HISTORY OF BLOOD BANKING

- In 1492, blood was taken from three young men and given to the stricken Pope Innocent VII in the hope of curing him. Unfortunately, all four died. Although the outcome of this event was unsatisfactory, it is the first time a blood transfusion was recorded in history.


EXERCITATIO
ANATOMICA DE
MOTV CORDIS ET SAN-
GVINIS IN ANIMALI-
BVS.
GVILIELMI HARVEI ANGLI,
Medici Regii, & Professoris Anatomiæ in Col-
legio Medicorum Londinens.
FRANCOFVRTI,
Sumptibus GVILIELMI FITZERI.
ANNO M. DC. XXVIII.

- The first research into blood transfusion dates back to the 17th Century when British physician William Harvey fully described the circulation and properties of blood in his *De Motu Cordis in* 1628. The first blood transfusions were also attempted around this time, although these were unsuccessful and proved fatal in humans.

- **The first successful blood transfusion** recorded was performed by British physician Richard Lower in 1665 when he bled a dog almost to death and then revived the animal by transfusing blood from another dog via a tied artery.

- In 1667, Jean-Baptiste Denis who was physician to King Louis XIV, performed the transfusion of blood from an animal to a human. Denis transfused the blood from a sheep to a 15-year old boy and later to a labourer, both of whom survived the transfusions.

- After being banned for more than 150 years, the use of blood transfusion was revived during the late 18th century.
- In 1818, the first successful **man to man blood transfusion** was performed by British obstetrician James Blundell. He successfully transfused human blood to a patient who had haemorrhaged during childbirth.

- In 1901, Karl Landsteiner, an Austrian physician **discovered the first human blood groups**, which helped transfusion to become a safer practice.

His work early in the 20th century won a Nobel Prize.

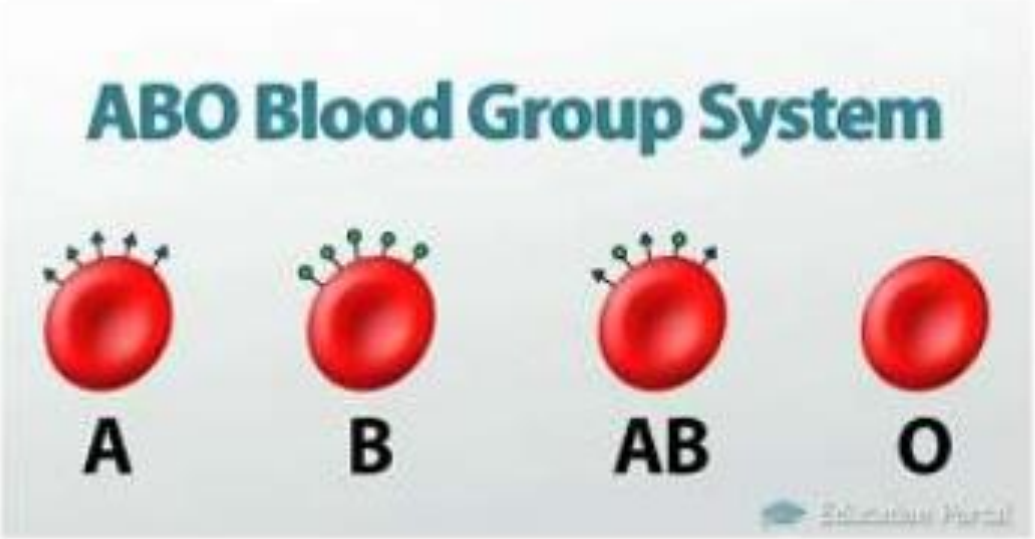
1901

Discovered by
Dr. Karl Landsteiner

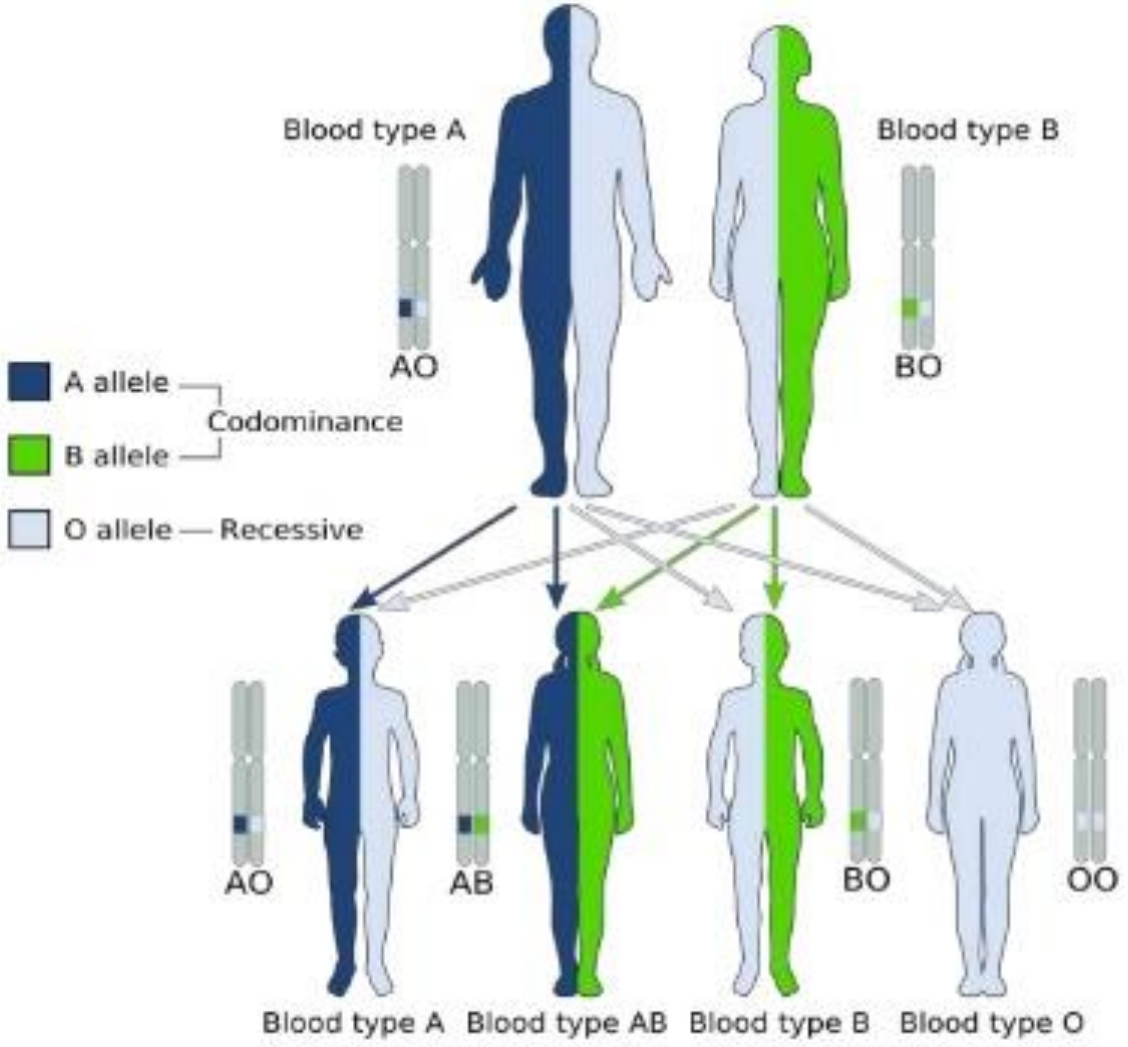
1940

Rh factor discovered along with Dr. Wiener

- In 1913, an American surgeon called Reuben Ottenberg suggested that patient and donor blood should be grouped and cross matched before a blood transfusion procedure. He conclusively demonstrated the importance of compatibility testing in his report of 128 cases of transfusion.



The inheritance pattern of blood groups was finally proved by Felix Bernstein in 1924.



In 1914, Albert Hustin reported the first human transfusion using citrated blood. He added sodium citrate and glucose to the blood to preserve it, and stop it from clotting.

- In 1936, Dr . Norman Bethune of Canada established the **first blood bank of the world** at Madrid in Spain during the Spanish Civil War .

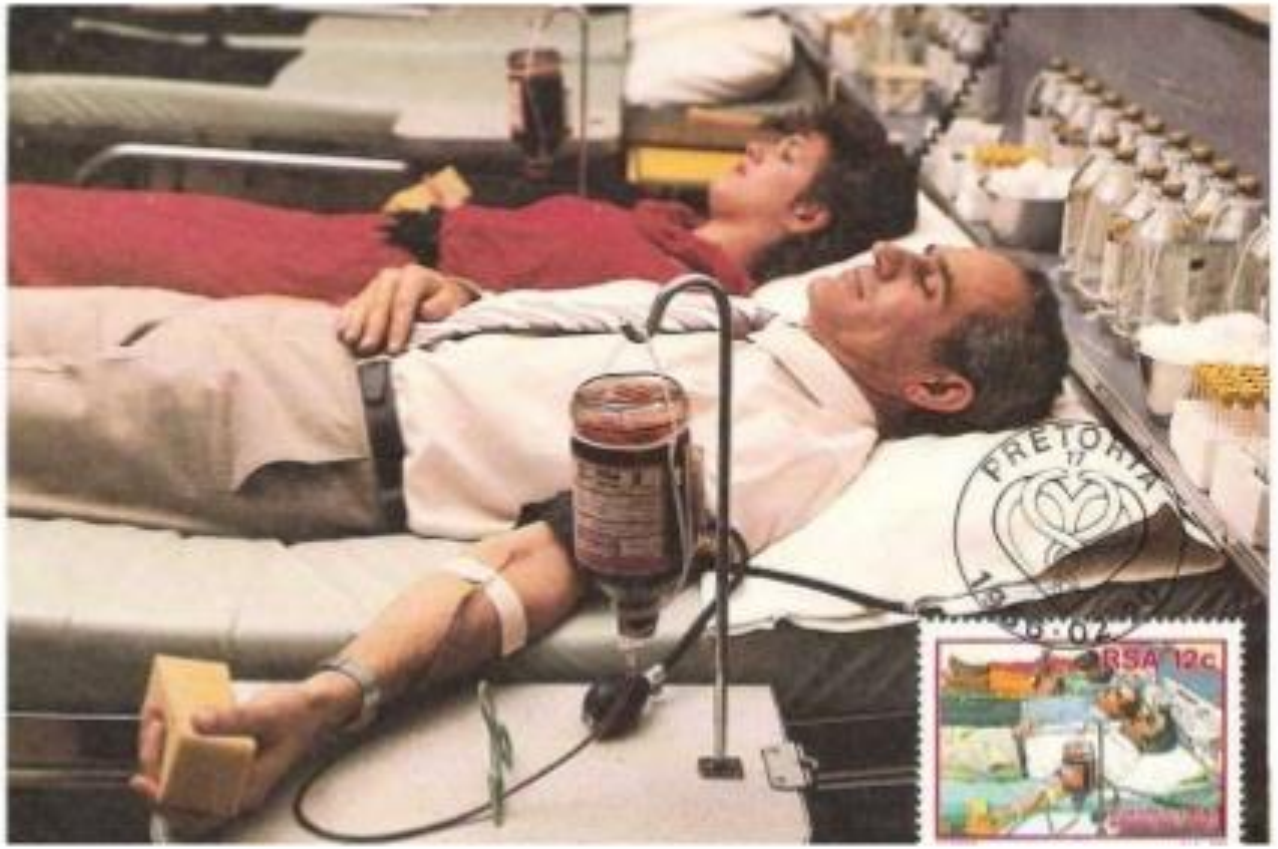


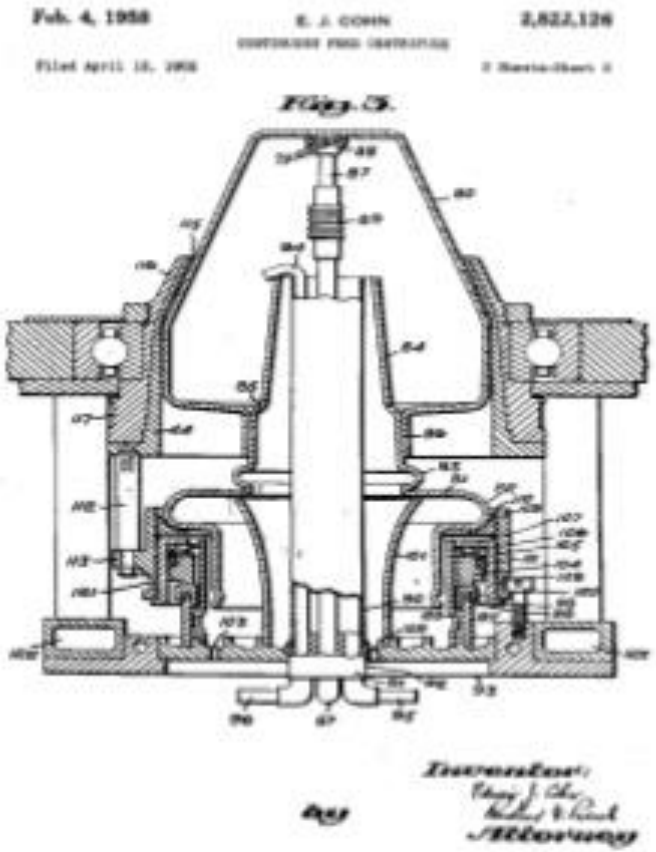
FIGURE 156.—Processed serum in cold storage at Calcutta Blood Bank, October 1944. Note British bottles, which were used by blood bank of India.

- In 1942, the **first Central blood bank of India** was established at Calcutta to meet the blood need of the war. It was situated at the site of the present All India Institute of Hygiene and Public Health, Calcutta.

FIGURE 73.—Edwin J. Cohn, Ph. D.

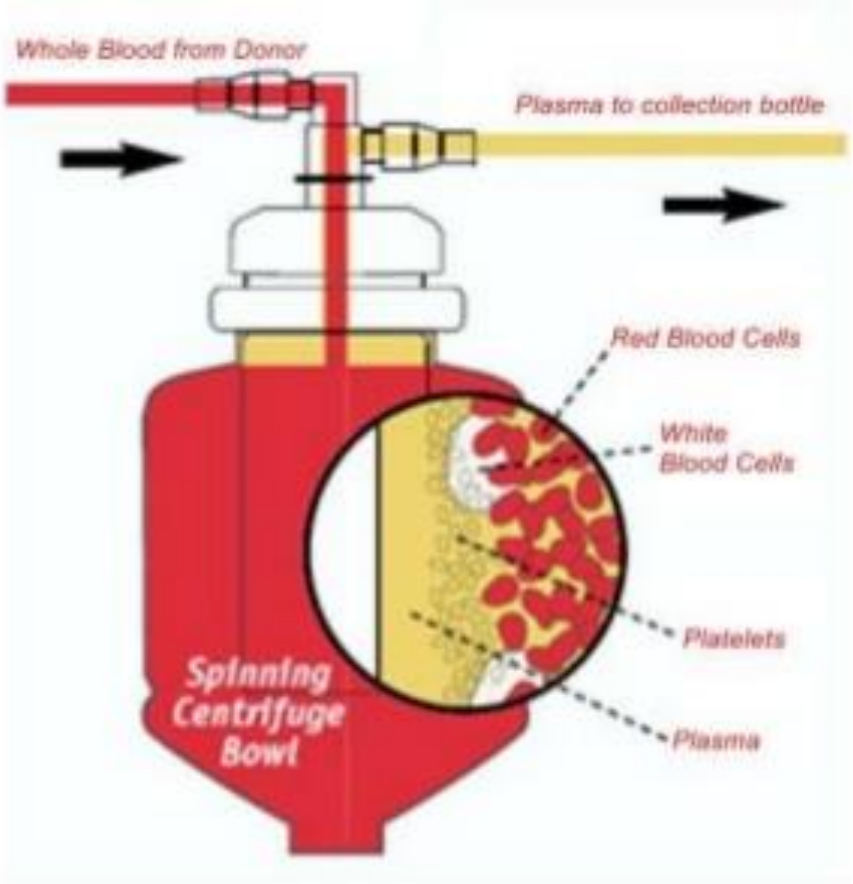
- In 1940, Edwin Cohn developed cold ethanol fractionation, the process of breaking down plasma into components and products. Albumin, gamma globulin and fibrinogen are isolated and become available for clinical use.

- Another advance in transfusion was development of the **first cell separator** in 1951 by Edwin Cohn; the cell separator allowed blood to be separated into red cells, white cells, platelets, and plasma.

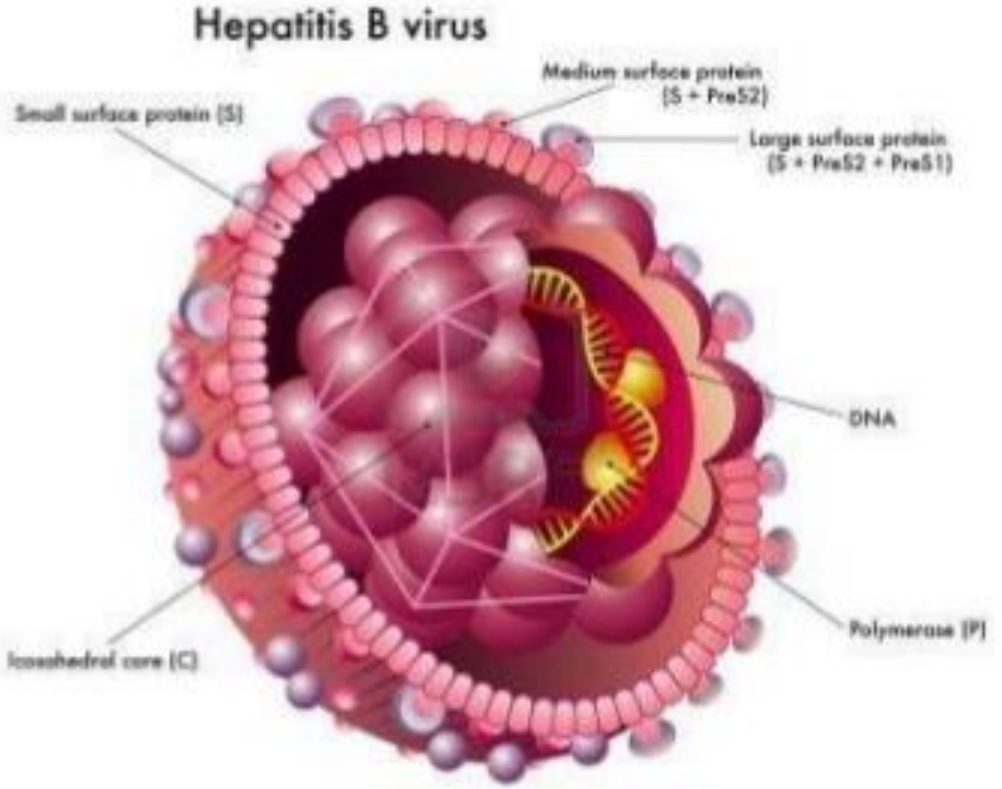


- Until the 1950s, blood was collected through steel needles and rubber tubing into glass, rubber-stoppered bottles, which were reused following washing and sterilization.
- In 1952, Carl Walter, a researcher under Harvey Cushing, and William Murphy described a system in which the blood was collected into a collapsible bag of polyvinyl resin.

