- **Blood bank:** : A place where blood is collected from donors, typed, separated into components, stored, and prepared for transfusion to recipients. It is a cornerstone of emergency and surgical medicine and is dependent on the clinical laboratory for ensuring the safe use of blood and its components.
- **Blood transfusions**: the introduction of blood or blood components from one person into the bloodstream of another. It can save life and improve health. It may result in acute or delayed complications. In addition, it carries the risk of transmission of infectious agents, such as HIV, hepatitis viruses, syphilis,

## Why it's done?

There are many reasons make people receive blood transfusions, including: **Major surgery**, **used to treat severe anemia resulting from the effects of chemotherapy**, **cancer**, **sickle cell disease**, **and thalassemia**. So it's essential for saving the lives for those persons.

Blood has several components, including red blood cells, white blood cells, plasma and platelets. **Whole blood** means the blood contains all its parts, but whole blood is rarely used for transfusion.

## Selection of Donation:

The questions are designed to identify the donor:

1- Name
2- Age (18-60)
3- Weight: At least 50 Kg.
4- Hb measurement must be more than 13.5 in men and 12.5 in women
5- Identification
6- Address housing
7- The latest donation (Must be at least 3 months).
8- Health: General good health (must be free from chronic diseases, don’t Taking antibiotics or drugs)
9- Diet (A meal is recommended at least four hours prior to donation. Drink plenty of fluids).
10- The physical measurement includes blood pressure, pulse, and temperature.

## ❖ Who is cannot donate blood?

People suffering from following infection that may be present in the donor and could be transmitted through transfusion to another person.

1- AIDS
2- Cancer
3- Hepatitis
4- Organ Failure: Kidney, lung or liver failure.
5- Parasites infection that cause malaria.

## ❖ Testing Donated Blood

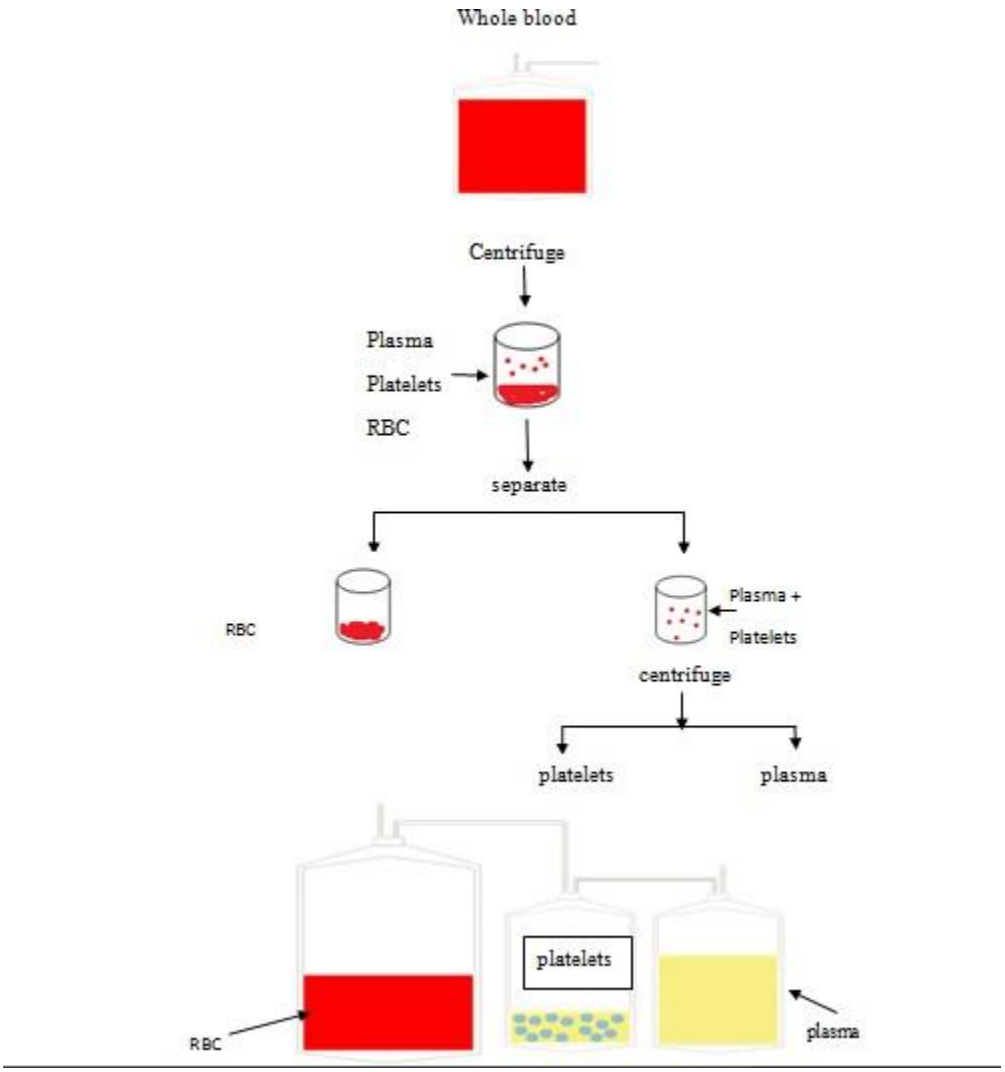
In the blood bank laboratory, certain tests must be performed on all donated blood. Each unit of donated blood is tested for:

