BLOOD BANK - IMMUNOHEMA

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HISTORICAL REVIEW

- **1492** - Transfusion from the three young men to Pope Innocent VII, happened but unfortunately all are unsuccessful.
- **1869** - attempt to nd a non toxic anticoagulant began
- **Braxton Hicks** - recommend sodium phosphate as the 1st example of blood preservatives
- **Karl Landsteiner** - in 1901 discovered the ABO blood groups.
- **Edward Lindermann** - carried out the vein to vein transfusion of blood using multiple syringes and special cannula.
- **Unger** - designed the syringe-valve apparatus that transfused from donor to patient.
- **1914** - Hustin uses sodium citrate as anticoagulant solution for transfusion
- **1915** - Leweisohn determined the minimum amount of citrate needed for anticoagulant.
- **1916** - glucose was tried as preservatives.
- **Rous & Turner** introduced the citrate-dextrose solution for preservation of blood.
- **Dr. Charles Drew** - developed the technique of blood transfusion & blood preservation that led to the establishment of the system of blood banks.
- **1941 -** Dr. Drew was appointed as director of the 1st American Red Cross blood bank.
- **1943 - Loutit & Mollison of England** introduced the formula for the preservative acid-citrate dextrose (ACD).
- **1957 -** Gibson introduced an improved preservative solution called citrate phosphate- dextrose, less acidic and replaced ACD as standard preservative.

BASICS OF BLOOD DONATION

- **AABB (American Association of Blood Banks)**
  - Provides International standards in Blood Transfusion Practices
- **DOH-NVBSP (National Voluntary Blood Services)**
  - Created thru National Blood Services Act of
  - 1994 or R.A. 7719
  - Provides domestic standards for Blood Transfusion Practices
- **PNBS (Philippine National Blood Services)**
  - Created in 2005
  - Formulated A.O. 2008-008 which provided rules and regulations governing blood service facilities

BLOOD TO ANTICOAGULANT RATIO

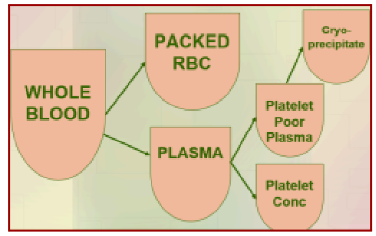
- **TRADITIONAL:**
  - 450 mL (±10%) Blood to 63 mL AC
  - 1:7
  - 429-583 grams (Blood + Bag)
- **MODIFIED:**
  - 500 mL (±10%) Blood to 70 mL AC

BLOOD VOLUME

- For 110 lbs (50 kg) donor – a maximum of 525 mL
- **Blood Volume of Normal Adult: 10-12 pints (5-6 L)**
- Normal Donor can replenish 1-pint donation in 24 hours
- Donor can donate whole blood every 8 weeks (2 months)

BLOOD COMPONENTS

- Whole Blood can be separated into components Packed RBC and Plasma thru Heavy Spin (5,000 x g for 5 mins in a refrigerated centrifuge)
- Plasma can be further separated into Platelet Concentrate and Platelet Poor Plasma thru Light Spin (2 to 3 mins at 3,200 rpm)
- Whole Blood/RBC can last for 21 to 42 days
- 30 days (1 month) – Philippine Standard
- Plasma can last for 1 year (frozen at -25°C)
- Platelet Conc – 5 days with constant agitation



DONATION PROCESS

- **Before Donation:** pep talk, donor's questionnaire, physical exam, check the hematocrit and hemoglobin.
- **During donation:** blood will be taken not more than 450 ml
- **After donation**: rest for 10-15 mins

Table 1–1 Current Donor Screening Tests for Infectious Diseases

TEST	DATE TEST REQUIRED
Syphilis	1950s
Hepatitis B surface antigen (HBsAg)	1971
Hepatitis B core antibody (anti-HBc)	1986
Hepatitis C virus antibody (anti-HCV)	1990
Human immunodeficiency virus antibodies (anti-HIV-1/2)	1992[1]
Human T-cell lymphotropic virus antibody (anti-HTLV-I/II)	1997[2]
Human immunodeficiency virus (HIV-1)(NAT)*,**	1999
Hepatitis C virus (HCV) (NAT) **	1999
West Nile virus (NAT)	2004
Trypanosoma cruzi antibody (anti-T. cruzi)	2007