- In **1981,** use of polyvinyl bags for collection, storage and transfusion of blood was legalised.



- In 1964, Plasmapheresis was introduced as means of collecting plasma for fractionation.

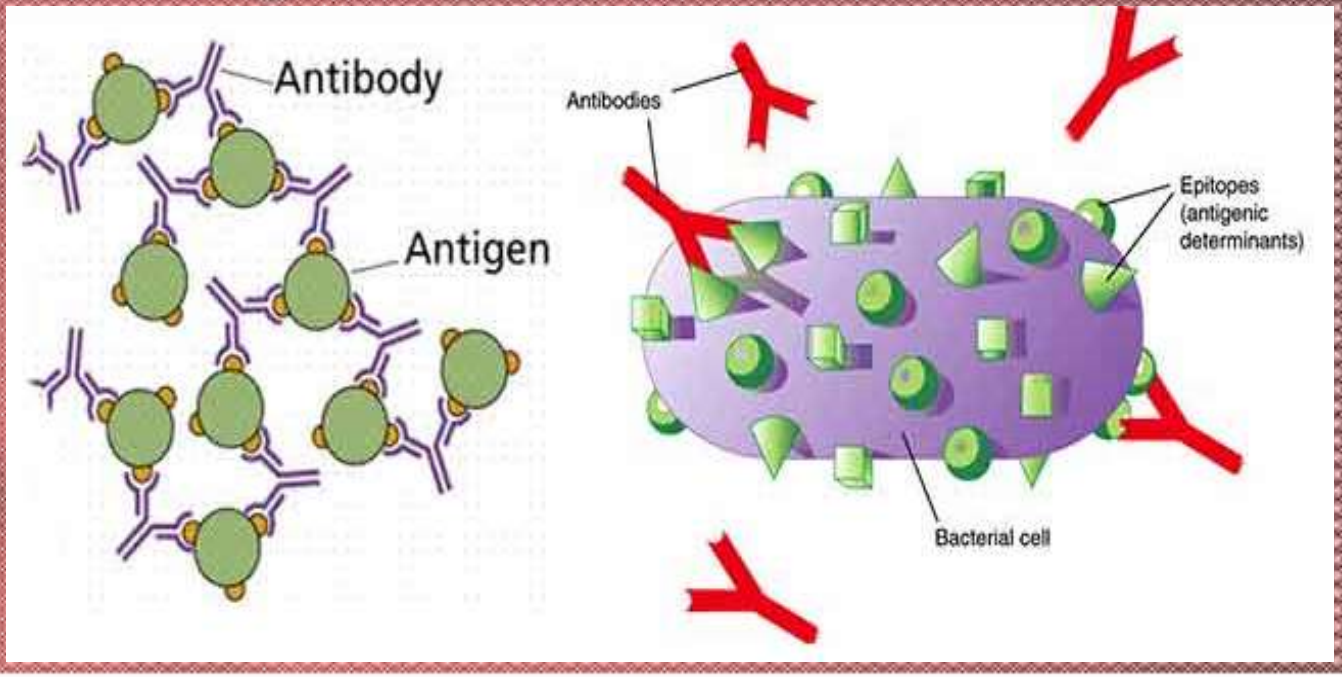


- In 1964, Infection of jaundice through blood transfusion was confirmed.
- In 1971, Hepatitis B surface antigen (HbsAg) testing of donated blood began.

- In 1986, first AIDS patient due to blood transfusion in Mumbai was reported.

Thank you!

Antigens and Antibodies



Antigen

- Any substance which, when introduced parentrally into the body stimulates the production of antibody specifically.
- This is a traditional definition since it had got some exception
  - Polio vaccine – oral administration
  - Some antigen may not produce antibodies
- **Specificity** is the hallmark of all immunological reactions

Function of Antigen

- **Immunogenicity :** induction of immune response
- **Immunological Reactivity:** specific reaction with antibodies or sensitized cells
- Based on this function antigen is classified as
  - **Complete antigen:** induce antibody formation and specific immune response
  - **Partial Antigen (Hapten):** a specific non-protein substance incapable of inducing antibody formation itself but elicit immune response when couple with **carrier protein**
    - **Complex hapten :** polyvalent – 2 or more antibody combining site
    - **Simple Hapten:** univalent

Structure of Antigen

- Smallest unit of antigenicity (antigenic detrminant) – **epitope.**
- Consist of 4-5 amino residues or monosacharide residues
- Posses a specific chemical structure, electric charge and spatial configration
- Capable of sensitizing of an imunocytes
- **Paratope:** area on antibody molecule on which epitope binds
  - This produces specificity of antigenic reaction

Biological Classes of Antigen

- Depending on the ability to produce antibody formation, antigen are classified as
  - **T cell dependent (TD):** structurally more complex
  - **T cell independent (TI):** simple, limited number of repeating epitopes
- Although antibody is produced by B-lymphocytes, but it requires cooperation of T-lymphocytes

Properties of Antigen

- **Size**
  - Antigenicity is related to molecular size
  - Larger molecules are highly antigenic where as **haptens** are low molecular weight having low antigenicity
- **Chemical Nature**
  - Antigenicity is directly proportional to degree of structural diversity
  - Amino acid> monosachharide> lipid> nucleic acid
- **Susceptibility**
  - Only substance metabolized by tissue enzyme to epitope fragments – potential to produce antigen
  - **Polystyrene** not antigenic
  - Also antigen rapidly broken down doesn't show Antigenicity properties
  - D-amino acids are not metabolized in the body where as L-amino acids do - **prevention of autoimmunity**

Properties of Antigen

- **Foreignness**
  - Described by **Ehrlich**
  - Normal body contain numerous antigens or antigen producing substance which may not produce antibody.
  - But introduction of this into other body produces immune response
  - This response is directly related to **degree of foreignness** – antigen from same species shows less Antigenicity than others
- **Specificity**

Specificity

- Antigen shows various types of specificity. They are mainly
  - Antigenic specificity
  - Species specificity
  - Iso-specificity
  - Auto-specificity
  - Organ specificity
  - Heterogenic specificity

Specificity

- **Iso-specificity**
  - Antigens found in the some but not all members of species
  - Blood grouping system is based on this
  - Genetically determined
- **Auto-specificity**
  - Sometime autologous antigens starts showing antigenicity
  - Autoimmunity disorders like Rheumatoid arthritis, Sjogren Syndrome

Antibodies

Introduction

- An antibody is an **immunoglobulin** (glycoprotein) molecule formed by the immune system in response to an antigenic stimulus.
  - Found in blood, bodily secretions or on mucous surface
  - Binds to the specific antigen responsible for its production, thereby inactivating it
- Immunoglobulins provides a structural and chemical concepts.
- "Antibody" is a biological and functional concept.
- All antibodies are **immunoglobulins**, but all immunoglobulins are not **antibodies.**

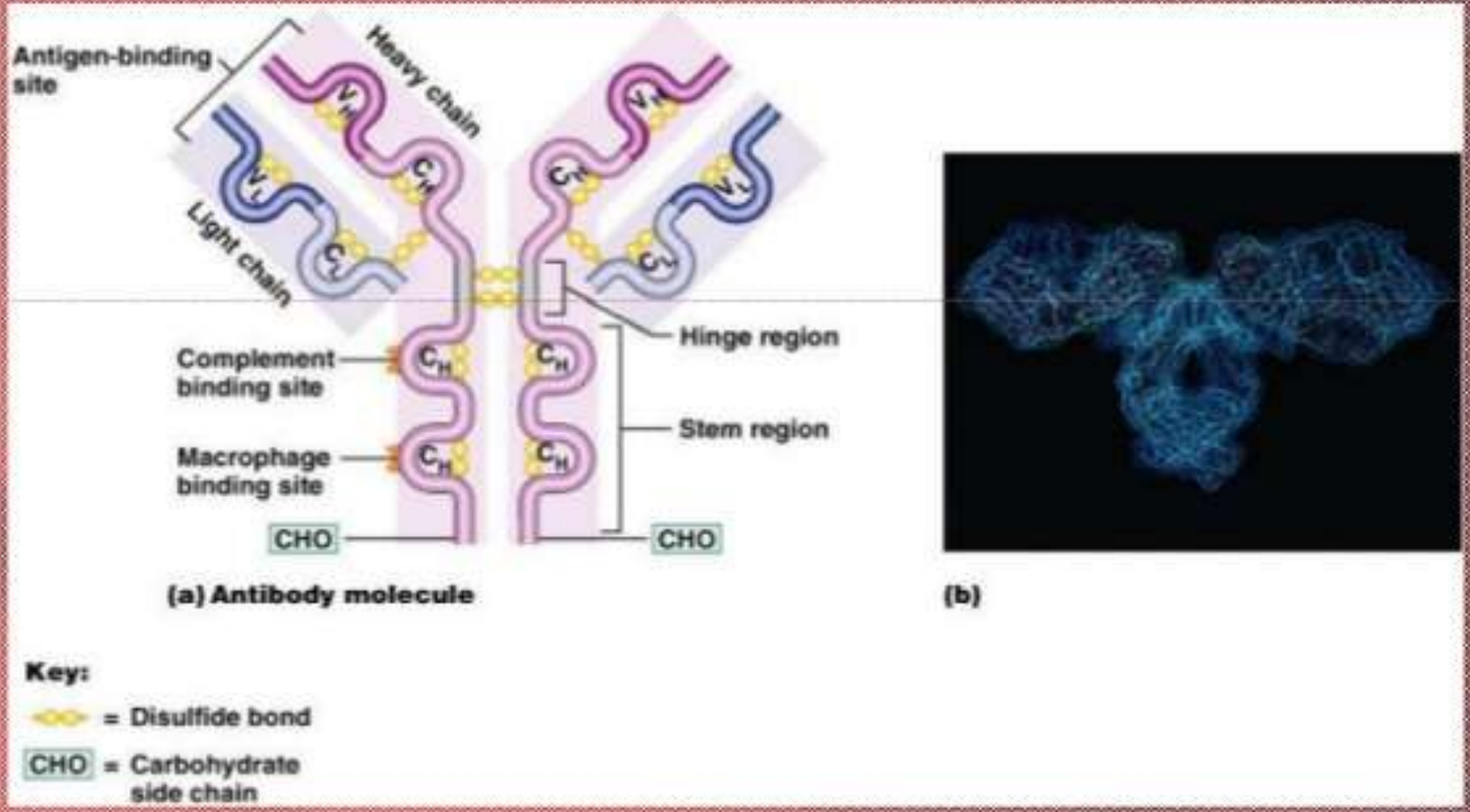
Antibodies

- Constitute the **gamma globulin** portion of blood proteins - 20-25 % of total serum protein.
- Are soluble proteins secreted by **activated B cells and plasma cells** in response to an antigen
- Are capable of **binding specifically** with that antigen

Antibody Structure

- Consists of **four looping polypeptide** chains linked together with **disulfide bonds**
  - Two identical **heavy (H) chains** and two identical **light (L) chains**
- The four chains bound together form an **antibody monomer**
- Each chain has a **variable (V)** region at one end and a **constant (C)** region at the other
- Variable regions of the heavy and light chains combine to form the antigen-binding site

Antibody Structure



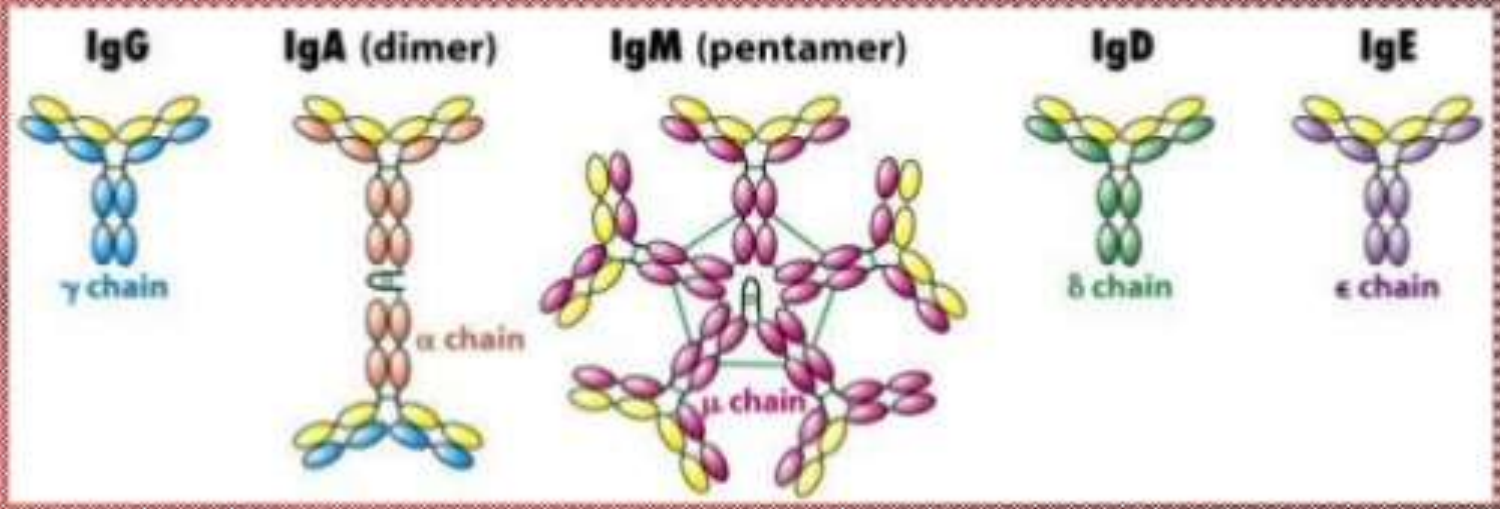
Antibody Structure

- Antibodies responding to different antigens have different **V regions**
- **C region** (either kappa or lambda unit) is the same for all antibodies in a given class
- C regions form the stem of the Y-shaped antibody and:
  - Determine the **class** of the antibody
  - Serve **common functions** in all antibodies
  - Dictate the cells and chemicals that the antibody can bind to
  - Determine how the antibody class will function in elimination of antigens

Classes of Antibodies

- Based on physiochemical and antigenic difference antibodies are classified as:
  - **IgG** : IgG1, IgG2, IgG3, IgG4
  - **IgA** : Serum IgA or Secretory IgA and IgA_1 or IgA_2
  - **IgM**
  - **IgD**
  - **IgE**

  *** Decreasing order of their concentration.



IgG

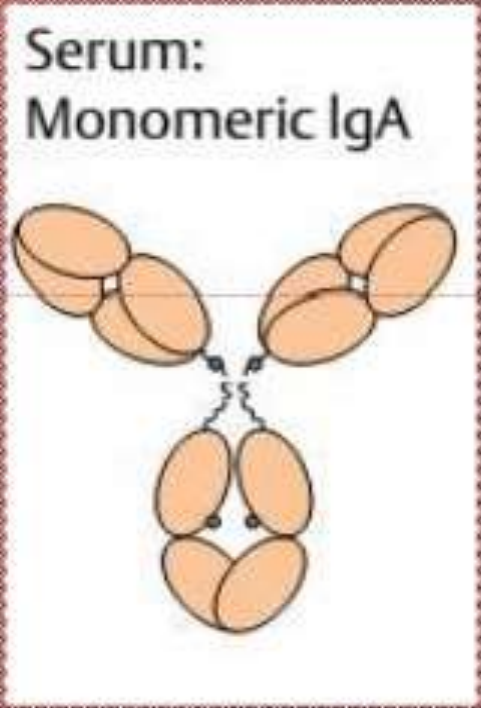
- Major serum immunoglobulin – 80 % of total
- Molecular weight – 7s
- It exist in polymerised form
- Distributed equally in intravascular and extravascular compartment
- Half life – 25 days
- Raised in chronic disease like Kala azar, chronic malaria or myeloma.
- Normal serum concentration: 8-16 mg per ml

IgG

- Only **maternal immunoglobulin** – normally transported across the placenta and provides **natural passive immunity** in the new born
- **Not synthesized** by **fetus**
- The function of IgG: general purpose antibody
  - Immunological reactions such as **complement fixation, precipitation and neutralization** of toxins and viruses
  - Protective against **infectious agents** – active in blood and tissues
  - Binds to MCO and enhances their **phaogocytosis**
  - **Surface recognition** – Fc fragments