- Determine the **donor's ABO blood group** and **Rh status**
- Hepatitis B
- Hepatitis C
- HIV types 1 and 2
- Syphilis

## ❖ Blood Collection Procedures

1. The donor sits in a reclining chair.
2. The site for drawing blood is selected and disinfected. A prominent vein is chosen for the venipuncture site.
3. The disinfectant is applied to the area around the vein to be used.
4. The needle used to draw the blood from the vein is gently inserted.
5. Blood fills the collection bag in a few minutes.
6. Just after the bag has filled, blood from the line is taken to fill several tubes for further testing.
7. The needle is removed and pressure is applied over the venipuncture site, then a bandage is placed for the next couple of hours.
8. The donor drinks some liquid to replace the lost blood volume.

## ❖ Blood Separation, Preservation and Storage




As can be seen in the above diagram, **a single donation of whole blood has supplied three separate components (packed red blood cells, platelets, fresh frozen plasma) that can potentially benefit three different patients.**

**Proper storage of whole blood and blood components is essential:**

- Both whole blood and PRBC's can be stored for up to 42 days at 1 - 6 degrees C.
- Platelets can be stored at room temperature for a maximum of 5 days.
- Fresh frozen plasma can be kept frozen for up to 1 year.

## Human blood groups

- A **blood type** (also called a **blood group**) is a classification of blood based on the presence and absence of inherited antigenic substances on the surface of red blood cells and also based on the presence or absence of antibodies in plasma.
- **Rhesus (Rh) factor**: is an inherited protein found on the surface of red blood cells. If your blood has the protein, you're Rh positive. If your blood lacks the protein, you're Rh negative.

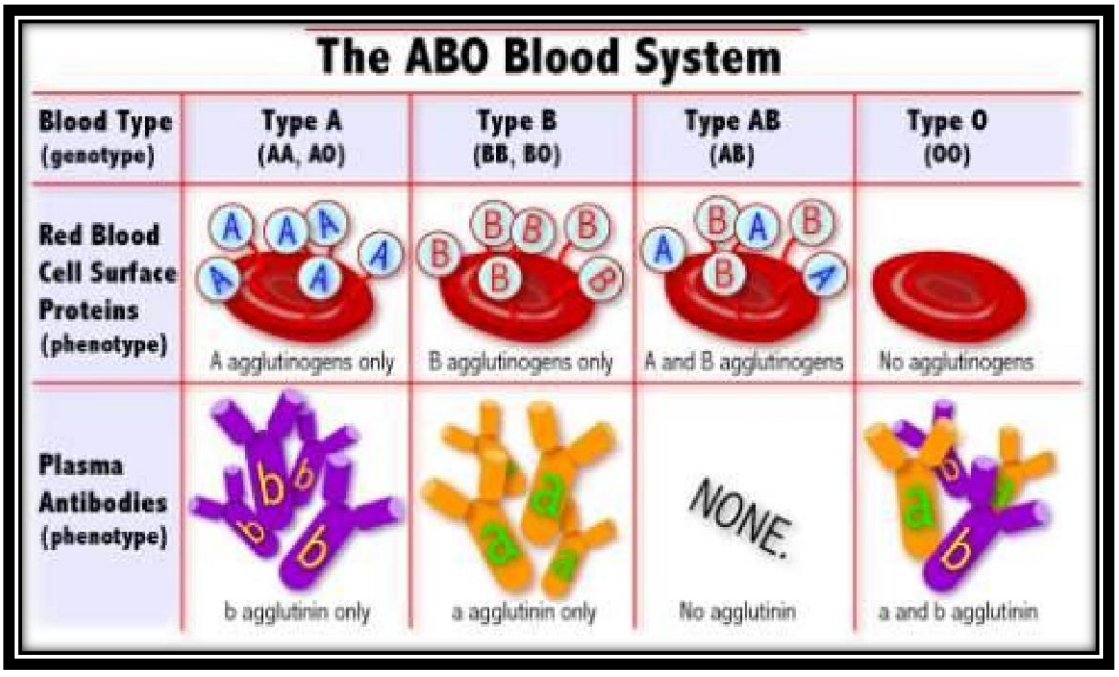
The ABO blood group antigens are one of the most important topics in blood transfusion to evaluate the compatibility of donor blood cells with recipient blood cells.

- **Blood type test is done:**

  - Before a person gets a blood transfusion or organ for transplantation.
  - Before surgery.
  - In pregnancy women.

- **The structure of Blood group antigens:**

Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane.
**For example,** the antigens of the **ABO blood** group are **sugars.** In contrast, the antigens of **the Rh blood** group are **proteins**.
**The figure below shows the red blood cell membrane and some of the blood group antigens attached to it.**



❖ **Determination of ABO blood groups:**

The ABO blood group is determined by the presence of A and B antigens on the surface of the red blood cells, and of anti - A or anti - B antibodies in the serum or plasma as below:

1. **Blood group A :**

Individuals have the A antigen on the surface of their RBCs , and blood serum containing Anti- B antibodies . Therefore , a group A individual can only receive blood from individuals of groups A or O ( with **A** being preferable ) and can donate blood to individuals of groups A or AB .