RBC BIOLOGY AND PRESERVATION

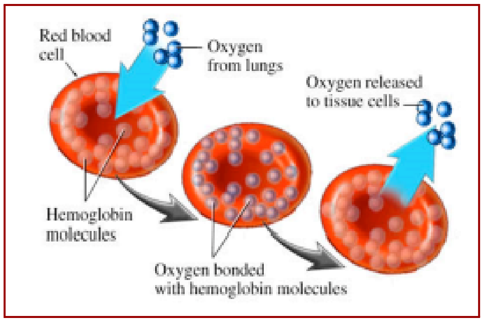
- **Crucial Areas for RBC Survival and Function:**
  - Normal Chemical Composition of RBC Membrane
  - Hemoglobin Structure and Function
  - RBC Metabolism
    - ➔ notes: defect in any or all of these areas will results in RBC surviving less than the normal 120 days

RBC (Erythrocytes)

- **Shape –** biconcave disc with large surface area
- Can change **shape**
- **No Nucleus/Organelles**
- Contains Hemoglobin
- Primary Function: Transport Oxygen from the lungs to the cells of the body & assist with CO2 removal
  - ➔ notes: RBC membrane represents a semipermeable lipid bilayer supported by a mesh like protein, cytoskeleton structure - phospholipid is the main lipid component of the membrane.
    - integral protein
    - peripheral protein
- Lack intracellular organelles necessary for cellular repair, growth, division
- **Short Life Span (~120 days)**
  - Aged RBC
  - Fragile - prone to rupture
- **Ruptured RBCs** are destroyed in spleen
  - Phagocytic WBCs "clear the debris"

MECHANISM OF TRANSPORT

- 4 Heme Molecules = 4 Oxygen Molecules
- Oxygenated Hemoglobin
  - Bright Red (systemic)
- Deoxygenated Hemoglobin
  - Blue (venous circulation)
- ➔ notes: Minutia - Every erythrocyte has more than 250 million hemoglobin molecules - which means each RBC can carry more than a billion oxygen molecules



CHANGES OCCUR DURING STORAGE

- **Shelf life =** expiration date
  - At end of expiration must have 75% recovery
  - At least 75% of transfused cells remain in circulation 24 hours AFTER transfusion

GOALS OF BLOOD COLLECTION

- Maintain viability and function
- Prevent physical changes
- Minimize bacterial contamination

STORAGE OF BLOOD

- Series of changes occur in vitro that alters the physiological properties
- To ensure that blood retains its in vivo environment involves
  - Anticoagulants
  - Preservative
  - Characteristics of plastic bag
  - Storage temperature
  - Shipping and transport conditions

ANTICOAGULANTS AND PRESERVATIVES FOR BLOOD PRODUCTS

- **PURPOSE:**
  - To ensure the viability and stability of the products
  - To inhibit growth of microorganisms
  - To prevent clotting of the product

CITRATE

- Calcium-chelating agent
- Prevents coagulation by interfering with calcium-dependent steps in the coagulation cascade

- **Acid-Citrate-Dextrose (ACD)**
  - Contains citric acid, sodium citrate, and dextrose
  - Shelf life of 21 days and now no longer use for red cells as other solution are available with extended shelf life of red cells
  - Acid pH does not help in maintaining 2,3-DPG levels
  - Used in apheresis procedure

- ➔ notes: 2,3 DPG is an organic phosphate normally found in the RBC that has a tendency to bind with hemoglobin and thereby decrease the affinity of hemoglobin for oxygen Diphosphoglycerate

- **Citrate-Phosphate-Dextrose (CPD)**
  - Alkaline pH helps in maintaining 2,3-DPG
  - Shelf life is extended to 28 days
  - CPD is not now commonly used

- **Citrate-Phosphate-Dextrose-Adenine (CPDA-1)**
  - Addition of adenine is associated with improved synthesis of ATP, allowing longer shelf life (35 days)

- **Other Solutions**
  - Adsol or AS-1- Baxter Healthcare
  - Nutricel or AS-3 – Pall Corp
  - Optisol or AS-5 – Terumo

- ➔ notes: preserving solutions that are added to the RBC after removal of plasma with or without platelet

ADDITIVE SOLUTION (AS)

- Primary bag with satellite bags attached
- One bag has additive solution (AS)
- Unit drawn into CPD anticoagulant
  - ➔ notes: advantages
    - 1. extend the shelf life of RBC's up to 42 days by adding nutrient
    - 2. allows for the harvesting of more plasma and platelets from unit
    - 3. Produces RBC concentrate of lower viscosity that is easier to infuse.