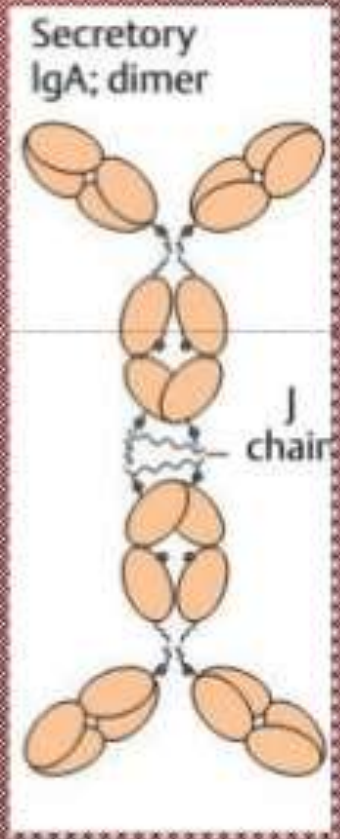
IgA

- 2nd most abundant class – 10-13 % of serum immunoglobulin
- Norma serum level: 0.6-4.2 mg/ml
- Half life – 6-8 days
- Found in colostrum, saliva and tears
- It occurs in two forms
  - **Serum IgA:** principally monomeric 7s molecule
  - **Secretory IgA:** two monomeric unit joined together by J-chain



IgA : Secretory

- Larger molecule than **serum IgA :** 11s
- Plasma cells situated near the **mucosal or glandular epithelium**
- It contain another glycine-rich polypeptide – **secretory component** or **secretory piece.**
  - Protect IgA from denaturation by bacterial proteases – sites such as intestinal mucosa



IgA

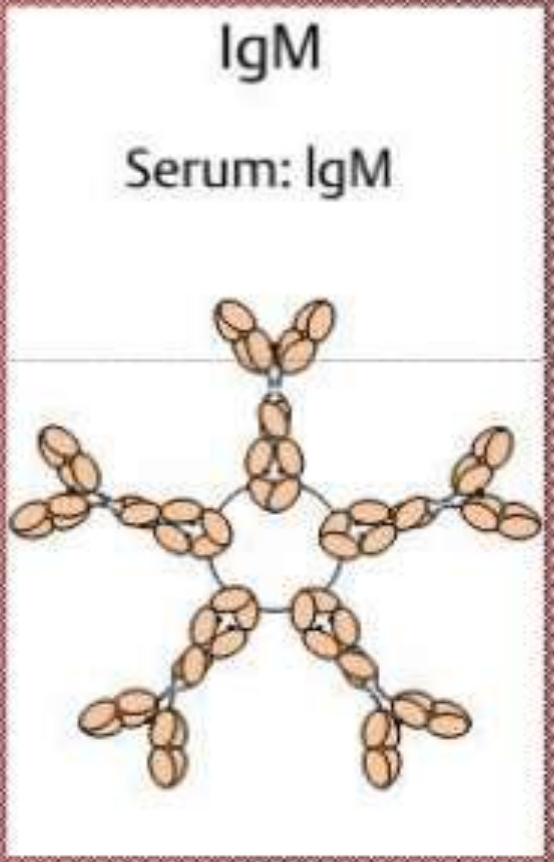
- **Function of IgA**
  - local immunity – against respiratory and intestinal pathogens
  - inhibits adherens of MCO to surface mucosa
  - Activate the alternative complement pathway
  - Promotes phagocytosis and intracellular killing of MCO

IgM

- 5-8 % of serum immunoglobulins
- Normal level 0.5 – 2 mg/ml
- Hlaf life – 5 days
- Heavy molecule – 19s – millionaire molecule
- Polymer of the four-polypeptide joined by **J-chain.**
- Most of IgM – intravascular (80%)
- Phylogenetically oldest immunoglobulin class – earliest immunoglobulin synthesized by fetus (20 weeks of age)

IgM

- Its **not transported** across the **placenta**
- Presence of IgM in fetus or newborn: **syphilis, rubella, HIV infection and toxoplasmosis**
- Relatively **short lived** – hence its serum demonstration indicates **acute infection**



IgM

- Its **highly potent** as compares to IgG
  - 1000 times : immune hemolysis
  - 500-1000 times : Opsonisation
  - 100 times : bacteriocidal
  - 20 times : bacterial agglutination
- Less active than IgG : neutralisation of toxins and viruses
- Largely confined – intravascular space
- Functions
  - Protection – blood invasion MCO
  - Monomeric IgM – major antibody receptor on the surface of lymphocytes for antigen recognition
- IgM deficiency - **septicemia**

IgD

- It resembles IgG structurally
- Serum concentration : 3 mg/100 ml mostly intravascular
- Half life : 3 days
- **Recognition receptor** for antigen on **B Lymphocytes** as that of IgM
- Also it causes stimulation of the B-cell – **activation and cloning** to produce **antibody** or supression

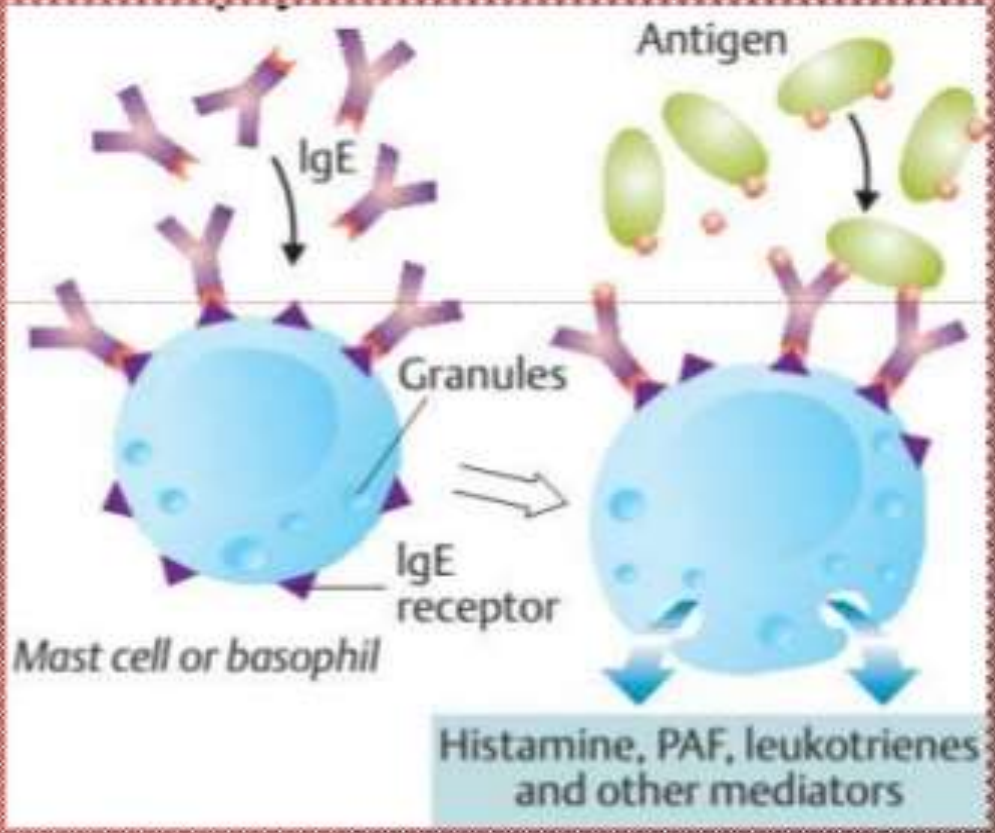
IgE

- Discovered by Ishizaka
- Molecular weight: 8 s
- Half life: 2 days
- Represents IgG structurally
- Heat labile but inactivated at 56^0 C in 1 hours
- Affinity for the surface of tissue cells – mast cells of same species
- Mediates **Prausnitz-Kustner** reaction
- Doesn't passes placental barrier or fix complement
- Extra-vascular in distribution

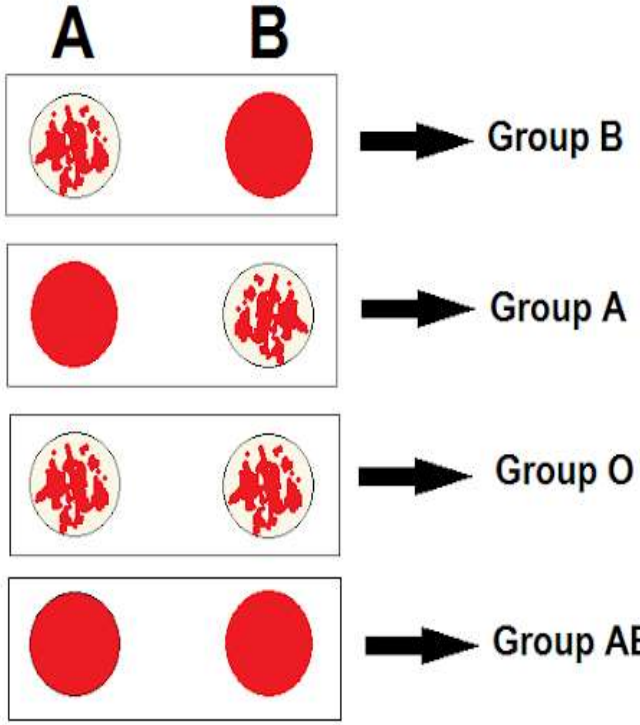
IgE

- There is very trace (few ng) serum level in normal condition, but elevated in
  - **Atopic (type 1 allergic):** asthma, hay fever and eczema
  - Intestinal parasitic infection
- IgE chiefly produced in the linings of respiratory and intestinal tracts
- **IgE deficiency** – undue susceptibility to infection.
- It produces
  - Anaphylacttic type of hypersensitivity
  - Protection against pathogen by mast cell degranulation and release of inflammatory mediators
  - Defense in helminthic infection

IgE


Antigen
IgE
Granules
IgE
receptor
Mast cell or basophil
Histamine, PAF, leukotrienes
and other mediators

BLOOD GROUPING



OBJECTIVES

- Importance of blood grouping
- Landsteiner's law
- Typing techniques
- Slide method
- Tube method
- Cross-matching

NEED FOR BLOOD GROUPING

- BLOOD TRANSFUSION
- HEMOLYTIC DISEASE OF NEWBORN
- PATERNITY DISPUTES
- MEDICOLEGAL USE
- SUSCEPTIBILITY TO VARIOUS DISEASES
- ROUTINE HEALTH CHECKUP

LANDSTEINER'S LAW

- If an antigen is present on a patient's red blood cells, the corresponding antibody will not be present in the patient's plasma under normal conditions.

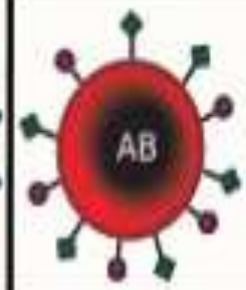
Reciprocal relationship between ABO antigens and antibodies

Antigens on RBCs	Antibody in plasma / serum	Blood group
A	Anti-B	A
B	Anti-A	B
AB	None	AB
None	Anti-A, Anti-B	O

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

ABO antigens & corresponding antibodies

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	A antigen	B antigen	 A and B antigens	None

UNIVERSAL DONOR AND RECIPIENT

- UNIVERSAL DONOR

  GROUP O

  - *Neither A or B antigens*

- UNIVERSAL RECEIPIENT

  GROUP AB

  - *Patient has no Anti A/Anti B present.*
  - *Cannot lyse any transfused cell*

ABO TYPING TECHNIQUES

- Slide test
- Tube technique
- Microplate
- Gel system

SLIDE GROUPING

ADVANTAGES:

- Preliminary typing tests
- Use during camps

DISADVANTAGES:

- Not routine test
- Less sensitive
- Drying of reaction giving to false positive results

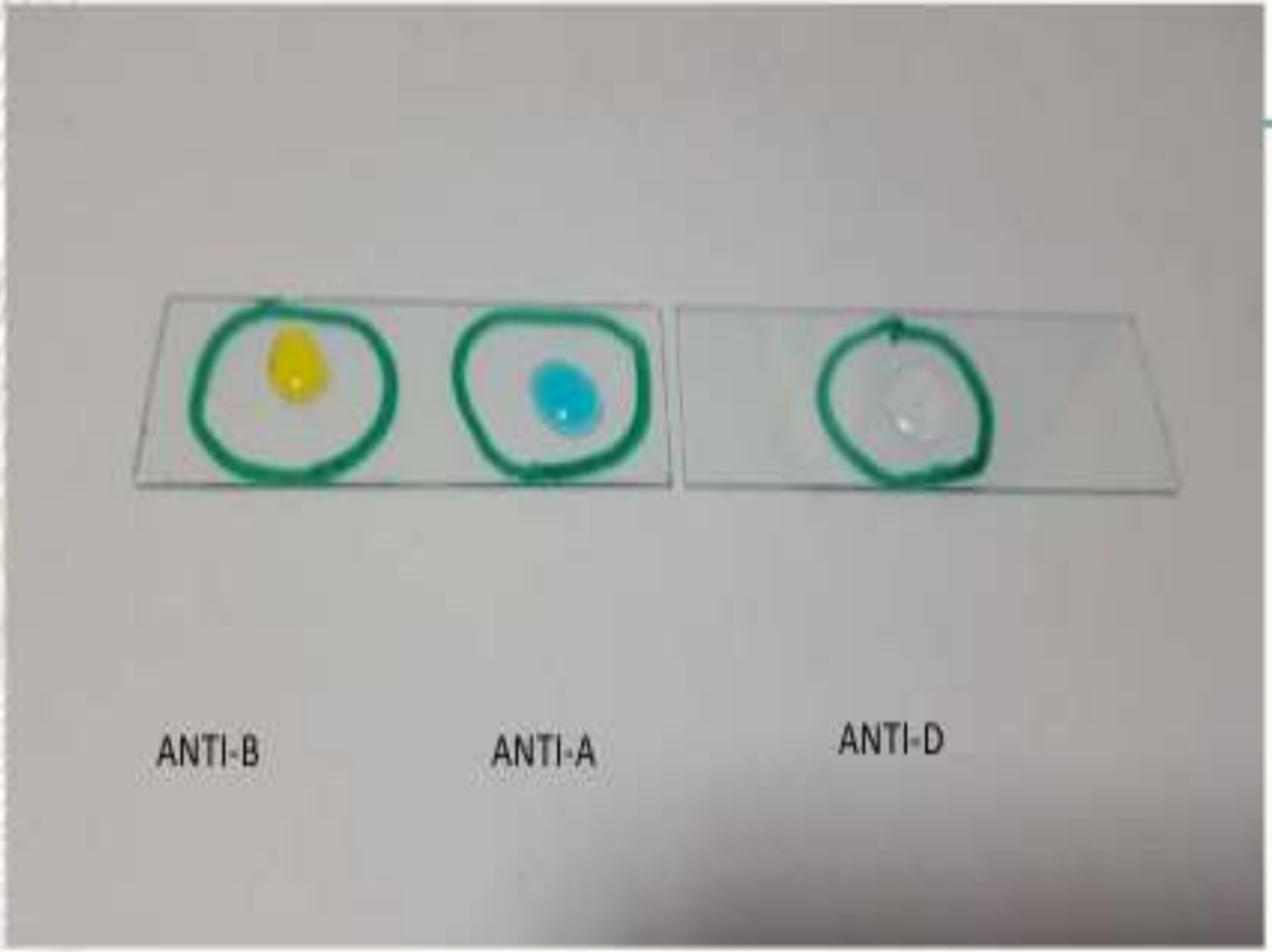
ANTISERA

SLIDE GROUPING

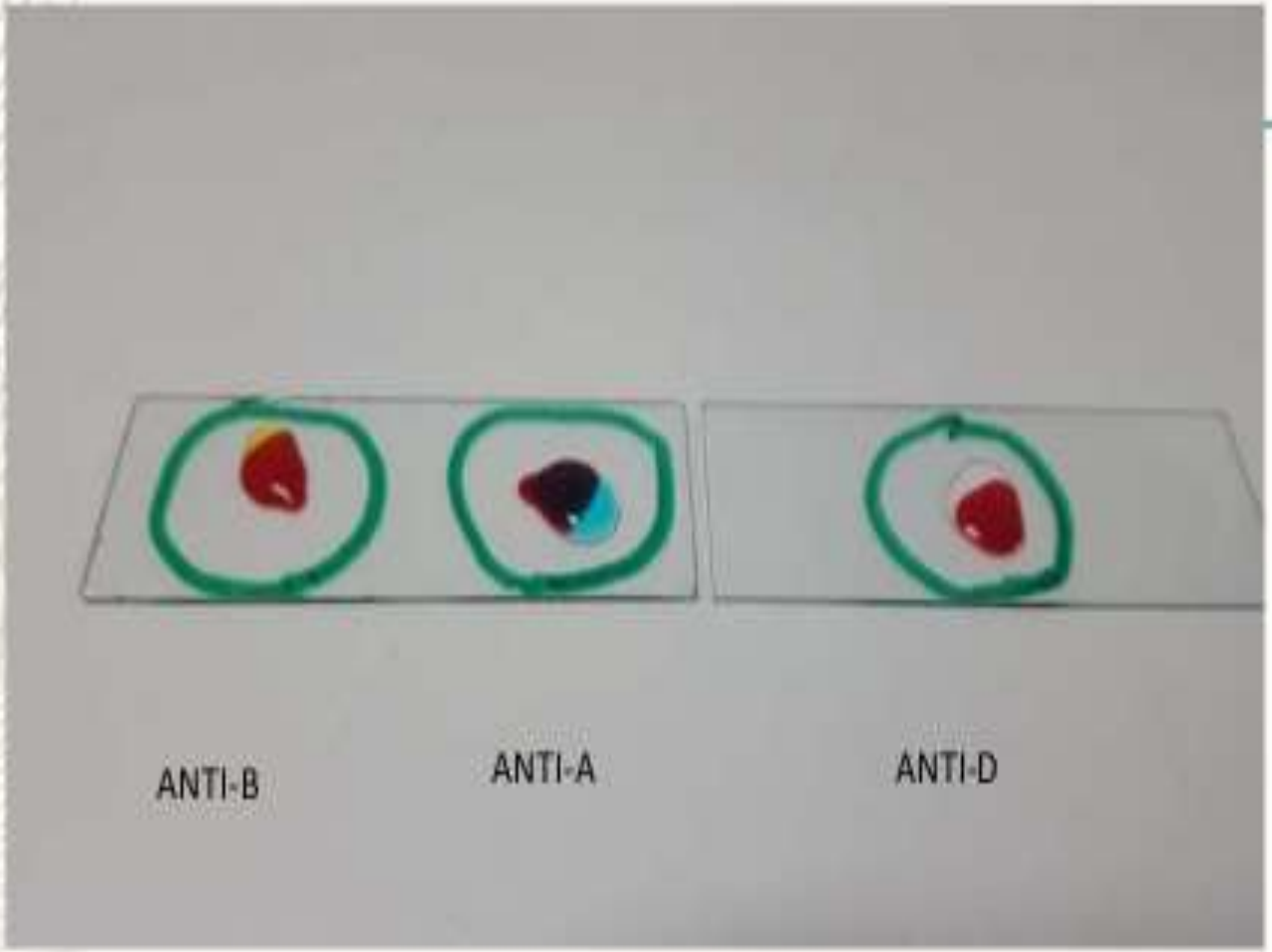
- Test should be done at room temperature or lower
- Tubes, slides should be dry and labeled properly
- Antisera should always be added before adding cells
- Results should be recorded immediately after observation
- Hemolysis is interpreted as positive result