2. **Blood group B :**

Individuals have the B antigen on their surface of their RBCs , and blood serum containing Anti-A antibodies . Therefore , a group B individual can only receive blood from individuals of groups B or O ( with B being preferable ) and can donate blood to individuals of groups B or AB .

3. **Blood group AB :**

Individuals have both A and B antigens on the surface of their RBCs , and their blood serum does not contain any antibodies against either A or B antigen . Therefore , an individual with type AB blood can receive blood from any group ( with AB being preferable ) , but can only donate blood to another group AB individual . (Universal recipient)

4. **Blood group O :**

Individuals do not have either A or B antigens on the surface of their RBCs , but their blood serum contains Anti- A and Anti-B antibodies . Therefore , a group O individual can only receive blood from a group O individual , but they can donate blood to individuals of any ABO blood group ( A , B , O , or AB ) . ( Universal donor ) .

| Blood Group | Antigens on cell | Antibodies in plasma | Transfuse with group |
|---|---|---|---|
| **A** | A | Anti-B | A or O |
| **B** | B | Anti-A | B or O |
| **AB** | A and B | None | AB, A, B or O |
| **O** | None | Anti-A & B | O |

**The common percentage of blood groups:**

| ABO Type | Percent% |
|---|---|
| O | 45% |
| A | 40% |
| B | 11% |
| AB | 4% |

**Methods for ABO and Rh grouping:**

- Tube method
- Slide method (white ceramic method)
- Microplate method
- New trends (Gel technology)

❖ **Slide method (white ceramic method) Principle:**

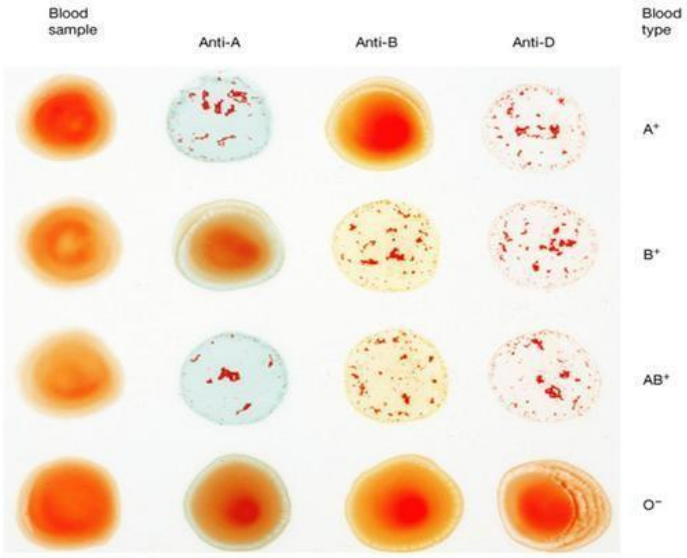
When red cells are mixed with various reagent antisera (soluble antibody), agglutination will occur on the slides containing cells positive for (possessing the antigen) the corresponding antigen. No agglutination will occur when the red cells do not contain the corresponding antigen.

**Material:**

1. Antibody A
2. Antibody B
3. Red blood cells
4. Slides
5. Applicator sticks
6. Pipets

**Procedure:**

1. On the section of slide labeled anti-A place one drop of antibody A.
2. On the section of slide labeled anti-B place one drop of antibody B.
3. Place one drop of cells in each antibody containing circle.
4. Carefully mix each solution with a separate applicator stick.
5. Tilt slowly for one minute.
6. Record results.


Blood sample
Anti-A
Anti-B
Anti-D
Blood type
A+
B+
AB+
O−

## Rh BLOOD GROUP SYSTEM

system for classifying blood groups according to the presence or absence of the Rh antigen, often called the Rh factor, on the cell membranes of the red blood cells (erythrocytes). 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 Rh blood group system was discovered in 1940 by Karl Landsteiner and A.S. Weiner.

The Rh antigens are encoded by two genes: ***RHD* and *RHCE***. ***RHD* e**ncodes for the **D antigen**, whereas ***RHCE*** encodes for the **Cc and Ee antigens**. There are 54 antigens that have been assigned to the Rh blood group system, Of the 54 antigens in the Rh blood group system, the most common and important are **D, C, E, c, and e.**

The significance of the Rh blood group is related to the fact that **the Rh antigens are highly immunogenic.** In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing hemolytic disease of the newborn,

HDN).

An individual is considered to be Rh positive if his or her red cells express the D antigen. The term *Rh negative* refers to the absence of the D antigen. The absence of the D antigen occurs in approximately 15% to 17% of individuals in white populations and is less frequent in other populations. In white populations, the absence of the D antigen is usually due to the deletion of the *RHD* gene. In Asian and black populations, the absence of the D antigen is usually due to an inactive RHD rather than a gene deletion.

## Antibodies

Most Rh antibodies are IgG, although some may be IgM. They are usually not capable of activating complement. Anti-D is one of the most common Rh antibodies because of the high immunogenicity of the D antigen. Anti-D can cause severe HDN and HTR.

