Lec 05
Blood Components & Plasma derivatives

Assist. Prof. Dr. Mudhir S. Shekha
Definitions

**Blood product**: Any *therapeutic substance* prepared from human blood

**Whole blood**: *Un separated* blood containing *anti-coagulant* preservative

**Blood component**: A *constituent* of blood, separated (component from whole blood *separation*) eg., red cells, platelets, FFP

**Plasma derivative**: Human plasma proteins prepared (*fractionation*) under pharmaceutical manufacturing conditions eg., albumin
Whole Blood

- Is human **blood** from a standard blood donation **collected** into bag containing **anticoagulant**
  (contains 63 ml of anticoagulant + 450 ml blood)

  Anticoagulant ratio is 1.4 ml:10ml blood (63ml / 450ml)

**anticoagulant**

1) Acid-Citrate-Dextrose (ACD)
2) Citrate-Phosphate- Dextrose (CPD)
3) Citrate,Phosphate,Dextrose,Adenine (CPDA-1)
WB Anticoagulants

- ACD & CPD preserve the unit → 21 days at 2-6°C.
- CPDA-1 (anticoagulant/preservative → 35 days).
- C = Citrate → to prevent clotting
- P = Phosphate → to maintain pH
- D = Dextrose → ATP generation
- A = Adenine-1 → substrate from which RBC produce ATP
- SAGM= Saline-Adenine-Glucose-Manitol → to improve RBCs storage viability till 42 days @ 2-6°C
• **Anticoagulant** - any substance that prevents blood clotting

• **Preservative** - any substance added to a specimen to prevent changes in the constituents of a specimen

• **storage lesion** refers to biochemical and biomechanical changes in red cells during **storage**
## History of development of anticoagulants

<table>
<thead>
<tr>
<th>YEAR</th>
<th>PERSON</th>
<th>ANTICOAGULANT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1869</td>
<td>Braxton Hicks</td>
<td>Sodium Phosphate</td>
<td>1st example of blood preservation research</td>
</tr>
<tr>
<td>1914</td>
<td>Hustin</td>
<td>Sodium Citrate</td>
<td>Citrate used 1st time</td>
</tr>
<tr>
<td>1915</td>
<td>Lewisohn</td>
<td>Sodium Citrate</td>
<td>Determined minimum amount needed for anticoagulation and demonstrated non toxicity in small amounts</td>
</tr>
<tr>
<td>1916</td>
<td>Rous and Turner</td>
<td>Citrate-dextrose</td>
<td>1st anticoagulant</td>
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<tr>
<td>1917</td>
<td>Robertson</td>
<td>Citrate-Dextrose</td>
<td>Used RT solution to build blood bank in Harvard medical unit</td>
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<tr>
<td>YEAR</td>
<td>PERSON</td>
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<tr>
<td>1943</td>
<td>Loutit and Mollison</td>
<td>(ACD)Acid citrate dextrose</td>
<td>Lowered the ph to 5 to make ACD</td>
</tr>
<tr>
<td>1957</td>
<td>Gibson</td>
<td>Citrate phosphate dextrose</td>
<td>Cells passively lose phosphate, so by adding phosphate this loss can be prevented</td>
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<tr>
<td>1957</td>
<td>Gabrio and colleagues</td>
<td>Inclusion of nucleotides</td>
<td>Important for red cell metabolism</td>
</tr>
<tr>
<td>1968</td>
<td>Shields</td>
<td>CPDA-1</td>
<td>Showed that Adenine (preservative) markedly improved WB storage</td>
</tr>
<tr>
<td>1970</td>
<td>Beutler</td>
<td>Concept of additive system</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>Hogman</td>
<td>(SAG)Saline, adenine, glucose</td>
<td>5 weeks storage without adverse effects of high ph</td>
</tr>
<tr>
<td>1980</td>
<td>Lovric</td>
<td>CP2D in primary bag with additive solution composed of SAG, sodium citrate, citric acid and sodium phosphate</td>
<td></td>
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<tr>
<td>1985</td>
<td>----</td>
<td>SAGM</td>
<td>Shelf life reduced to 42 days</td>
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</table>
BLOOD COMPONENTS

• Human blood consists of **plasma** in which **cells** are **suspended**

• Blood separated into different parts:
  1. Packed red blood cells
  2. washed PRBC
  3. leukodepleted PRBC
  4. Pooled or aphaeresis platelets
  5. Fresh frozen plasma
  6. Cryoprecipitate
  7. Granulocytes
  8. Factor IX conc.