BLOOD SAMPLE

FIRST ADD ANTISERA TO SLIDE

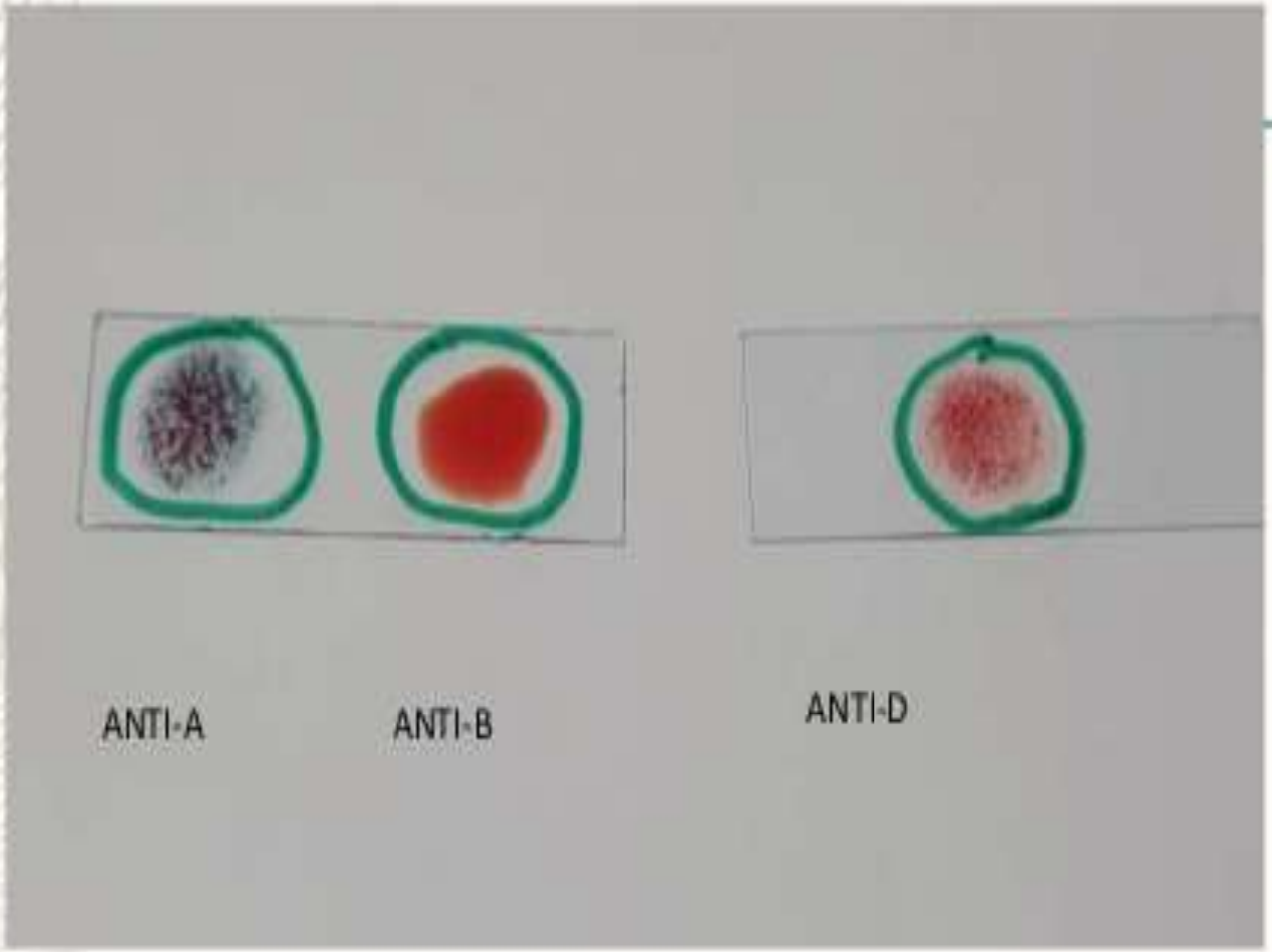


SAMPLES ADDED TO SLIDES

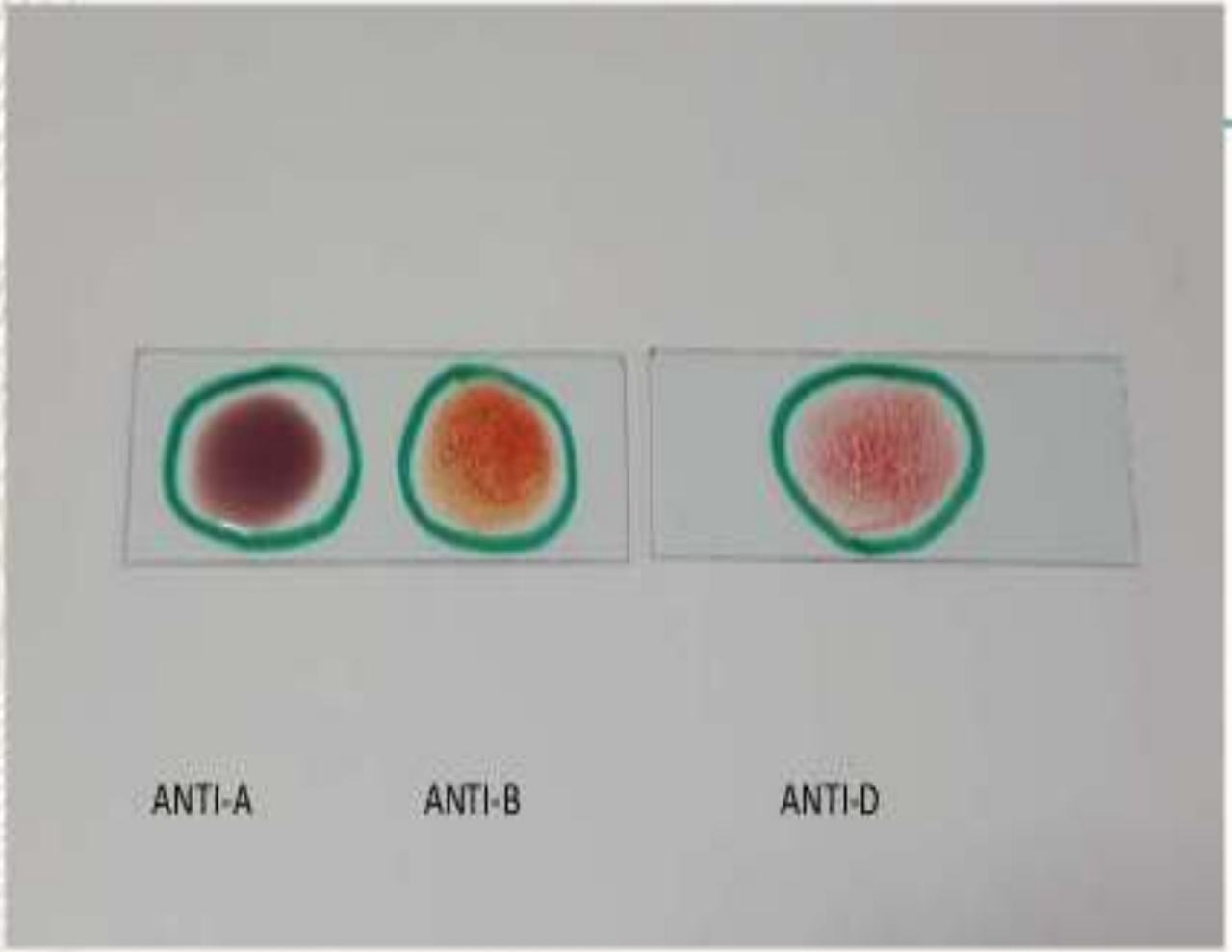


OBSERVE FOR AGGLUTINATION

sample 1



Sample 2



Test Tube Method

Recommended method (Gold standard)

- Allows longer incubation of antigen and antibody mixture without drying
- Tubes can be centrifuged to enhance reaction
- Can detect weaker antigen / antibody

Two steps in ABO grouping

Cell grouping (Forward grouping)

- Tests the patients red cells with known Anti-A & Anti-B to determine the antigen expressed

Serum grouping (Reverse grouping)

- Test the patients serum with known A & B cells to determine the presence of antibody

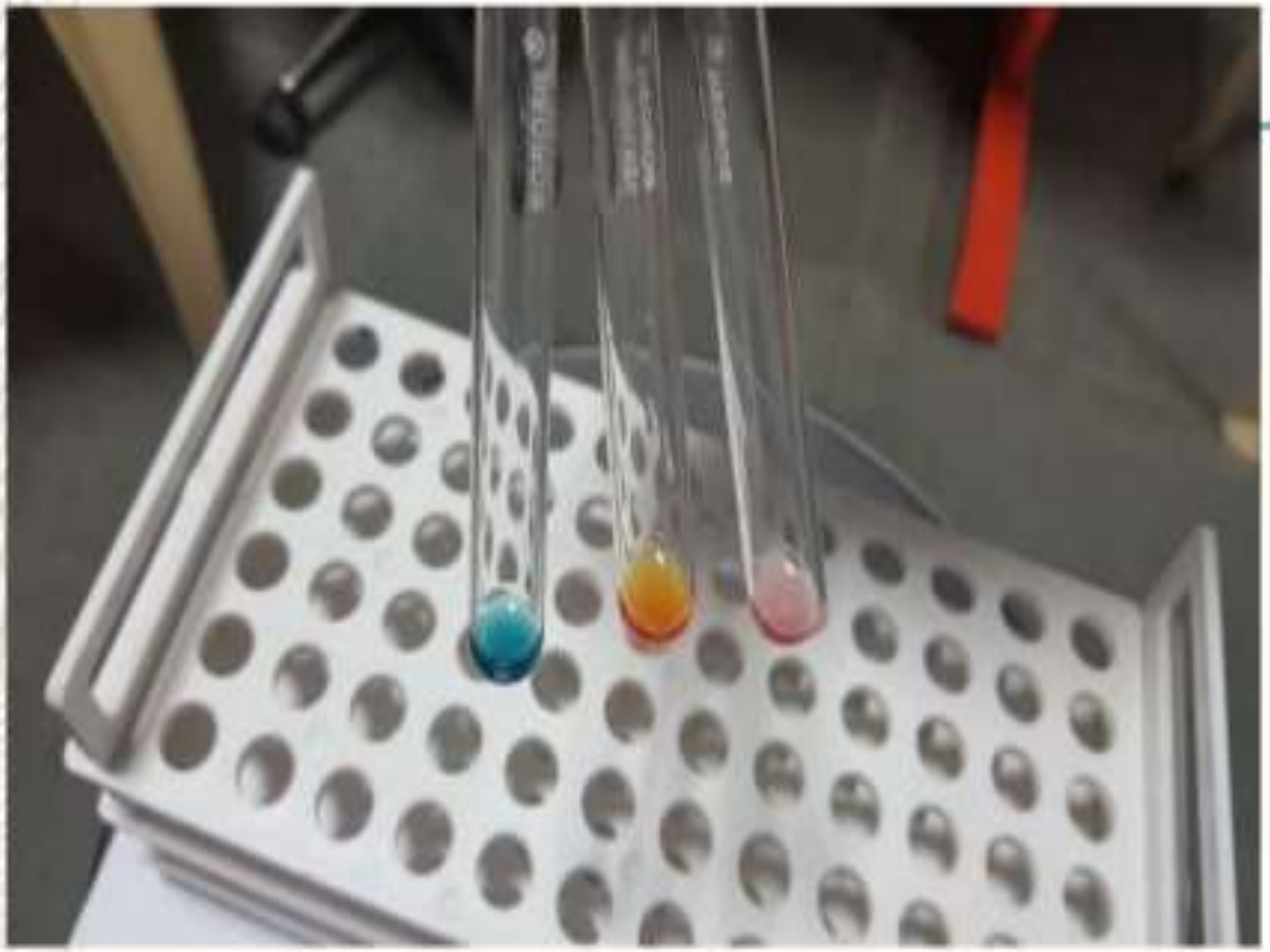
CELL GROUPING (Forward grouping)

- Prepare 2-5% suspension of test sample in normal saline
- Set three tubes , label them as A, B, D
- Add two drops of anti A , anti-B, anti D in three different tubes
- Add one drop of 2-5% cell suspension (Ratio of 2:1)
- Mix contents well and centrifuge at 1500 rpm for 1 minute
- Observe for hemolysis
- Gently disperse cell button and check for agglutination
- Confirm negative results under microscope