## Weak D Phenotype

Some red cells that express the D antigen require prolonged incubation with the anti-D reagent or application of the antiglobulin test for agglutination to occur. These red cells are considered to be D antigen-positive and are described as *weak D,* formerly termed *Du*. The weak D phenotype is thought to occur by one of three mechanisms: **(1) inheritance of an *RHD* gene encoding for a weakened expression of D, (2) interaction of the D allele with other genes, and (3) inheritance of an *RHD* gene missing some epitope.**

## Clinical Significance

• Rh incompatibility between donor and recipient results in hemolytic transfusion reactions.

• Rh incompatibility between mother and fetus results in hemolytic disease of the newborn(HDN)

## Rh (D) Typing Techniques

The different methods available are:

• Slide technique

• Tube technique

• Microplate method

• Microtyping System

# Hemolytic Disease of the Newborn

**Hemolytic disease of the newborn** is also called **erythroblastosis fetalis**. This condition occurs when there is an incompatibility between the blood types of the mother and fetus.

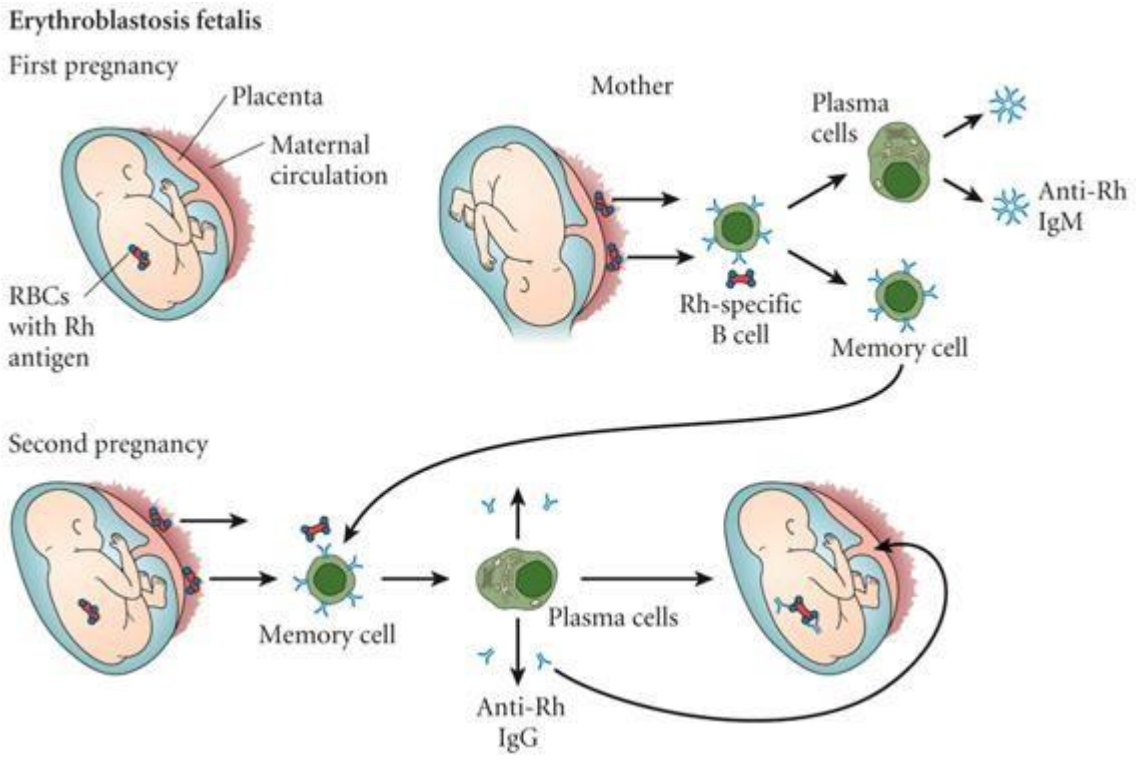
**What causes hemolytic disease of the newborn (HDN)?**

HDN most frequently occurs when an Rh negative mother has a baby with an Rh positive.The mother's immune system Recognize the baby's Rh positive red blood cells as "foreign." theimmune system responds by developing antibodies to fight and destroy these foreign cells.

The mother's immune system then keeps the antibodies in case the foreign cells appear again, evenin a future pregnancy. The mother is now "Rh sensitized."

In a first pregnancy, Rh sensitization is not present. Usually, it only becomes a problem in a futurepregnancy with another Rh positive baby.

During the second pregnancy, the mother's antibodies cross the placenta to fight the Rh positive cells in the baby's body. As the antibodies destroy the red blood cells, the baby can become sick. **The condition is called hemolytic disease of the newborn.**



**Complications of HDN:**

Complications of hemolytic disease of the newborn can range from mild to severe. As below

- **Mild anemia, hyperbilirubinemia, and jaundice.**
- **Severe anemia with enlargement of the liver and spleen.** When these organs and the bone marrow cannot compensate for the fast destruction of red blood cells, severe anemia results and other organs are affected.
- **Hydrops** (fluid throughout the body's tissues, including in the spaces containing the lungs, heart, and abdominal organs), which can lead to heart failure from too much fluid
- **Kernicterus** is the most severe form of hyperbilirubinemia and results from the buildup of bilirubin in the brain. This can cause seizures, brain damage, deafness, and death.