- Remove platelet rich plasma within 72 hours
- Add additive solution to RBCs, ADSOL, which consists of:
  - Saline
  - Adenine
  - Glucose
  - Mannitol

- Extends storage to 42 days
- Final Hematocrit approximately 66%

SALINE-ADENINE-GLUCOSE-MANNITOL (SAGM)

- After taking blood donation in CPD and separating red cells from plasma and platelets SAGM is added to the packed red cells
- The resulting red cells have flow characteristics equivalent to plasma reduced blood and a storage life of 35-42 days
- Other advantage is by removing maximum amount of plasma from blood for the manufacture of factor VIII and albumin

Table 1–3 Approved Anticoagulant Preservative Solutions

NAME	ABBREVIATION	STORAGE TIME (DAYS)
Acid citrate-dextrose (formula A) *	ACD-A	21
Citrate-phosphate dextrose	CPD	21
Citrate-phosphate-double-dextrose	CP2D	21
Citrate-phosphate-dextrose-adenine	CPDA-1	35

Table 1–5 Additive Solutions in Use in North America

NAME	ABBREVIATION	STORAGE TIME (DAYS)
Adsol (Baxter Healthcare)	AS-1	42
Nutricel (Pall Corporation)	AS-3	42
Optisol (Terumo Corporation)	AS-5	42

- Notes: AS1 & AS5 contain mannitol which protect against storage related hemolysis. AS3 contains citrate and phosphate

STORAGE CHANGES IN BLOOD

- In vivo, red cells are carried and protected by the plasma, which helps:
  - Regulate temperature
  - Control pH
  - Provide adequate glucose supply
  - Remove metabolic waste

- In protected environment life span is 110-120 days
- Lowering of temperature and preservatives help in reducing the changes but changes do occur and is known as “STORAGE LESION

pH

- Glycolysis is slowed at temperature of 1 to 60C
- Glycolysis results in the production of lactate and decrease in pH
  - Day 0 (CPD) pH = 7.20
  - Day 21 (CPD) pH = 6.84

ATP

- ATP is closely associated with red cell viability
- Loss results in rigidity and decrease deformability
- ATP is also needed for Na+/K+-ATPase pump
- ATP levels on day 35 of storage is 45% in CPDA-1
  - ➔ notes: ATP energy source of the cells ATPase pump is the transport of Sodium out of the cells and potassium inside the cell. When RBC ATP are depleted, Calcium, and sodium are allowed to accumulate intracellularly, and Potassium and water are lost. Resulting in dehydrated, rigid cells resulting in a decrease in RBC survival.

2,3 DPG LEVELS

- Decrease in pH of stored blood results in low 2,3-DPG levels
- With low levels there will be left shift of oxygen dissociation curve and increase quantity of oxygen
- After transfusion the levels return to normal values within 24 hours
  - ➔ notes: 2, 3 DPG binds with deoxyhemoglobin and facilitate hemoglobin transport to the tissue

Na+ and K+ levels

- Na+/K+ leak through the red cells
- Cells lose potassium and gain sodium
  - ➔ notes: RBC volume and water homeostasis are maintained by controlling the intracellular conc. Of Sodium and potassium
  - ATP is required for the transport of Sodium out of the cells & Potassium into the cells.
  - when ATP is depleted it allows the accumulation of Na++ intracellularly and K+ extracellularly, resulting to dehydrate

PLASMA HEMOGLOBIN

- Hemolysis results in raised plasma Hb

STORAGE LESION

- Biochemical changes which occur at 1-6C
- Affects oxygen dissociation curve, increased affinity of hemoglobin for oxygen.
  - Low 2,3-DPG, increased O2 affinity, less O2 released.
  - pH drops causes 2,3-DPG levels to fall
  - Once transfused RBCs regenerate ATP and 2,3-DPG

- Few functional platelets present
- Viable (living) RBCs decrease
- Significant for infants and massive transfusion.

