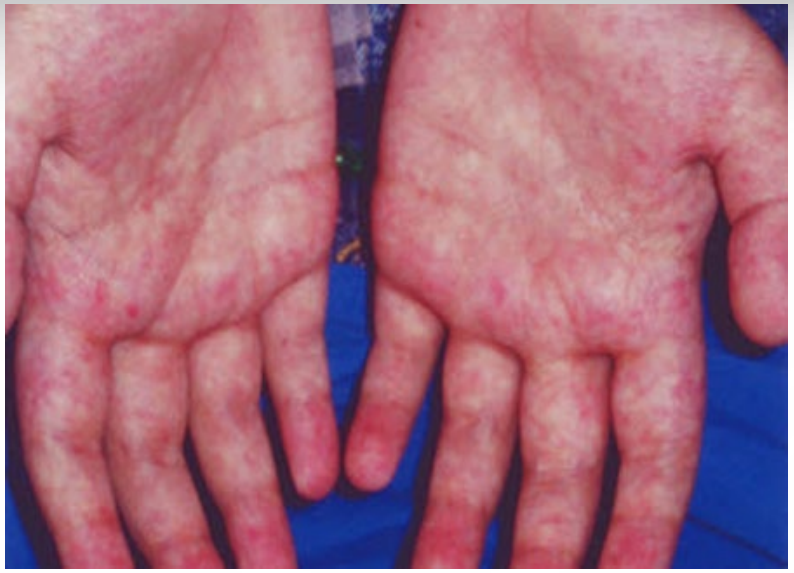
1. Graft-versus-host disease (allogeneic transplant only)
2. Stem cell (graft) failure
3. Infections; bacterial, viral and fungal
4. Hemorrhage.
5. Veno-occlusive mostly of the liver and heart.
6. ABO incompatibility and DIC
7. Cataracts and Infertility
8. New Malignancies.
9. Death

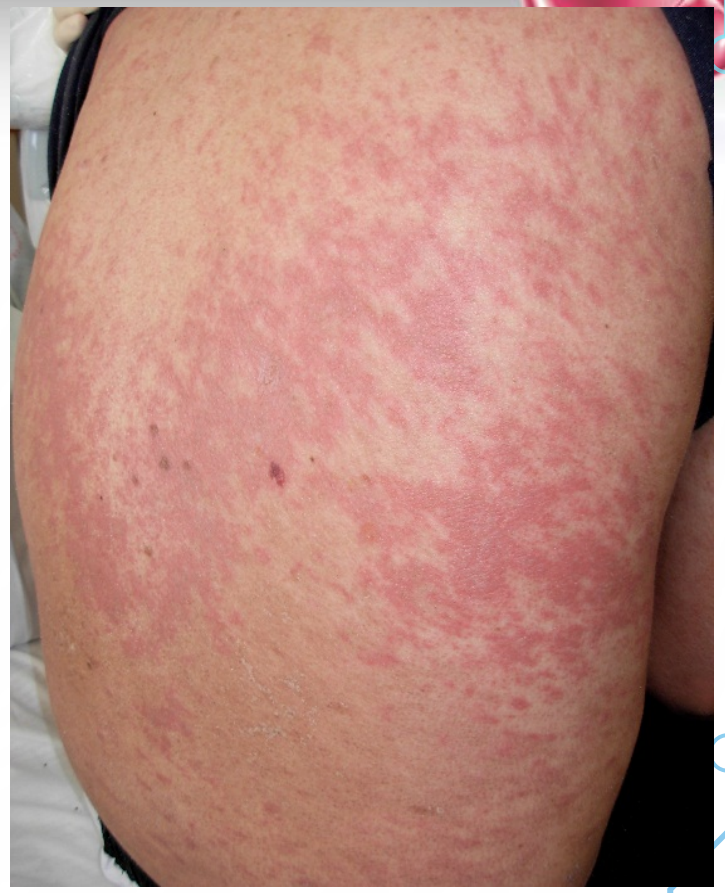
GRAFT-VERSUS-HOST DISEASE

Graft-versus-host disease (GVHD); Caused by an immune reaction of the donor-derived immune cells (T-lymphocytes) against recipient tissues

- **It can be caused, in addition to SCT (BMT), by massive blood transfusion**
- Its either in acute or chronic form
- Increased with the age and in certain degree of HLA mismatched transplantation.

- The immune reaction attacking different organs' tissue mainly;
- Skin (different types of rash), liver and GIT





- Its life-threatening, especially in the acute form needs high dose of corticosteroids
- In chronic form may also affect joints and secretory glands and treated by immunosuppressive drugs


THANK
YOU!