**What are the symptoms of hemolytic disease of the newborn?**

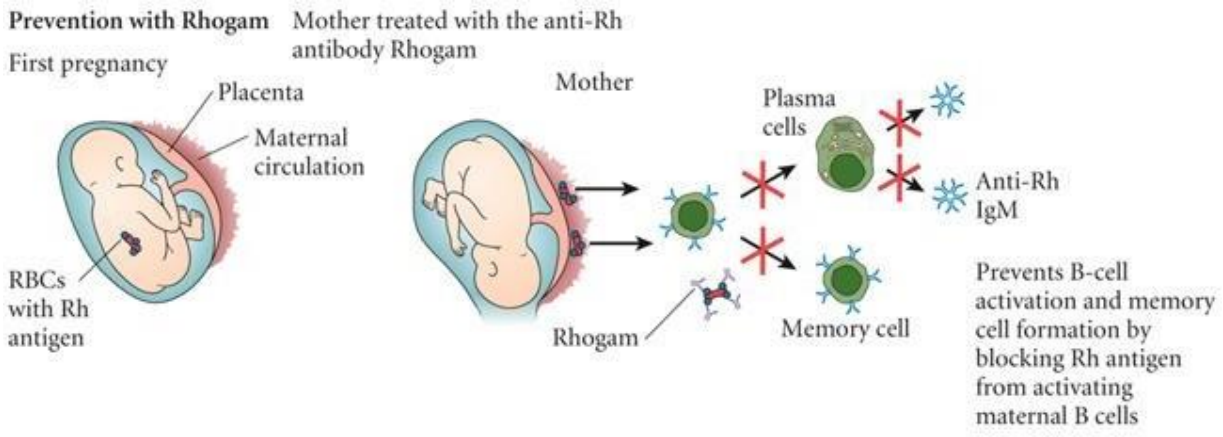
- A pale coloring may be evident, due to anemia.
- Jaundice or yellow coloring of skin and eyes may be present. jaundice can develop quickly,usually within 24 to 36 hours.
- The newborn may have an enlarged liver and spleen.

**Prevention of hemolytic disease of the newborn**

HDN is a very preventable disease. Nearly all women with Rh negative blood are identified in early pregnancy by blood testing. If a mother is Rh negative, she is usually given a drug called Rh immunoglobulin (RhIg), also known as RhoGAM.

**Rh immunoglobulin (RhIg):** This is a specially developed blood product that can suppress an Rh negative person immune system to form antibodies against Rh positive cells. It may also be used when Rh negative people are given Rh positive blood. It is given by injection into muscle or a vein.

Many women are given RhoGAM around after the baby is born within 72 hours, if her baby is Rh positive. If her baby is Rh negative, she does not need another dose.




## How is hemolytic disease of the newborn diagnosed?

Once a baby is born, diagnostic tests for HDN may include the following:

- Complete blood count and immature red blood cell (reticulocyte) count
- Bilirubin level
- Blood typing
- Coombs test

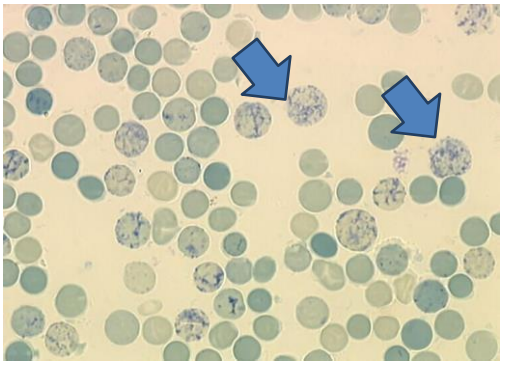
## Reticulocyte Count

**Reticulocytes** are immature red blood cells, typically composing about 1% of the red cells in the human body. Reticulocytes develop and mature in the bone marrow and then circulate in the blood stream as mature red blood cells.

## Procedure

1- Mix 1 ml of blood with (1) drop of dye solution (specific for reticulocyte count (new methylene blue or brilliant cresyl blue)
2- Incubate in water bath for 1/2 h.
3- After incubation, resuspend the cells by gentle mixing and make films on glass slides in the usual way. When dry, examine the films without fixing. In a successful preparation, the reticulofilamentous material should be stained deep blue and the non-reticulated cells stained with pale greenish blue.

Examin under microscope



# Complication of Blood Transfusion

Blood transfusion is a common procedure that usually goes without complications. But there are some risks. Some transfusion reactions happen during the transfusion, while others may take several weeks to develop.

- **Immediate** → Immunological
  → Non- Immunological

- **Delayed** → Immunological
  → Non- Immunological

## Immediate- Immunological

1- **Hemolysis**: RBC incompatibility.

2-**Febrile reaction**: Antibody against donor's WBC.3- **Anaphylaxis**: Antibody to IgA.

4- **Urticaria**: Antibody to plasma proteins. (Allergic reaction) to IgE plasma proteins.

## Immediate – non Immunological

1- **Marked fever:** due to Bacterial contamination.
2- **Heart failure:** due to volume over load of blood.
3- **Hemolysis:** physical distraction of RBC due to freezing or overheating.

## Delayed – Immunological

1- **Hemolysis:** Antibody to RBC antigens (weak Ag need long time to induce the immune system).
2- **Graft versus host disease:** Lymphocyte reaction against recipient tissue. **symptoms: rash, fever, diarrhea, and liver dysfunction within weeks after transfusion**
3- **Post transfusion purpura:** development of antiplatelets antibody.