• There are more than 20 different products available
Whole blood

- Red cells
- Granulocytes
- Plasma
- Platelets

(Fresh) frozen plasma (FFP)
- Cryoprecipitate
- Stored Plasma

Fractionated products
- F VIIa
- F VIII
- F IX
- Immuno-globulins
- Albumin
Purpose Blood Components Separation

• Decrease risky effects of blood transfusion.

• Giving patients specific component required.

• Allow a longer survival for components.

• More than one patient will use the unit.
There are two types of centrifugation:

- **Light spin;** short time, low RPM (2000 rpm at 20\(^\circ\)C for 11 min)
- **Heavy spin;** Longer spin, high RPM (3500 rpm at 20\(^\circ\)C More than 11 min)
**Blood Components**

- **Blood components**
  - **Oxygen carrying components**
    - Red cell concentrates (RCC)
    - Leukocyte poor blood
    - Frozen-thawed red cells
  - **Platelet products**
    - Platelet rich plasma (PRP)
    - Platelet concentrates (PC)
  - **Plasma products**
    - Fresh frozen plasma (FFP)
    - Frozen plasma (FP)
    - Cryoprecipitate
    - Stored plasma

- **Plasma Derivatives**
  - **Coagulation Factor concentrates**
    - Factor VIII concentrates
    - Factor IX complex concentrates & others
  - **Oncotic agents**
    - Albumin
    - Plasma protein fraction (PPF)
  - **Immune serum Globulin**
    - Hepatitis B Ig (HBIG)
    - Varicella-zoster Ig (VZIG)
    - Rh Ig (RhIG)
    - Tetanus Ig (TIG)
A- Blood components that carry oxygen

- Increase the **oxygen** carrying **capacity** of the blood → increasing the circulating **red blood cell mass**.

1- Packed red blood cell (PRBCs)

- **RBCs** have **higher** specific **gravity** than **plasma**, it moves to lower portion of the bag by centrifugation. WB (Light spin) → Two products:
  1) PRBCs
  2) Platelet Rich Plasma (PRP)

Whole Blood Unit

After centrifugation

**WB** separates **into**

**plasma & platelets**

**& PRBCS**
1- Red blood cell concentrates

• After centrifugation → **removing** ≈ 200 ml of **plasma** from whole blood RBCs + **100 ml** of residual **plasma**.

• In CPD-A can be stored for 35 days at 4°C. Expir→opened → 24 hr

• **EFFECT**: restoring oxygen carrying capacity

• **INDICATIONS**: anemic conditions with hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Whole Blood</th>
<th>Red cell concentrate</th>
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<tbody>
<tr>
<td><strong>Total Volume</strong></td>
<td>500 ml</td>
<td>300 ml</td>
</tr>
<tr>
<td><strong>Volume of red cells</strong></td>
<td>200 ml</td>
<td>200 ml</td>
</tr>
<tr>
<td><strong>Volume of plasma</strong></td>
<td>300 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
<td>40 %</td>
<td>70 %</td>
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</table>

• High **hematocrit** (55 - 65 % ) → **viscous** → infuse **slowly**. The Rate of infusion increased by adding **saline**

• Other fluids should not used → **Calcium** containing fluids (eg. Ringer’s lactate) should not be added → May cause **clotting**
  • **Glucose** solutions → can cause **clumping**

• Only **saline** can be added to blood
2- Leukocyte poor blood

- All leukocytes → dead

- Indication
  - To prevent febrile reactions from leukocyte antibodies
  - To treat immunocompromised patients
  - To restore RBCs to patients who have had two or more non-hemolytic febrile reactions

- WBCs can be removed by discarding the buffy coat (inverted centrifugation). Or by washing RBCs or by using filters

- Leukocyte Reduction Filters (maintains closed system)
3- Frozen-thawed red cells

- The RBC's are first **incubated** in a 40% **glycerol** solution which acts as an "**antifreeze**" within the cells. The units are then placed in special **sterile containers** in a **deep freezer**. **Cryopreserved** units are **thawed** and washed free of glycerol prior to use as saline suspended RBC's.

- Red cells can be frozen with use of **cryopreservation** techniques. preserved → at -65°C for up to **10 years**. & -120°C in low glycerol for **3 years**. both can be used after **thawing** for **24 hrs** if stored at 2-4°C

- Expensive& recommended → **rare blood types** and **auto-transfusion**.