TUBE METHOD

SAMPLE ADDED

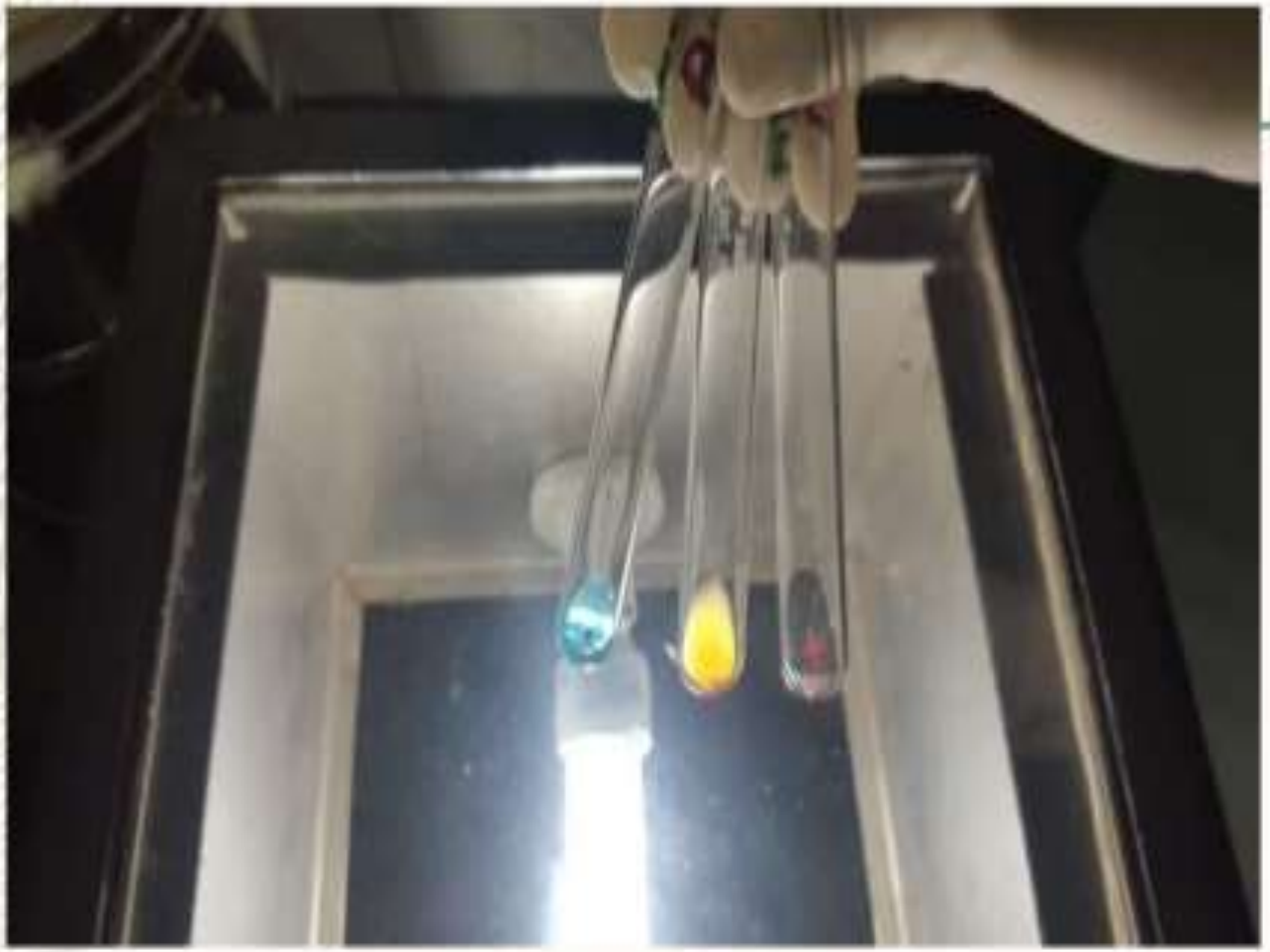
MIX TUBE CONTENTS



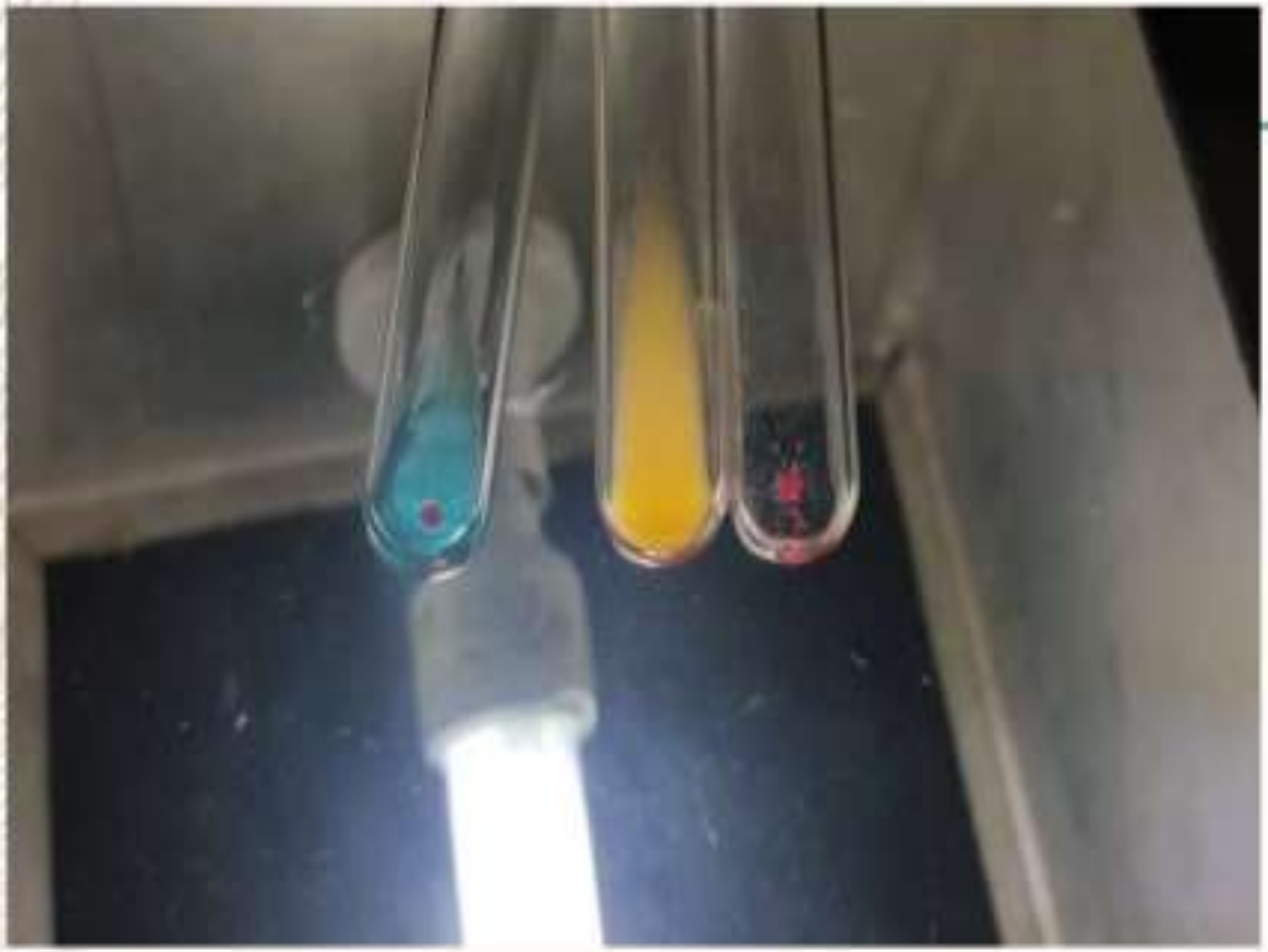
CENTRIFUGE LOADING

CENTRIFUGE AT 1500 RPM

RH VIEWING BOX



GRADING AGGLUTINATION



SERUM GROUPING (REVERSE GROUPING)

- Prepare 2-5% suspension of pooled cells A,B,O
- Label three tubes A cells, B cells and O cells
- Place two drops of serum in each tube
- Add one drop of cell suspension (A cell to A tube, B cell to B tube and one drop of O cell to O tube
- Centrifuge tubes at 1500 rpm for 1 minute
- Gently disperse for agglutination
- Negative results check by microscope

2 vol of test serum/plasma

+

1 vol of 5% suspension of reagent red cells in respective tubes

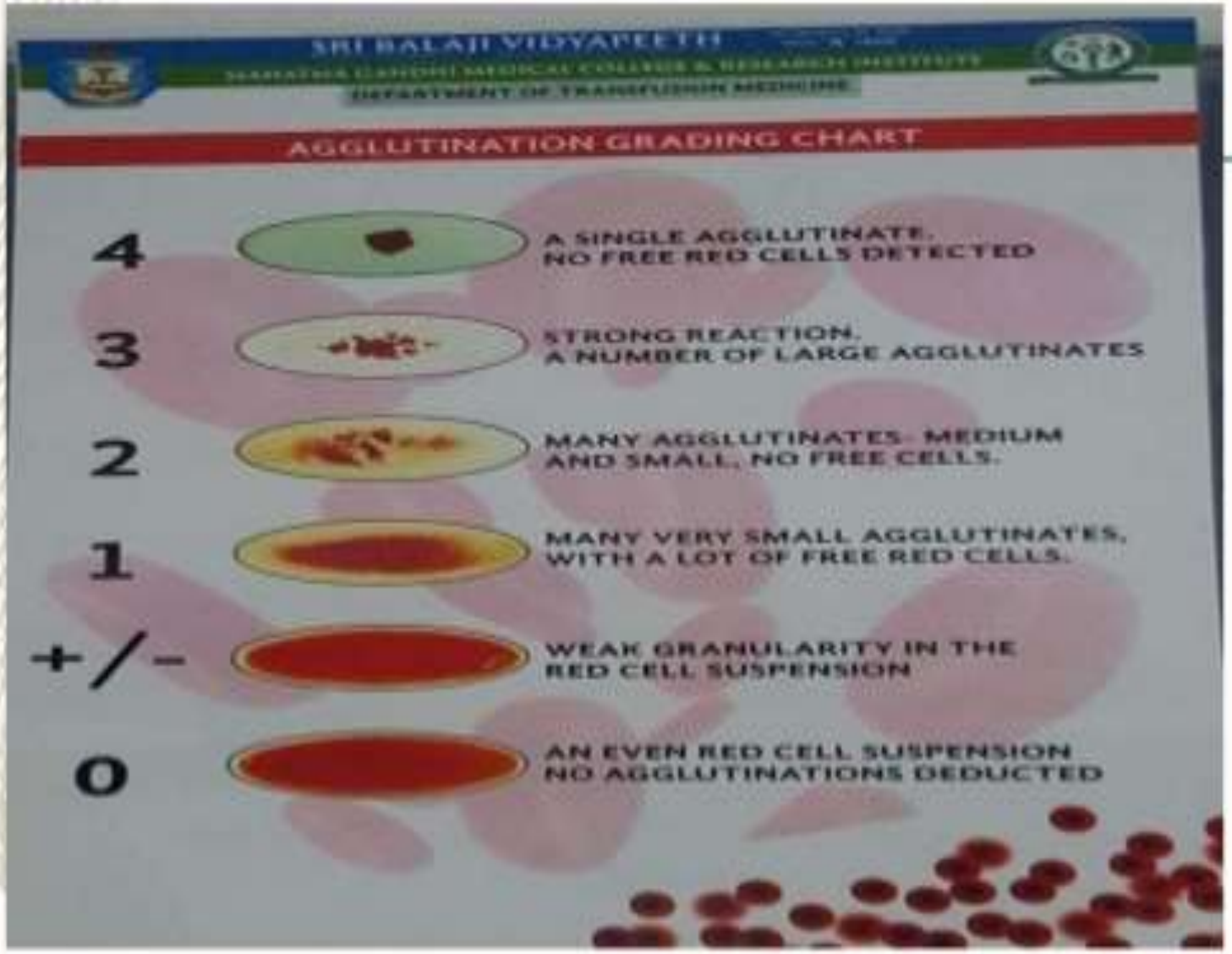
Reverse Grouping

Shake & leave at room temp (20-24°C) for 5 min

Centrifuge at 1000 rpm for 1 min

Centrifuge & record the results similarly as for cell grouping

GRADING OF AGGLUTINATION



Rh Blood Group System

- This system also discovered by Karl Land Steiner(1940)
- Second important blood group system
- The main cause of hemolytic disease of new born(HDN)

Rh Blood Group

- Consists of 49 antigens
- Most significant are D,C,E,c and e
- Commonly used term Rh factor refer to D antigen on RBCs surface

Types of Rh Blood Group System

- **Rh Positive:**

  Posses Rh antigen on surface of RBCs

- **Rh Negative:**

  Lack Rh antigen on surface of RBCs

Determination of Blood Group on the basis of Agglutination

Sr.No	Anti-A	Anti-B	Anti Rh-D	Blood Group
Slide 1	√	×	√	A +ve
Slide 2	×	√	√	B+ve
Slide 3	√	√	√	AB+ve
Slide 4	×	×	√	O+ve

Interpretations

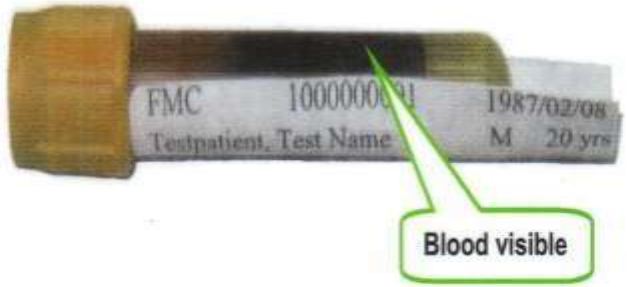
- If agglutination is observed when blood is mixed with Anti Rh-D reagent, then the individuals is said to have “+ve” Rh factor.
- If no agglutination is observed when blood is mixed with Anti Rh-D reagent, then the individuals is said to have “-ve” Rh factor.

Collection and Handling of Blood

Patient's specimen:

- A properly labeled sample is essential so that the results of the test match the patient.
- Specimen container must be labeled with **FULL DETAILS** of patient's ID.
- The key elements in labeling?

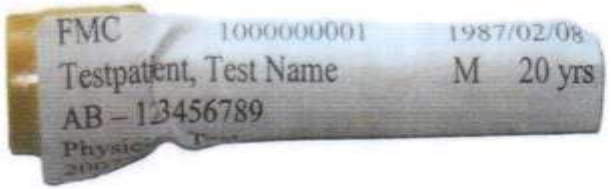
CORRECT



Patient name next to the lid.

Label so blood is visible, and lab can see blood volume, hemolysis, etc.

INCORRECT



Do not apply the label over the lid

Do not wrap the label around the tube like a flag

Do not apply the label with the tube lid on the right side

- **Blood can be collected from 3 different sources:**

  I. Venous blood.

  II. Arterial blood.

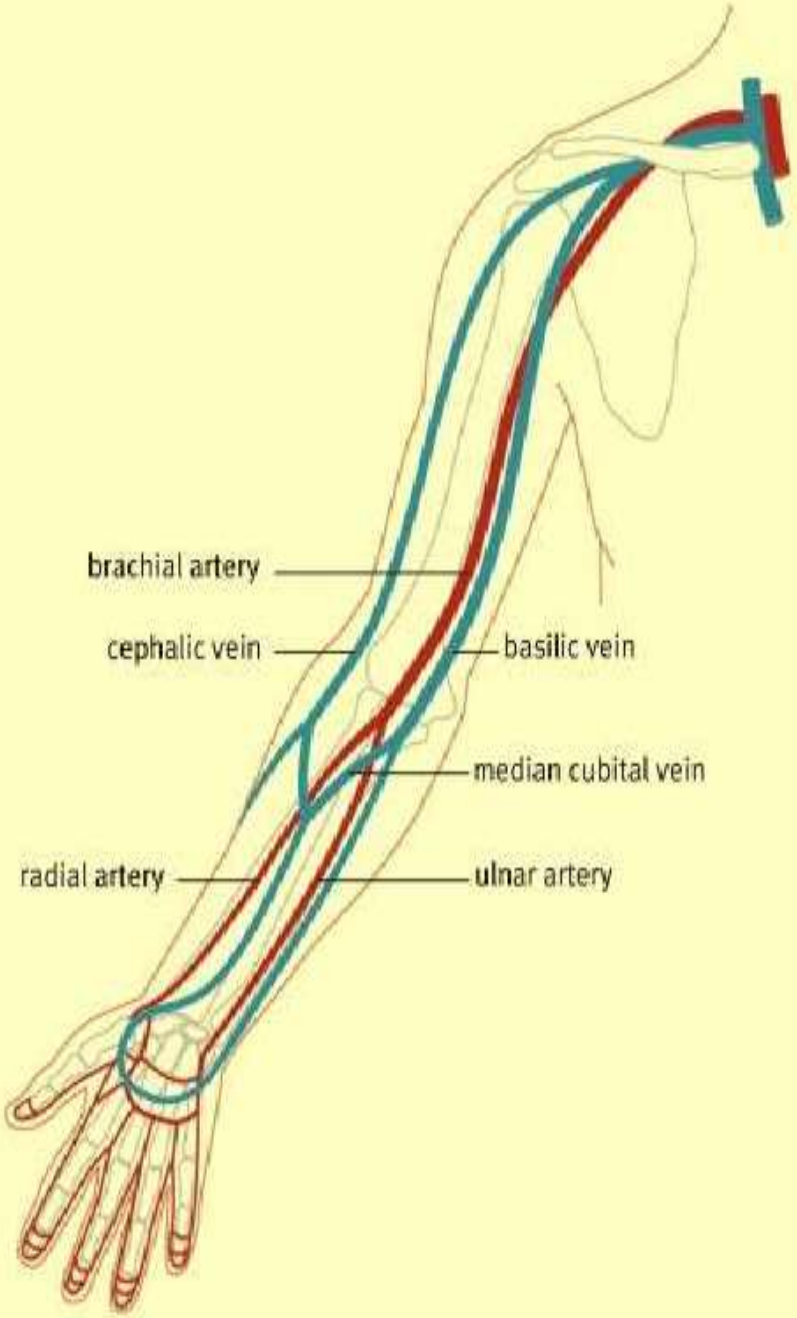
  III. Capillary blood.

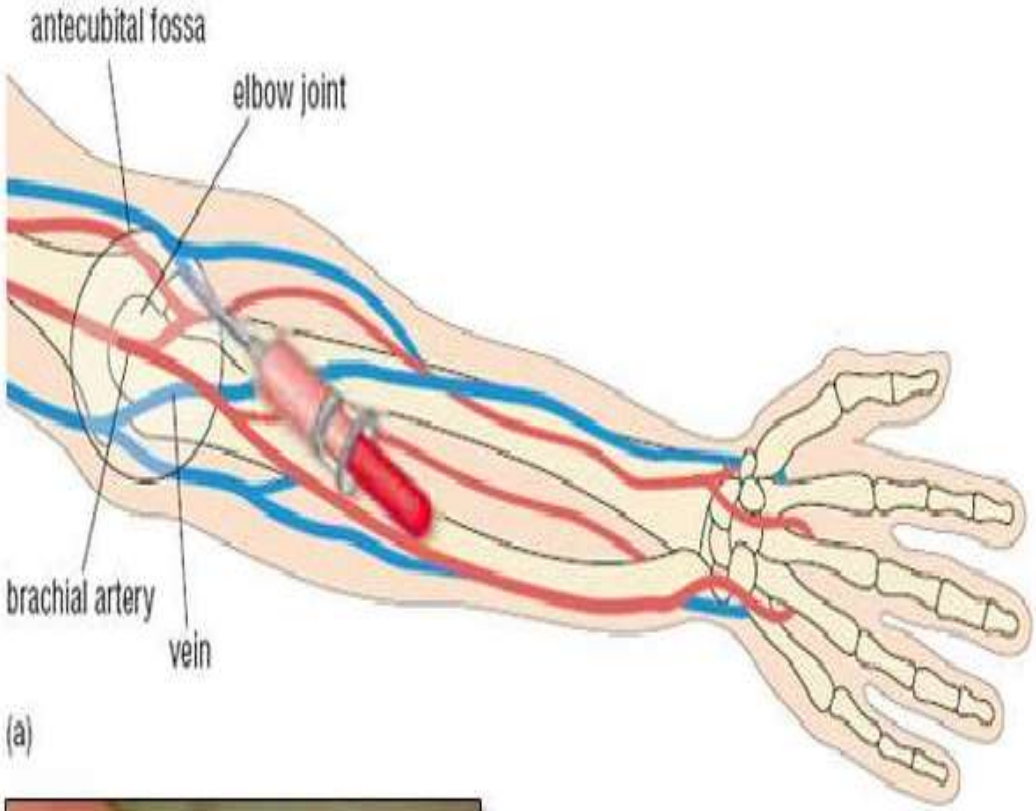
I. Venous blood

- **Most commonly required ….WHY??**

- ✓ Because most majority of routine tests are performed on venous blood.
- ✓ Blood can be taken directly from the vein.
- ✓ The best site for venous collection is the deep veins of the ante-cubital fossa.