## Delayed –non Immunological

1- **Iron over load:** due to multiple transfusions.
2- **Hepatitis:** transfusion of HCV and HBV.
3- **AIDS:** transfusion of HIV.
4- **Protozoal infection:** Malaria parasites.

### Ferritin test

Ferritin, a major iron storage protein, is essential to iron homeostasis and is involved in a wide range of physiologic and pathologic processes.
It is clear that low ferritin values less than reference range are usually representative of body iron deficiency. On other hand, patients with ferritin levels that are higher than the reference range may be indicative of conditions such as iron overload, infections, inflammations, collagen diseases, hepatic diseases, neoplastic disease and chronic renal failure.

**Procedure:**

1. set a Test Device on a dust-free clean place.
2. Check/insert ID Chip onto the instrument.
3. Take out one tube of Detection Buffer from refrigerator and leave it at room temperature for 20 minutes or longer.
4. Draw 30 µL of serum, plasma or Control with a transfer pipette and add it to the tube containing Detection Buffer.
5. Mix well the specimen with Detection Buffer by tapping or inverting the tube.
6. Take 75 µL of sample mixture with a pipetteand load it onto the well of disposable Test Device.
7. Leave the Test Device at room temperature for 10 minutes before inserting the device into the holder.
8. To start scanning, insert test device onto the holder of i-CHROMATM Reader and press "SELECT" button.

Make sure to push the device all the way in. The instrument will automatically start to scanthe Test Device immediately.

9. Read the results on the display screen of i-CHROMA TM Reader.

**Reference Range:**

**30 ~ 350 ng/mL for male**
**20 ~ 250 ng/mL for female.**

# Coombs Test

**A Coombs test** (also known as antiglobulin test or AGT) is used inimmunohematology and immunology.

There are two types of Coombs tests:

1-Direct Coombs test (DCT) or direct antiglobulin test (DAT) .

2- Indirect Coombs test or indirect antiglobulin test or (IAT).

Direct Coombs test is used to demonstrate the sensitization of RBCs in vivo with IgG antibodies and/or complement components (C3b, C3d andC4).

The Application of this test:

1-Hemolytic transfusion reactions .

2-Hemolytic disease of the fetus and newborn.

3Autoimmune hemolytic anemia (AIHA).

4-Red blood cell sensitization .

5-Antibodies directed against certain

drugs that bind to the RBC membrane

The Procedure:

1- Blood sample is taken (1ml)
2- The RBCs are washed (with normal saline / 3ml) to removing thepatient's own plasma, repeated three times.
3- Incubated with antihuman globulin (also known as "Coombs reagent")for 30 minutes.

**The result**: If this produces **agglutination of RBCs,** the direct Coombstest is positive, a visual indication that antibodies (and/or complement proteins) are bound to the surface of red blood cells.

## The indirect Coombs test:

Detects antibodies that are present unbound in the patient's serum. This test is performed to detect presence of Rh-antibodies or other antibodiesin patient's serum in case of the following:

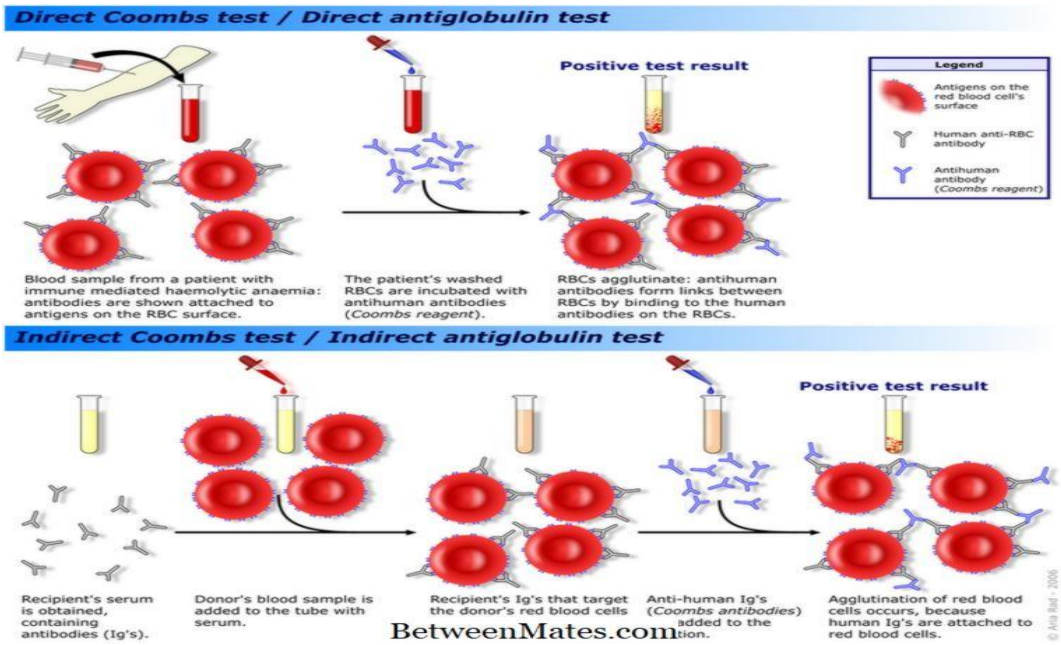
a. To check whether an Rh-negative women has developed Anti Rh antibodies.