**Deglycerolized RBCs**
- RBCs that have had the glycerin removed
- Thawed at 37°C
- A blood cell processor washes the cells with varying concentrations of saline
- Considered “open”, expires in 24 hrs.
- See [https://www.youtube.com/watch?v=lsq8QEcry1E&t=10s](https://www.youtube.com/watch?v=lsq8QEcry1E&t=10s)
4- Washed RBCs

• Washing of red cells removes 70 – 95% of leucocytes and there is associated 15 – 20 ml of RBC’s but aim effective in removal of plasma proteins and microaggregates.

• HEMATOCRIT: < 70%

• Indications:

  - Patients having recurrent attacks of Febrile non-haemolytic transfusion reactions and urticarial reactions.
4- Washed RBCs

- Patients who have developed antibodies to plasma proteins (anaphylaxis)
- IgA deficient patients who has developed anti IgA (IgA deficiency prevalent in 1 in 700 persons)
- (Paroxysmal nocturnal hemoglobinuria) PNH / removal of complement factors
5- Irradiated RBCs

- Gamma radiated (2500 rad for 4 min) to **destroy T-Lymphocytes** to prevent **graft versus host** disease (fatal 90%) in severely immunocompromised, Stem cells /Bone marrow transplant Lymphoma & intrauterine transfusions. Donor units from a blood relative, HLA-matched donor unit, Immunodeficiency, Premature newborns, Chemotherapy and irradiation

Matching **donors** with **patients**.

![Before Irradiation](image1.png) ![After Irradiation](image2.png)
6- Synthetic oxygen carrying agents

• Perfluorochemical (e.g. Fluosol-DA) & hemoglobin-based oxygen carriers
  • Fluorinated hydrocarbons
  • Freely dissolve oxygen
  • Poor soluble in plasma
  • Side effects:
    • Hypotension
    • Disseminated intravascular coagulation
  • For Athletes (heart disease, stroke, heart attack, blood clot.)

• Chemically modified hemoglobin
  • Free Hb has a very short half life?
  • Chemically modified to:
    • increase intravascular survival
    • and to make it more effective in carrying oxygen
B- Platelets

- Important in maintaining hemostasis
- INDICATIONS: Cancer patients, Bone marrow recipients & Postoperative bleeding
- How platelets are processed → Requires 2 spins:
  - Soft – separates RBCs and WBCs from plasma and platelets
  - Heavy
    - platelets in platelet rich plasma (PRP) will be forced to the bottom of a satellite bag
    - 40-60 mL of plasma is expelled into another satellite bag, while the remaining bag contains platelet concentrate
Preparation of platelet concentrate

RBCs → PRP → Plasma → Platelet concentrate
B- Platelet Products

- Platelet Rich Plasma (PRP)
  - Gentle **centrifugation** of whole blood
  - Supernatant **transferred** to the 2\textsuperscript{nd} bag

- Platelet Concentrates
  - Prepared from PRP by a 2\textsuperscript{nd} centrifugation
  - Removal of all but 50 ml of plasma
  - Contain approx. $6 \times 10^{10}$ platelets
  - 60 – 80\% Plts present in whole blood unit

- good survival 8-10 days & Storage→ in plastic bag on 20 - 22 °C with gentle shaking
Whole blood unit

Centrifuge using **LIGHT** spin

Express Platelets Rich Plasma (PRP) into satellite bag

Take PRP and centrifuge again now using **HEAVY** spin

Express PPP (Platelet poor plasma) into satellite bag & freeze at -18ºC

Final products: PRBCs, Platelets concentrate, FFP
B- Platelet Products

• **Contamination** by WBCs & RBCs is usually **small**

• But there is enough to induce **alloimmunization**

• Platelet concentrates from Rh **+ve** should **not be administered** to Rh **–ve** women

• Storage at 22°C, therefore care to prevent contamination
1. Pooled platelets

- The platelets are collected from a number of donations (4 separate donors) these are then pooled together to form one unit (>160mls)
- Leucocyte depleted and suspended in predominately platelet additive solution (PAS) and a small amount of plasma
2. Apheresis platelets

• Apheresis involves removing the donor’s blood, spinning it in an apheresis machine, collecting the platelets and returning the other parts of the blood to the donor.