Vascular anatomy of the arm

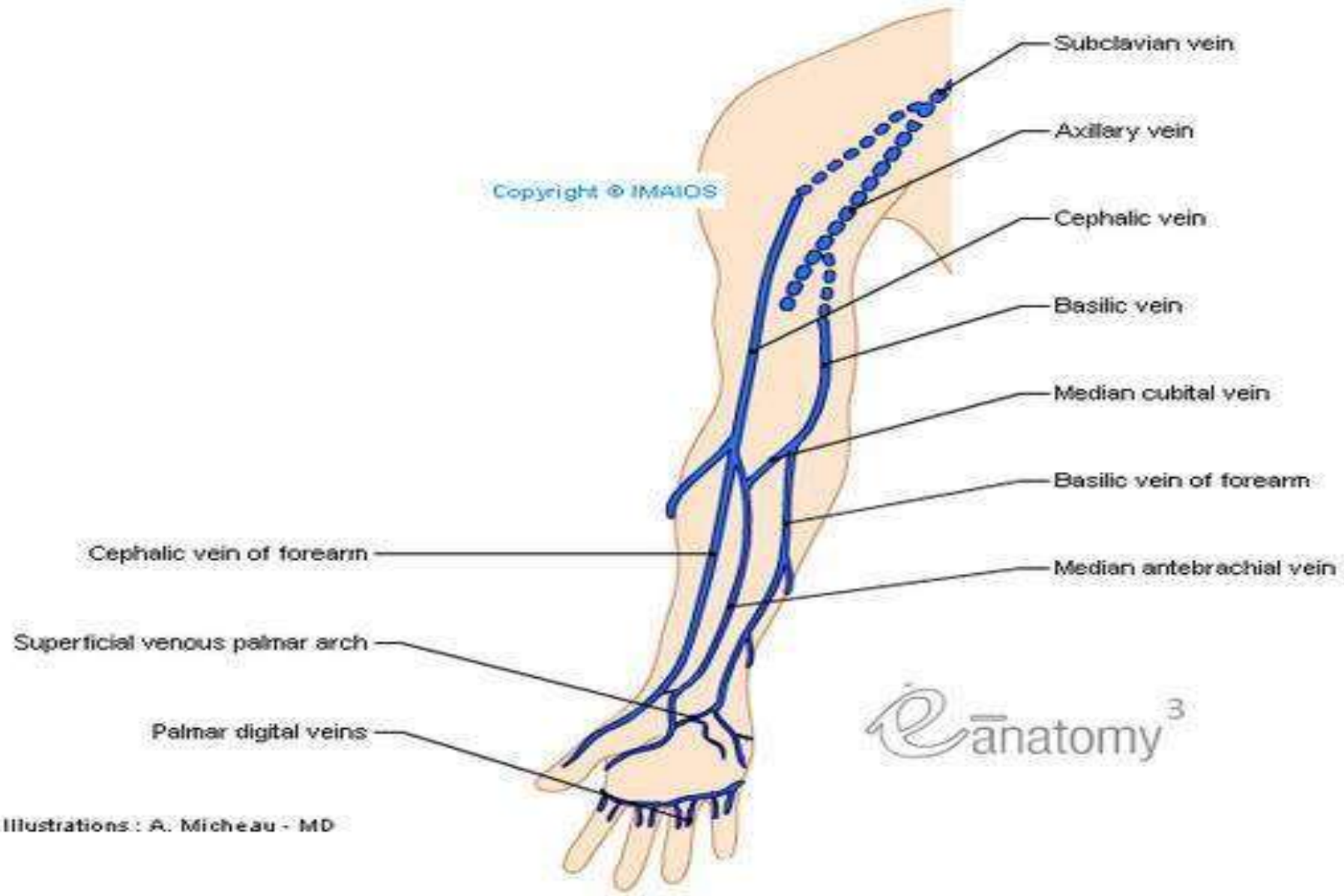




(a)

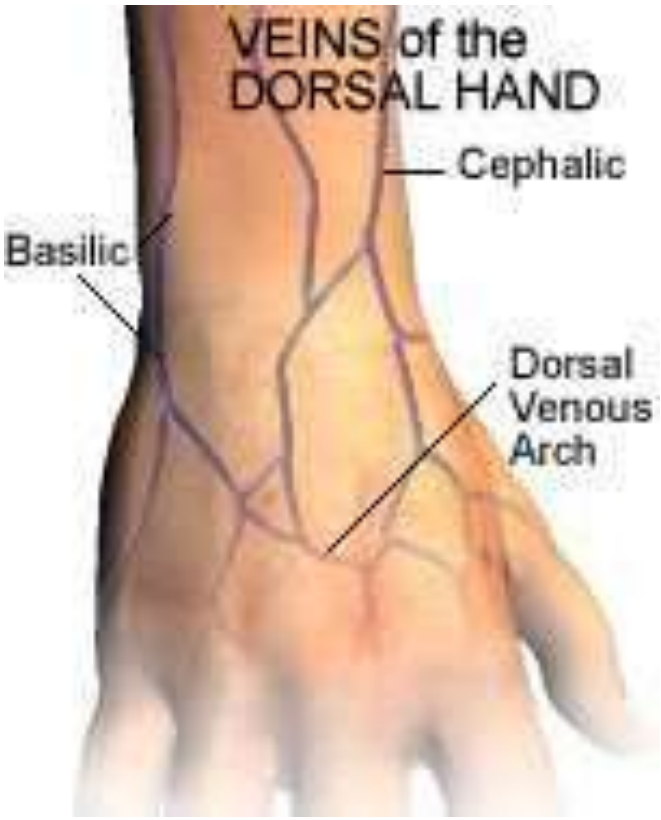
(b)

1. Forearm vein

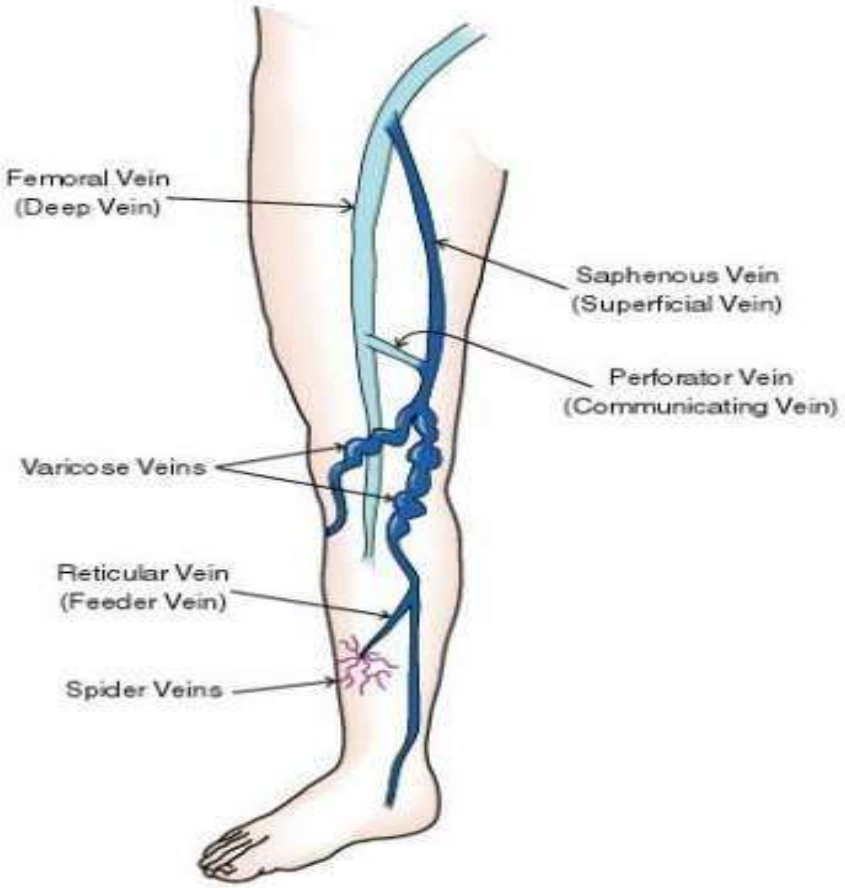


- If difficult to obtain from the ante-cubital fossa we can draw blood from following various site:

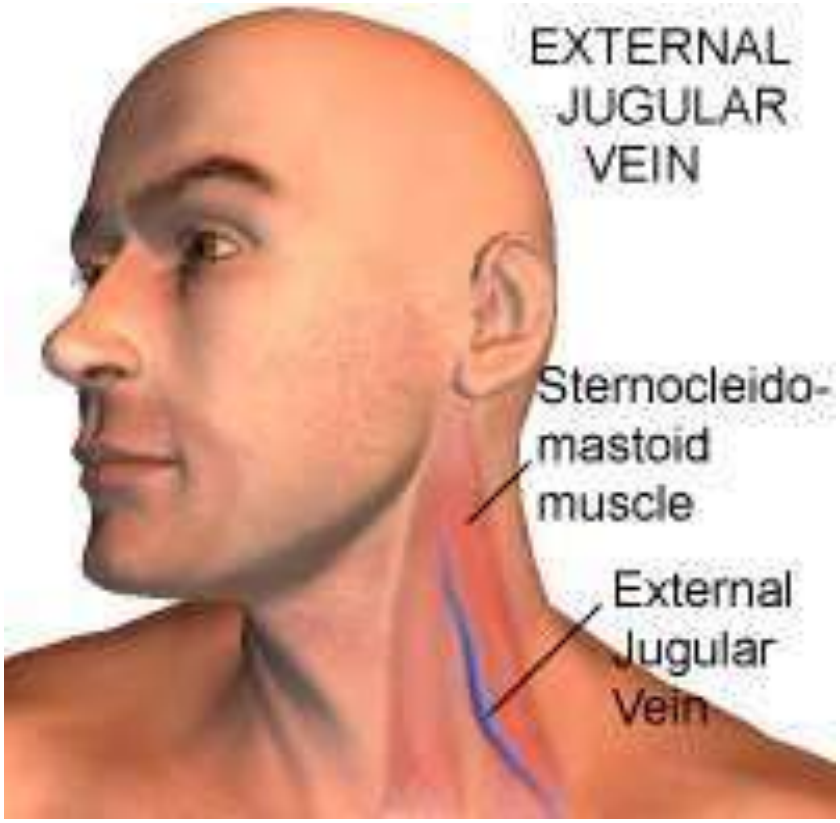
2. Dorsum of hand vein



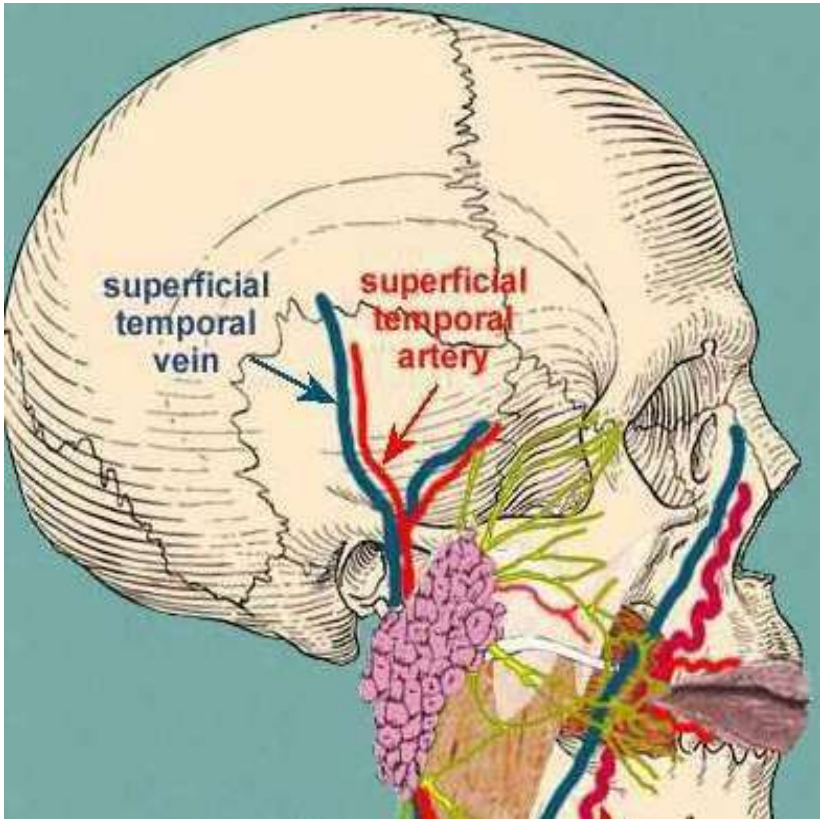
3. Femoral vein



4. Jugular vein



5. Scalp vein



- These sites -other than the forearm- require extra caution and expertise for collection of blood.

Blood collection tools

- **Material:**

- Tourniquet.
- Vacutainer or syringe.
- Alcohol swab.
- Bandage/ medi-plast.

Blood Collection tubes

- Plastic tube with a rubber stopper include color coded.
- Contain anticoagulants and/or other chemical additives.
- Plain tubes contain no anticoagulants.
- All tubes must be mixed thoroughly.

ANTICOAGULANT TUBE

- **EDTA** (Ethylene Diamine Tetra-Acetate) liquid:
  - Types: Na and K2 EDTA (1.5-0.25mg /ml)
  - Functions by forming Ca salts to remove Ca.
  - Uses: most hematology studies. such as: CBC, PCR and HbA1c.
  - Requires full draw (invert 8 times).

- **Light Blue :**

  - Sodium citrate (1:9 ratio).
  - Anticoagulant: 32g/l.
  - Action: Remove Calcium.
  - Uses: Coagulation studies and platelet function.

- **DARK GREEN**

- Sodium Heparin or Lithium Heparin anticoagulant.
- Action: inactivate thrombin and thromboplastin.
- Uses:

- For Lithium level use Na Heparin anticoagulant
- &for Ammonia level use Na or Lithium Heparin

- **RED (Plain tube):**

- No preservative/anticoagulant.

- Uses: usually for blood bank tests, toxicology and serology

SST/ Gold top tube:

- SST (Serum Separator Tube)
- No additives.
- Clotting accelerator and separation gel.
- Uses: Chemistry, Immunology, and Serology.

PST /Light Green

- Plasma Separating Tube with Lithium Heparin
- Uses: Chemestries

- **BLACK:**

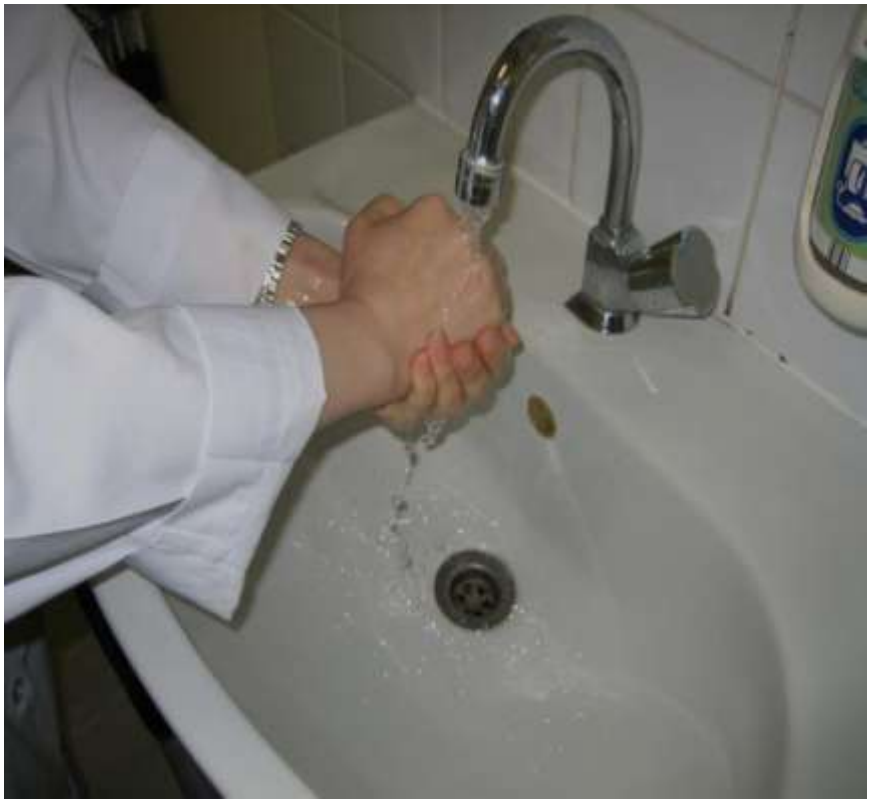
- Na citrate 1:4.
- Action: Remove calcium.
- Uses: Westergren sedimentation rate (ESR).

- **ESR Tube**

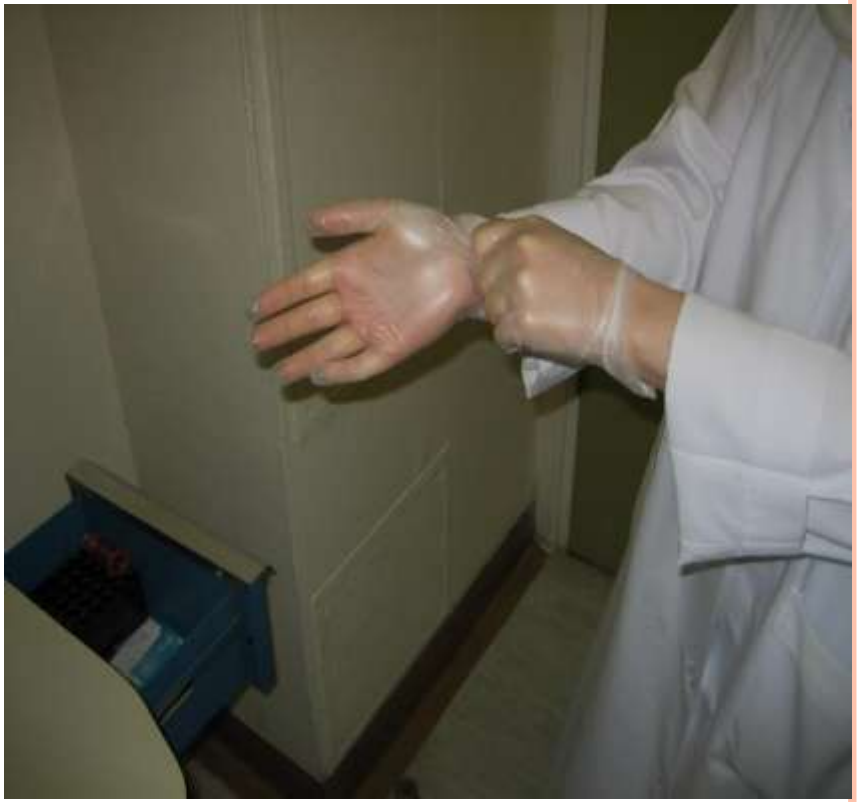
- Additive: 3.8% sodium citrate

PHLEBOTOMY PROCEDURE

Wash hands

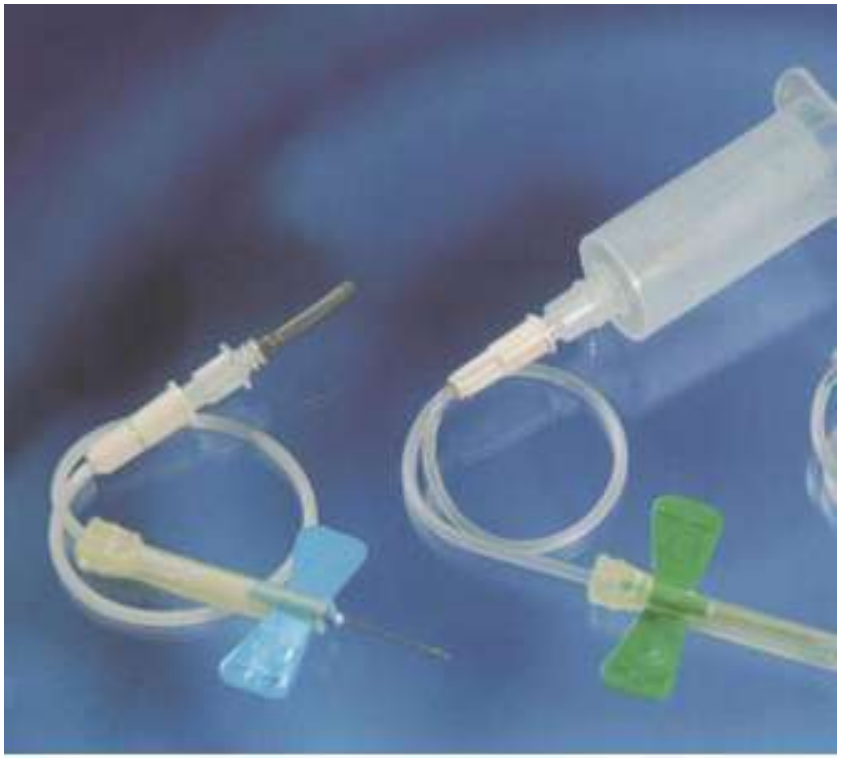


Apply gloves



Procedure:

1. Appropriate syringe and/or needle should be selected.
2. If multiple specimens are to be collected its better to use butterfly needle.