**b.** Transfusion of Rh positive blood

### Procedure

1- Serum is extracted from the blood sample taken from the patient.
2- Then, the serum is mixing with RBCs of known antigenicity; that is, RBCs with known reference values from other patient blood samples. 3- Incubate with AHG for 30 minutes.

**The result** If agglutination occurs, the indirect Coombs test is positive. Because the AHG was bind to Ab and the agglutination appear.



# Types of Anticoagulants

**Anticoagulants** are chemical substances that are added to blood to prevent coagulation. For various purposes, a number of different anticoagulants are available. The following solid or liquid anticoagulants are mixed with blood immediately after sample collection:

**1.Ethylenediamine tetra acetic acid (EDTA)** EDTA has become the standard hematology anticoagulant because of its very efficient and complete anticoagulation and its lack of effect on the size (morphology) or number of blood cells in the specimen. Its disodium or tripotassiumsalt are used.

**Uses**: It is the preferred anticoagulant for cell counts, morphological studies, platelet counts and platelet function tests since it prevents plateletaggregation.

**Mode of action**: Its effect by tightly binding (chelating) ionic calcium thus effectively blocking coagulation. The amount of EDTA necessary for the complete chelation of Calcium **1.5±0.25mg** of **Na2** or **K3 EDTA** per 1ml of blood (e.g. 0.02ml of 10% solution of K3EDTA is used for 1ml of blood). This concentration does not appear to adversely affect any of the erythrocyte or leucocyte parameters.

**2- Trisodium Citrate**

Sodium citrate combines with calcium, thereby preventing the conversion of prothrombin to thrombin, and coagulation does not occur. 9 volumes of blood are added to 1 volume of the sodium citrate solution and immediately well mixed with it. Sodium citrate is also the anticoagulant for the erythrocyte sedimentation rate (ESR); for this, 4 volumes of venous blood are diluted with 1 volume of the

sodium citrate solution.

### 3- Balanced or double oxalate

Salts of oxalic acid by their ability to bind and precipitate calcium as calcium oxalate serve as suitable anticoagulants for many hematologic investigations. Three parts of ammonium oxalate is balanced with two parts of potassium oxalate (neither salt is suitable by itself, i.e., ammonium oxalate causes cellular swelling and potassium oxalate causes erythrocyte shrinkage). It is used in the proportion of 1-2mg/ml of blood.

### 4- Heparin

**Heparin** is an excellent natural anticoagulant extracted from **mammalian liver or pancreas.** It is more expensive than the artificial ones and has a temporary effect of only 24 hours. Heparin prevents clotting by inactivating thrombin, thus preventing conversion of fibrinogen to fibrin. It is the best anticoagulant when absolute minimal hemolysis is required (e.g., osmotic fragility test and hematocrit determination). It is used in the proportion of **0.1-0.2mg** of the dry salt for **1ml** of blood.

**Anticoagulant/preservative solutions for blood storage: -**

**Citrate-phosphate-dextrose (CPD) (storage blood for 21 days) Citrate**: Anticoagulants by binding to Ca2+ (reducing [Ca2+] to zero) **Phosphate** : Buffers which also provides phosphate source for metabolism,

**Dextrose**: Provides energy source for continued glycolysis and ATPproduction

- **CPD-adenine(storage blood for 35 days) Adenine**: Provides substrate for ATP synthesis * Addition of adenine to CPD prolongs shelf-life to 35 days

- **SAG-M** * Saline, adenine, glucose, and mannitol ,Mannitol prevent

hemolysis of RBC

- **ADSOL** Adenine, dextrose, sorbitol, sodium chloride and mannitol maintains red cell viability for blood transfusion for 6 weeks. It would be useful to know about its preservation qualities over longer periods. * Shelf-life = 42days

# Cross matching test

**Cross matching test** is referring to the testing that is performed prior to a blood transfusion in order to determine if the donor's blood is compatible with the blood of a recipient, or to identify matches for organ transplants.

## Purposes of this test:

The cross match is routinely used as the final step of pretransfusion compatibility testing. It serves two purposes:

(1) Serve as a final check of ABO compatibility between donor RBCs and patient plasma or serum

(2) To detect clinically significant antibodies that may have been missed by the antibody-screening test.

## Types of test:

1- **Major**: using serum of patients with RBC of donor.
2- **Minor**: using RBC of patient with plasma of donor. But it is not use in routine test for all patients.

## Procedure of cross matching:

Collect 5 ml of patient's blood, (2ml) with EDTA tube and (3ml) with plain tube for serum obtaining & 2 ml of donor blood.

1- Do blood grouping (blood type) and Rh test for patient and donor blood (or blood bottle).
2- Collection patient's serum.
3- Prepare RBC suspension (5%) by Taking 2 ml of donor and patient's blood, washing by normal saline.
4- Prepare 3 tubes, two (A, B) for matching, and the other (C) for patient.
5- Add 2 drops of patient's serum to all tubes.
6- Add 2 drops of donor RBC suspension to tube A&B.
7- Add 2 drops of patient's RBC suspension to tube C.
8- Add 3 drops of bovine albumin to all tubes.
9- Keep tube A at room temperature for 45 min.
10- Incubate tube B&C at 37c for 30 min.
11- After incubation, washing tubes three times by normal saline.
12- Add 2 drops of anti-human globulin to all tubes and putting it in centrifuge at 1000rpm for 30 sec.