- **Summary of biochemical changes:**
  - pH decreases
  - 2,3 DPG decreases
  - ATP decreases
  - Potassium increases
  - Sodium decreases
  - Plasma hemoglobin increases

Table 1–2 RBC Storage Lesion

CHARACTERISTIC	CHANGE OBSERVED
% Viable cells	Decreased
Glucose	Decreased
ATP	Decreased
Lactic acid	Increased
pH	Decreased
2,3-DPG	Decreased
Oxygen dissociation curve	Shift to the left (increase in hemoglobin and oxygen affinity; less oxygen delivered to tissues)
Plasma K^+	Increased
Plasma hemoglobin	Increased

REJUVENATION SOLUTIONS

- Red cells stored in the liquid state for less than 3 days after expiration date
- can be rejuvenated for 1 to 4 hours at 37 degrees Celsius

REJUVESOL

- Is the only FDA-approved rejuvenation solution
- **Consists of PIPA:**
  - Phosphate
  - Inositol
  - Pyruvate
  - Adenine

RED CELL FREEZING

- Primarily done for autologous units and storage of rare blood types
- Involves addition of cryoprotective agent to red cells that are less than 6 days old
- Glycerol is the most commonly used
- Glycerol is added to red cells slowly with mixing to permeate red cells
- Rapidly frozen and stored in freezer with:
  - High Concentration Glycerol 40% (commonly done)
  - Low concentration Glycerol 20%

Table 1–7 Advantages of High-Concentration Glycerol Technique Used by Most Blood Banks Over Low-Concentration Glycerol Technique

ADVANTAGE	HIGH GLYCEROL	LOW GLYCEROL
1. Initial freezing temperature	−80°C	−196°C
2. Need to control freezing rate	No	Yes
3. Type of freezer	Mechanical	Liquid nitrogen
4. Maximum storage temperature	−65°C	−120°C
5. Shipping requirements	Dry ice	Liquid nitrogen
6. Effect of changes in storage temperature	Can be thawed and refrozen	Critical

BLOOD SUBSTITUTE

- **Categories:**
  - Hemoglobin-based oxygen carrier
  - Perfluorochemicals (PFS)

REPLACEMENT FLUIDS (PLASMA SUBSTITUTE)

- Balanced Salt solutions - a solution of NaCl with electrolyte composition resembling ECF
  - NSS (0.9% NaCl)
  - Ringer's lactate
  - Hartmann's solution
- Colloid Solution

PLATELET PRESERVATION

PLATELETS

- Cellular fragments derived from the cytoplasm of megakaryocytes
- Do not contain NUCLEUS, although mitochondria contains DNA
- Released and circulate approx 9-12 days
- Small, disk-shaped cells, 2-4 µm
- NV: 150,000 – 350,000/ µL
- <10,000 – HEMORRHAGE
- >50,000 – minimize chance of hemorrhage
  - 30% - reserved in spleen
  - 70% - in circulation

ROLE OF PLATELETS

- Initial arrest of bleeding by platelet plug formation
- Stabilization of hemostatic plug
- Maintenance of vascular integrity

BASIC REQUIREMENT FOR PLATELET STORAGE

- Platelets should be stored at 22-24ºC (controlled temperature): Aging process slows down at 22ºC as compared to 37ºC
- Continuous gentle agitation must be maintained on a at bed agitator: if interrupted leads to rapid fall in pH due to lactate and decrease in O2 consumption pH should be above 6.0
- Plasma (50-60 ml) is needed for storage
- **Plasma provides a physiological environment for platelet storage. It provides:**
  - Bicarbonate buffer
  - Glucose for metabolic substrate
  - Inhibitors of coagulation activation

- In 1981, platelet storage was extended from 3 days to 5 days at room temperature due to improved platelet storage bags that allowed more efficient gas exchange.
- In 1984 this was further extended to 7 days. However, with the extended storage, an increased number of platelet transfusion-associated sepsis reactions were reported.
- In 1986, the storage time for platelets was changed back to 5 days.

PLATELET ADDITIVE SOLUTION (PAS)

- PAS are synthetic mediums introduced to replace a significant portion of plasma volume in a platelet component
- First developed in 1980s and continued to be improved over the following years
- PAS being used in Europe since 1991