Attach needle to holder

Place tube into holder

3. Tourniquet should be applied on the upper arm.

4. Sterilize puncture area with a spirit/alcohol swab and allow it to dry.

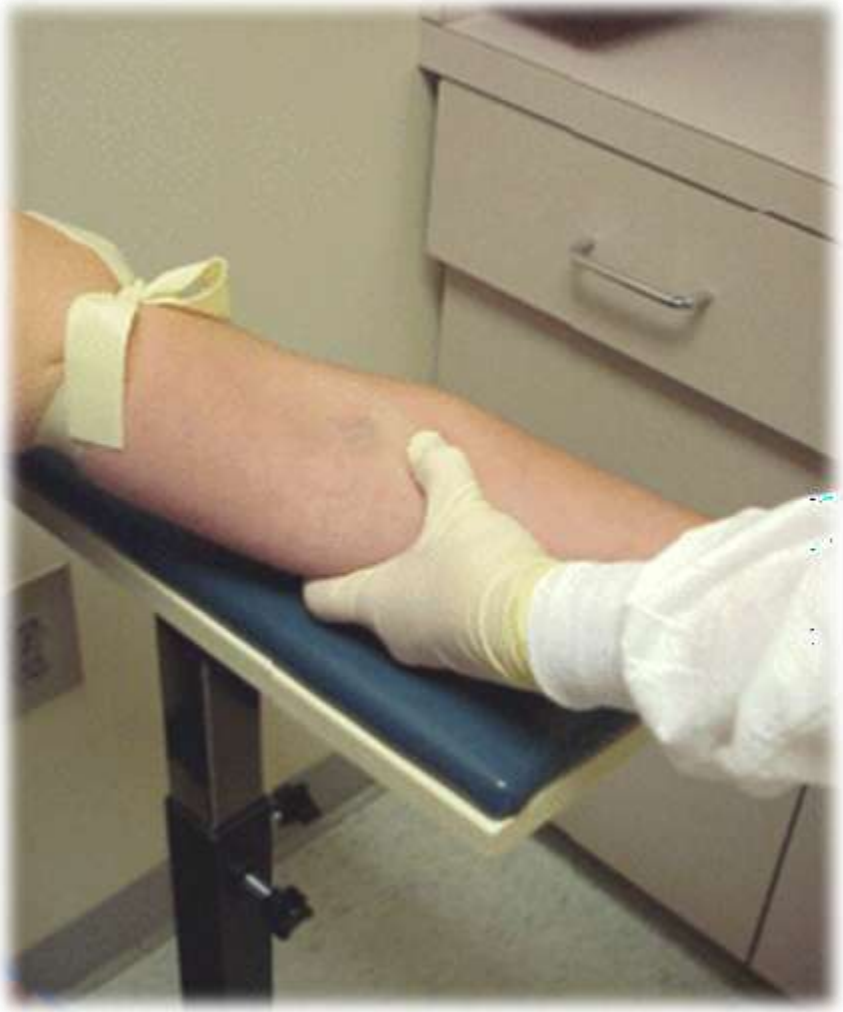
5. Visualize and palpate the vein.

6. Don’t enter the vein directly and vertically.

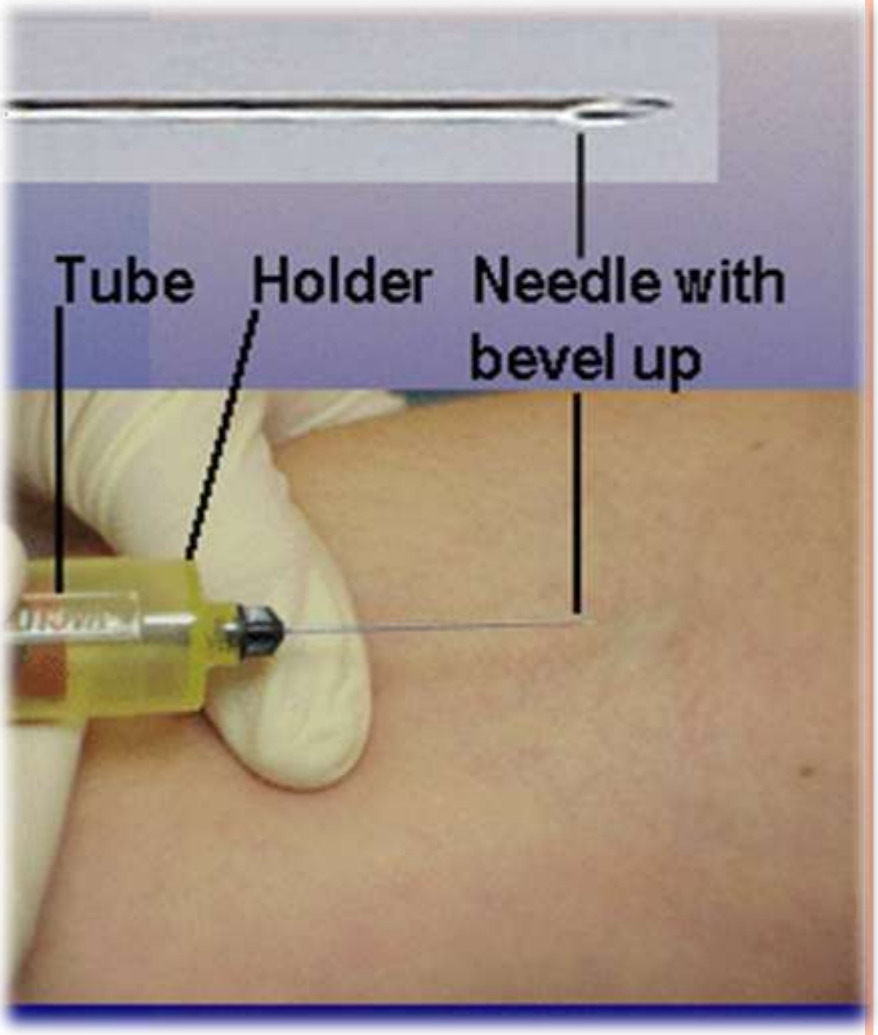
Why?

Because there is more chance of puncturing the other side of the venous wall by this way.

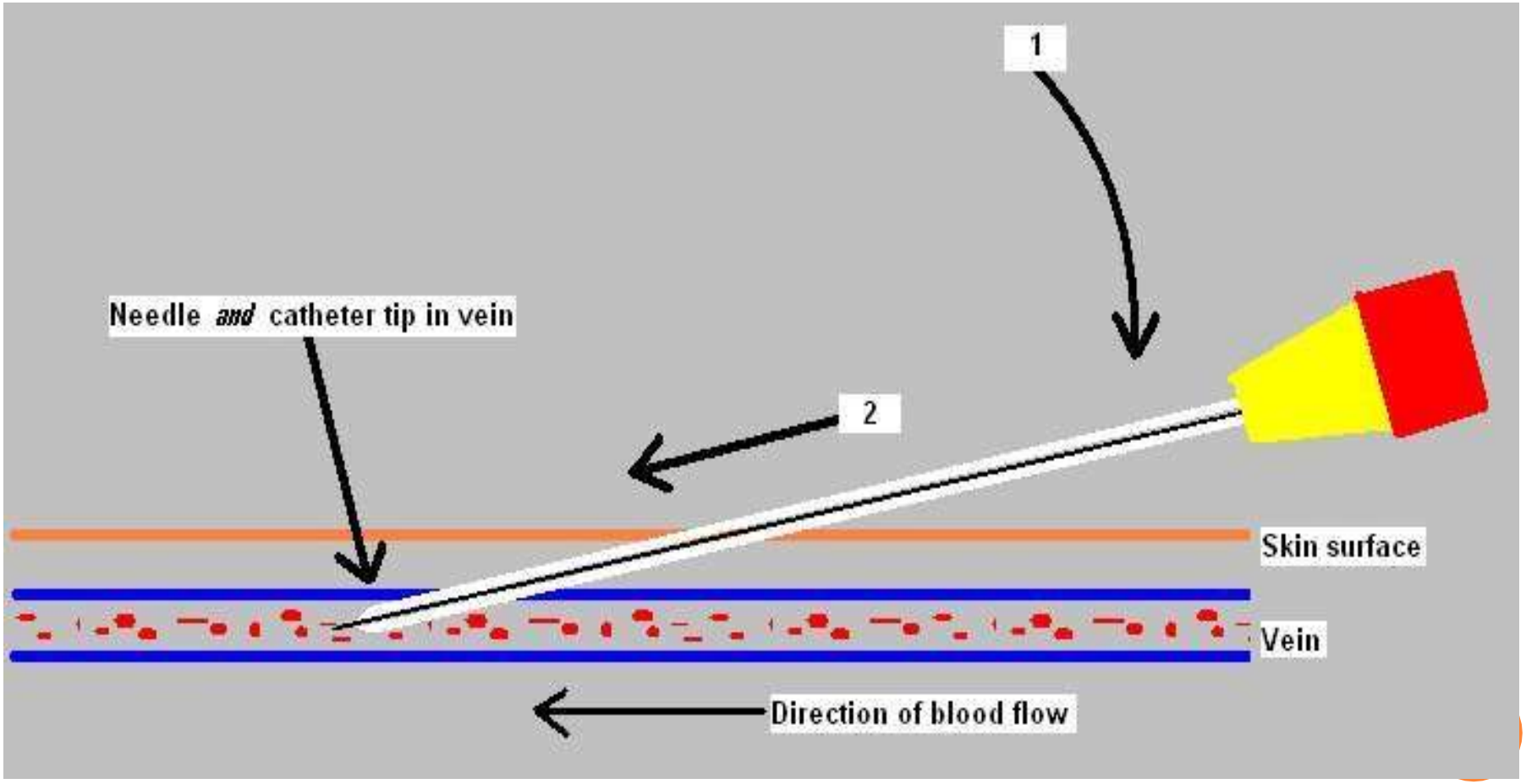
Hold vein in place



Insert needle

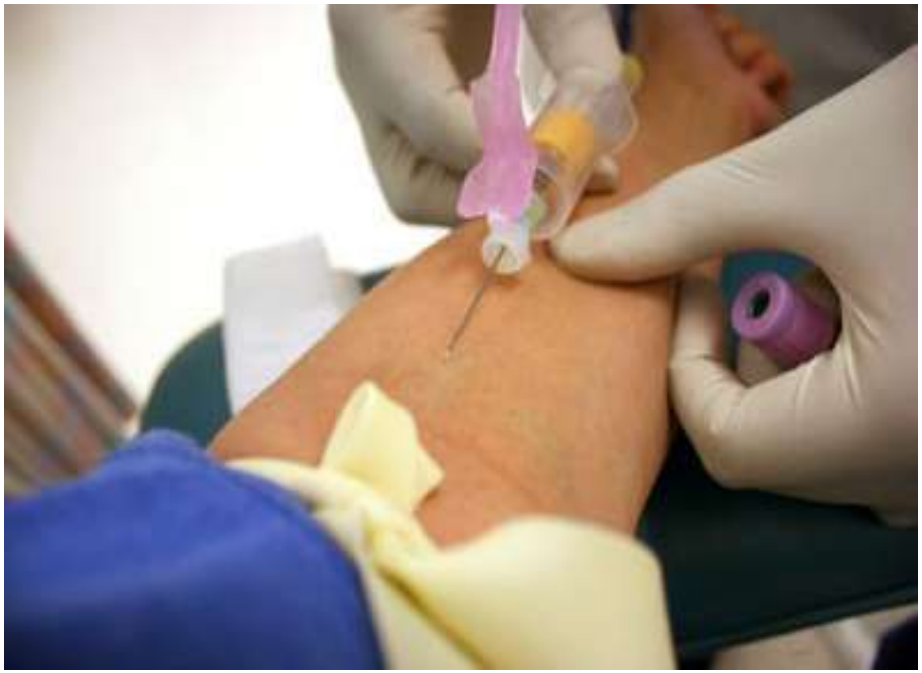


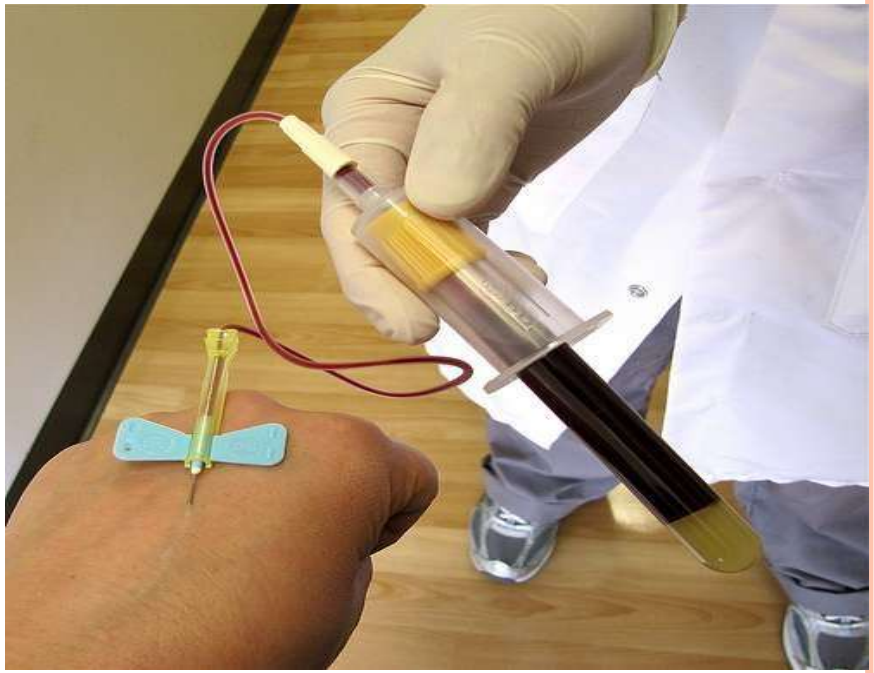
VENIPUNCTURE



7. Draw blood according to required tests.
8. Withdraw the needle. Loosen the tourniquet.
9. Press down on the gauze, applying adequate pressure.

10. Apply a piece of band or medi-plast.
11. Dispose of contaminated material in designated container.
12. Put blood into a suitable container.





Order of Draw:

1. **Blood culture tube** (Yellow-black stopper)
2. **Plain tube** (Red stopper or SST)
3. **Coagulation tube** (light blue stopper).

& the Last draw with Additive tubes in this order:

1. Heparin (Green stopper)

2. EDTA (Lavender stopper)

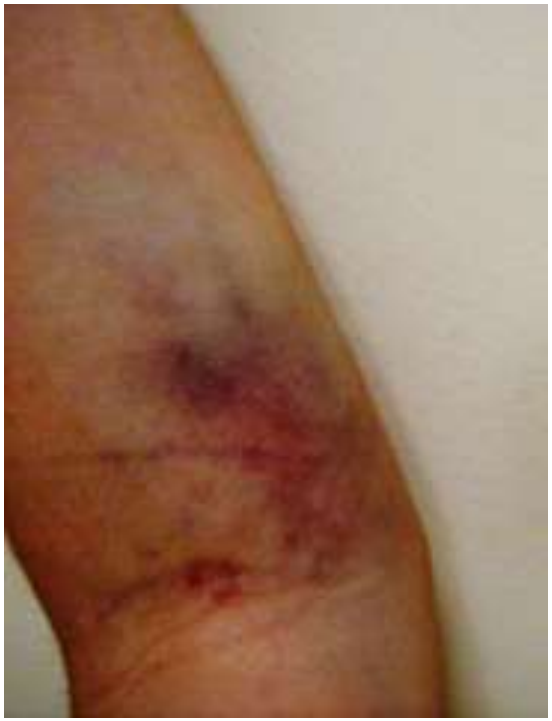
3. Oxalate/flouride (light gray stopper)

Tubes with anticoagulants/additives must be mixed thoroughly with collected blood.

Precaution:

- Venipuncture area must be cleaned/sterilized properly.
- Tourniquet should not be applied for a long time and not more than 1 min.

To avoid stasis of blood



CONT: VENOUS BLOOD

- Blind attempts to puncture the vein should not made.
- Subcutaneous manipulation of the needle to enter a vein should not be done as it causes a lot of pain.
- Once the needle is withdrawn, pressure should be applied and maintained for 1-2 minutes.
- If you can’t control the pressure this will cause **Ecchymoses** i.e. extravasation of blood.