• Collected from a single donor via apheresis (adult bag 100–400mls). Apheresis donations are often available in paediatric splits of 4 or 8 (40–60mls)

• Leucocyte depleted and suspended in plasma
C- Plasma Products

- Platelet poor plasma can be separated into a number of products
  1. Fresh frozen plasma
  2. Platelet concentrate
  3. Frozen plasma
  4. Cryoprecipitate
  5. Stored plasma
1- Fresh frozen plasma (FFP)

• Prepared from whole blood within **6 hours** of collection

• **Rapid freezing** of plasma preserves (CPD) the labile coagulation factors at maximum levels → like fresh plasma → all clotting factor / VIII, V, too → labile factors/ complement plasma proteins

• Don't contain **cellular elements**

• **200** ml volume

- **Freeze at -18°C** for **1 year** from collection date.
  - -30 C° → 2 years

- Or freeze at **-70°C** for up to **7 yrs**

- **Cross match** is **not** required, but of coarse should be ABO compatible.
Indications of FFP

1. **severe bleeding** in unknown factor deficiency (Bleeding, Abnormal clotting

2. **complex coagulation factor deficiency** / DIC, severe liver lesions, liver transplantation, massive transfusion, blood replacement after bleeding & Severe burns

3. **congenital factor deficiency**, if no missing factor concentrate /Antithrombin III, C1-esterase inhibitor, Factor V / 4 TTP - plasma exchange / 3 L / day.
2- Platelets Concentrate (PC)

- Platelet Rich Plasma (PRP) centrifuged using (heavy spin), this will produce:
  1) Fresh frozen plasma (FFP)
  2) Platelets concentrate (PC)

- PC are stored at room temperature on platelet agitator → prevent clumping

- PC stored for 5 days at 20-24°C.

- Each unit should raise the platelet count by 5000/µL

- Indications:
  1. To prevent bleeding due to thrombocytopenia or platelet dysfunction
  2. To a patient undergoing an operation, if the platelet count is less than 20,000/µL
3- Frozen Plasma (FP)

- **Separated** from whole blood within **24 hours of collection**
- Contains at least **50 % of original factor VIII & factor V**
- Adequate source for treatment of mild to moderate **coagulation factor deficiencies**
- **200 ml volume**
- Storage at **-30°C** for up to **12 months**
4- Cryoprecipitate

- Produced from **freshly** separated **plasma** by freezing at -70\(^\circ\)C followed by thawing at 4\(^\circ\)C
- **Flocculent precipitate** is rich in **factor VIII**, **fibrinogen** and **fibronectin**
- Once **thawed**, mixture is **centrifuged** to sediment the **cryoprecipitate** & all but **5 to 10 ml of supernatant plasma** is removed
- Contains **250 mg fibrinogen**
- **80 clotting units of factor VIII**
- Stored at **-30\(^\circ\)C** for **12** months
• Increase of 2% of factor **VIII** level for each bag of cryoprecipitate infused

• **Supernatant plasma removed** is called **stored plasma**
  • Must be used within 5 weeks if stored at 4°C
  • Lasts for 2 years at -30°C

• **Indications:**
  • Hemophilia A
  • Von Willebrand disease (VWD)
  • Congenital or acquired fibrinogen defects (i.e., dysfibrinogenemia)
5- Stored plasma

- **Plasma** separated from **whole blood** after **24** hours of storage at **4°C**
- Can also be **derived** from **cryoprecipitate** production
- Contain reduced levels of labile coagulation factors **V, VIII & fibrinogen**
- It is indicated for patients requiring **volume expansion** or **protein replacement** when labile clotting factors are not required
- Plasma products do **not** require **crossmatch** prior to use but should be **ABO compatible**
## Summary
### Blood Components

<table>
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<tr>
<th>Blood Component</th>
<th>Centrifugation</th>
<th>Storage</th>
<th>Indication</th>
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</table>
| **1) PRBCs**    | WB Light spin=2000rpm-20ºC -11min. PRBCs + PRP | 2-6ºC +SAGM 42d | • Anemia  
• Newborn exchange transfusion |
| **2) PC**       | PRP heavy spin=3500rpm-20ºC -11min. PC + FFP | R.T 3-5 d | • Bleeding  
• Operation if plt. Less than 20000/µl |
| **3) FFP**      | -18ºC  
-65ºC | 1 year  
7 years | • Clotting factor deficiencies  
• Severe burns |
# Summary

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</table>
| 4) Cryo    | a. WB special heavy spin= 3500rpm at 4°C - 11min. → RBC + Plasma  
    b. Plasma → store at -18 °C then thaw at 4 °C then heavy spin at 4°C | -30°C  
    1 year | • Hemophilia A  
    • Von Willebrand disease |