13- Then reading the result:

- Presence agglutination in tube B means incompatible
- Non agglutination in tube B means compatible
- Agglutination in tube **A** only refers to presence cold Ab in patient's blood and the blood can transferred to patient only after warmed to 37c.
- Agglutination in tube **C** means that patient infected with **Autoimmune Hemolytic Anemia** the blood can transferred to patient if tube B is –ve.

## Conclusion:

## In case of compatibility

There is no reaction between Abs of patient's serum and Ags of donor RBC, after washing, the Abs are removed. When adding AHG (that function was to bind with Abs of patient's serum) which removed by washing so the result show non-agglutination.

## In case of in compatibility

Abs of patient's serum bind (agglutinate) to Ags of donor RBC and after washing, the Abs cannot remove, because it is binding to it's Ags. After adding AHG it bind to Abs of patient's serum that binding firstly with Ags of donor RBC. So agglutination appears clear and the result is incompatibility.

## Note:

In an emergency, when there is not enough time for blood typing and cross matching, O red blood cells may be given, Rh-negative. (O-) type blood is called the universal donor because it has no A,B antigens for a patient's antibodies to combine with. In contrast, AB blood type is called the universal recipient because it has no A,B antibodies to combine with the antigens on transfused red blood cells. If there is time for blood typing, red blood cells of the recipient type (type-specific cells) are given. In either case, the cross-match is continued even though the transfusion has begun.

## Cross match procedure

Collect 5 ml of patient blood

3ml for serum collection

2ml in EDTA tube for RBC collection.

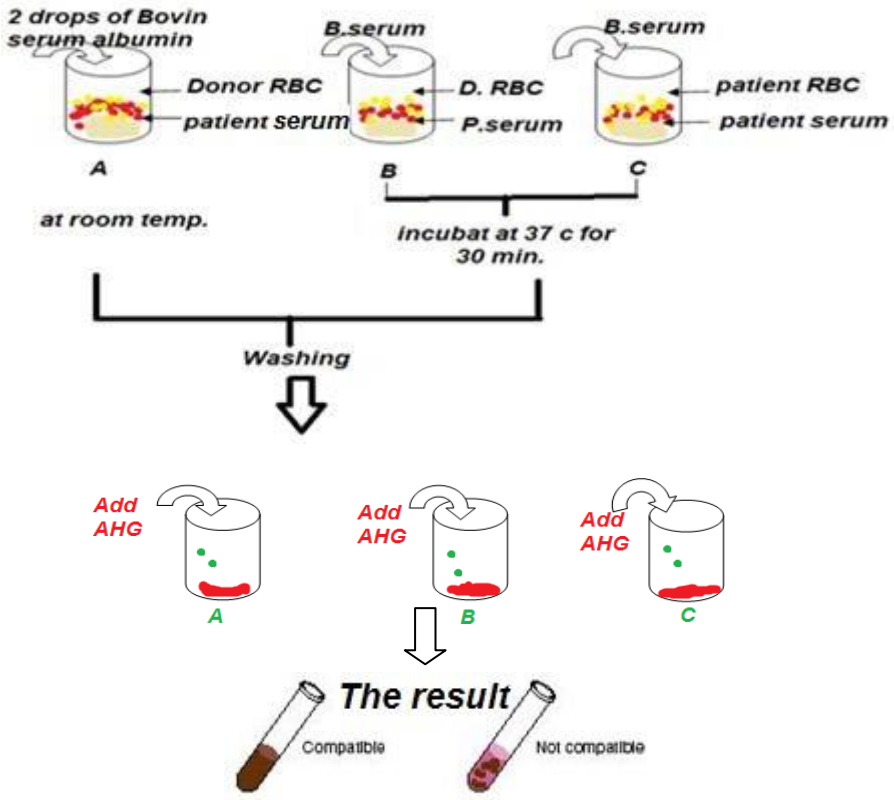
Washing RBC

Prepare 5% RBC suspension

(50µl RBC+950µl normal saline)

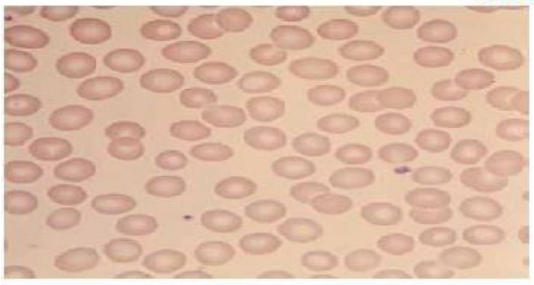
2ml of donor blood

For Prepare 5% RBC as:

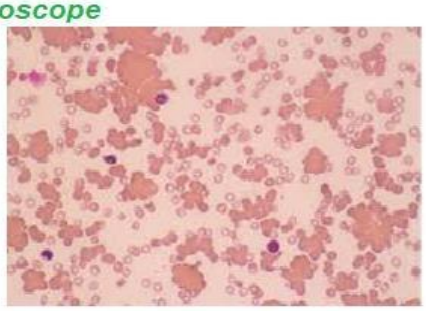



**The result of tube B**

*Under microscope*



*compatible - non agglutination*



*Non compatible - Agglutination*