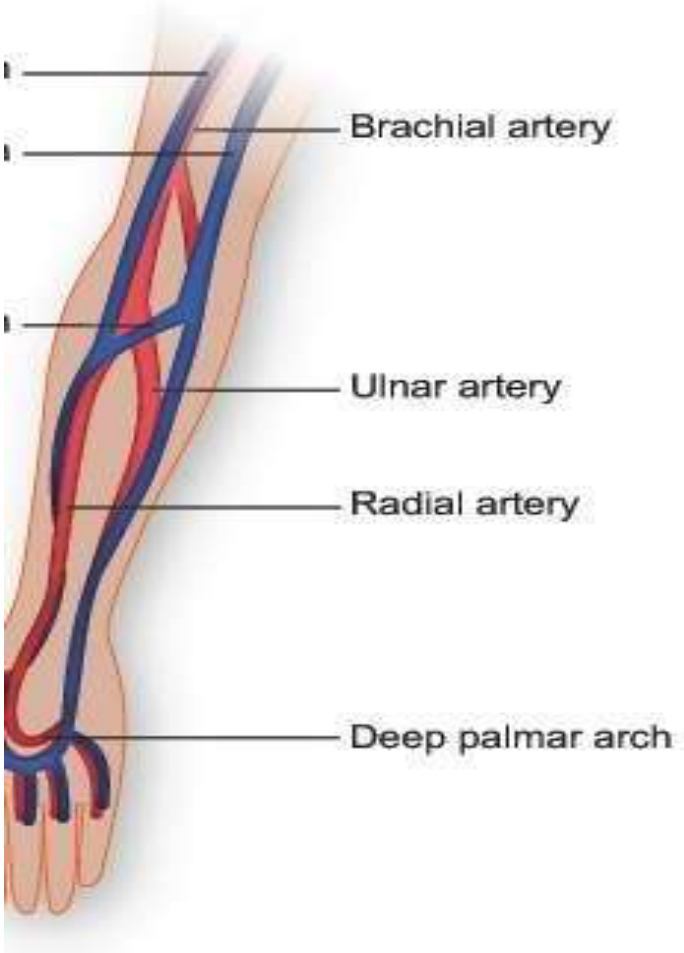
II. Arterial blood

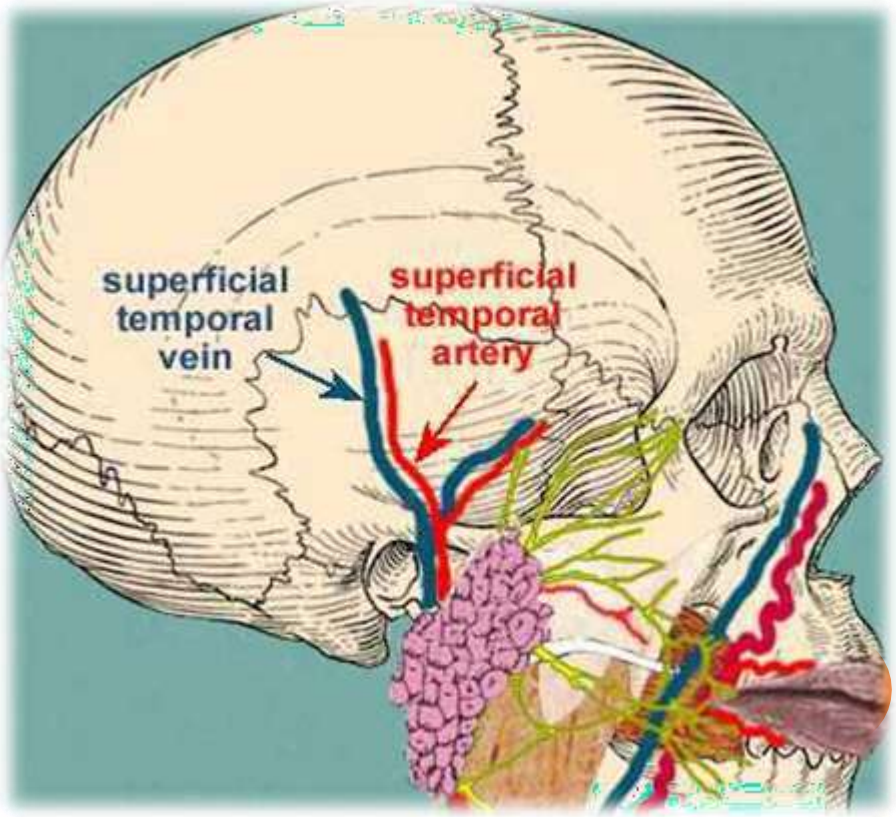
- Specially required for estimation of blood gases (ABG): PH, CO2 and O2
- Collect quickly, fill completely and seal both ends immediately
- No air bubbles
- Put in ice water

Arterial blood can be obtained from a superficial artery such as:

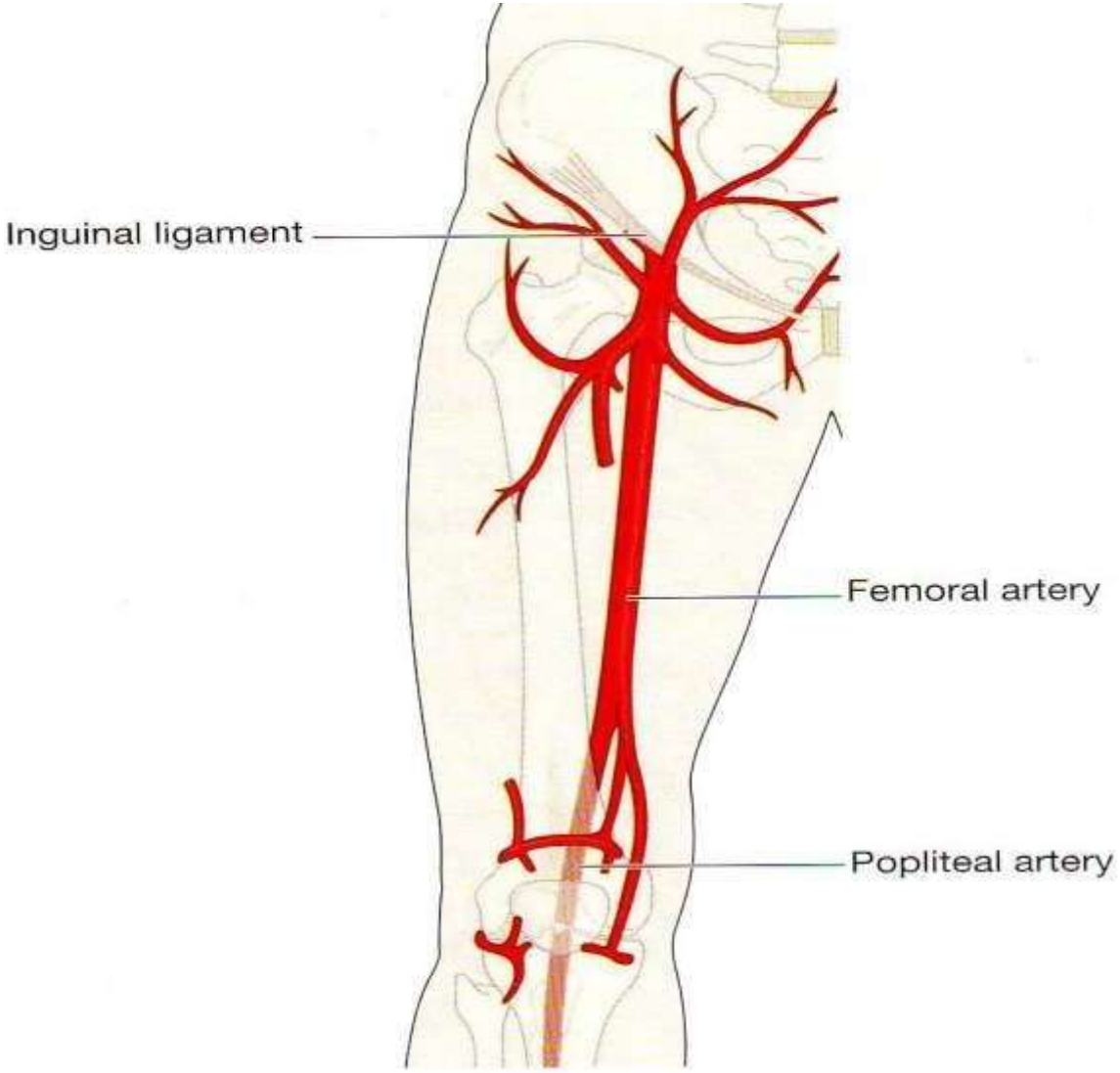
1. Radial and brachial artery

2. Temporal artery





3. Femoral artery

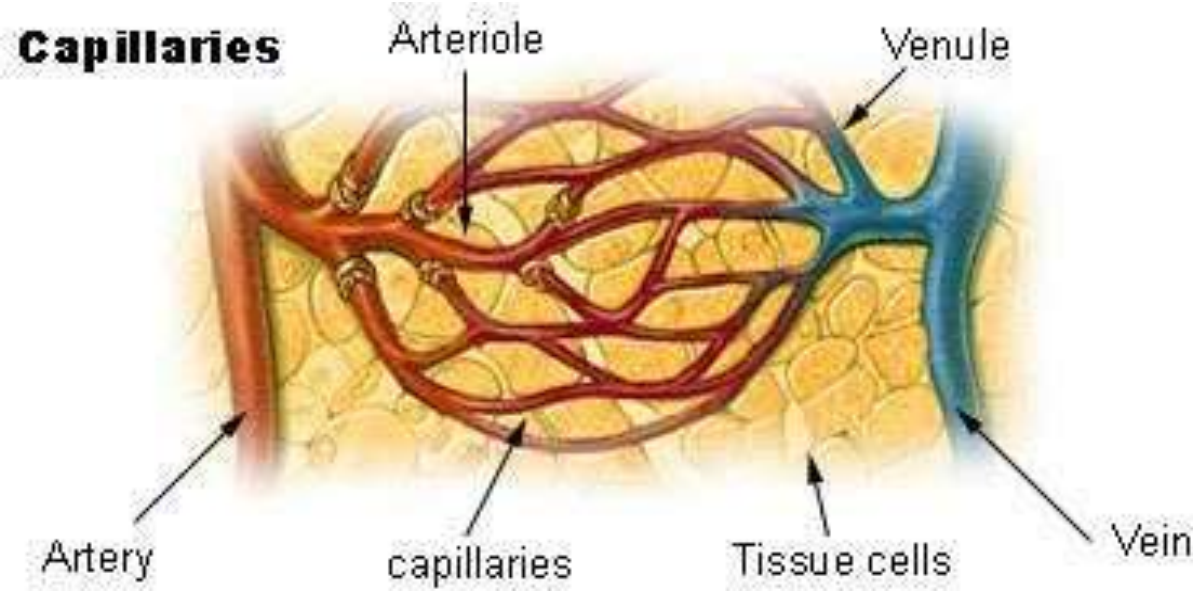


The femoral artery and its main branches.

III. Capillary blood

- To draw only a small amount of blood in a microtube or strip for blood sugar and bleeding time tests.

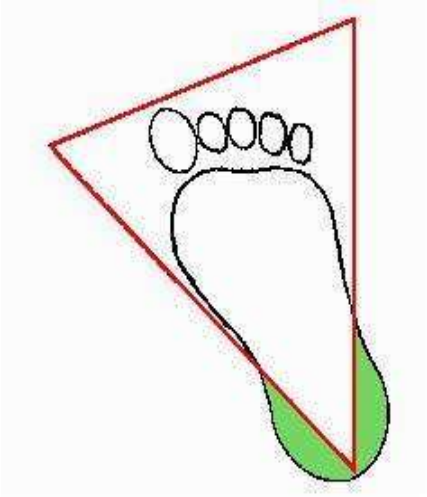
- For infants and young children.



CAPILLARY TUBE

Blood can be obtained from:

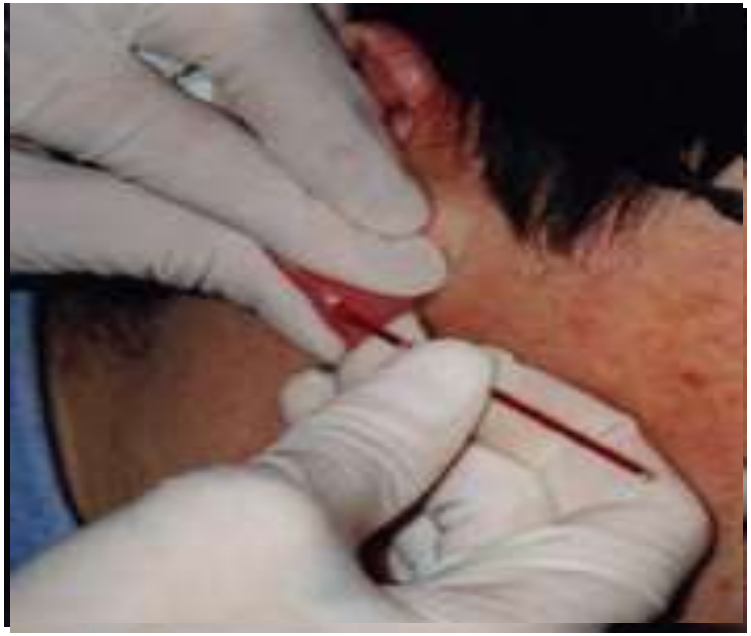
1. Heel pulp



Automatic lancet device

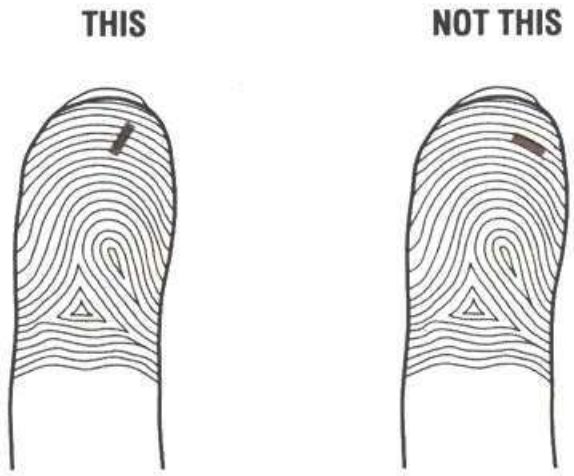
2. Finger pulp

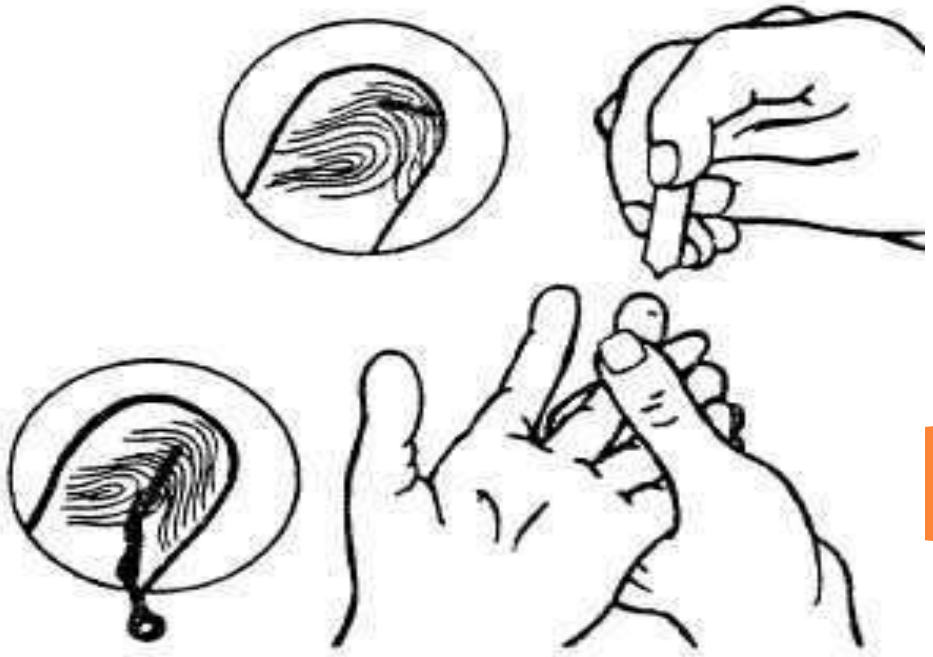
3. Ear lobule



How to collect capillary blood?

- Select the least used finger.
- Cleanse the site with alcohol swab.
- Puncture <u>across the grain of the skin</u>, then transfer blood to a strip or small container.

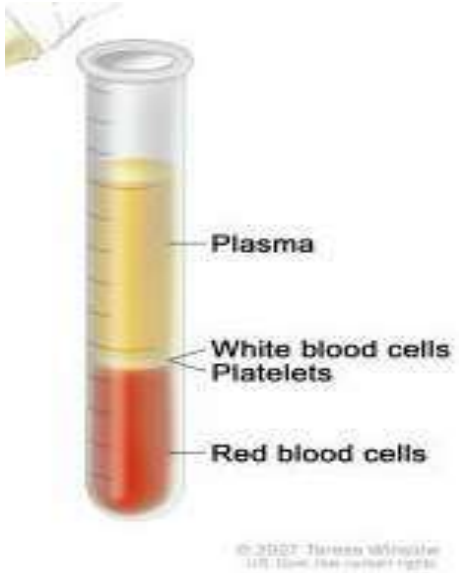




2. interpretation

A. **Plasma:**

- A fluid obtained from anticoagulated and centrifuged blood (at 5000 rpm) where all formed elements are removed.



- Plasma usually is required for Coagulation Profile and Fibrinogen Assay.

B. Serum:

- It is the liquid that remained after blood has clotted naturally in a plain tube.

Donor Selection

Objectives

- Donor selection
- Types of blood donors
- Donor registration
- Medical and physical examination
- Lab investigations
- Donor record register

Introduction

Donor Selection Criteria Donor selection determines the eligibility of a donor to donate blood and blood components.

Protect the donor

- Ensures that it is safe for the donor to donate

Protect the recipient

- Ensures that any risk of transfusion transmitted
- Infection or other adverse effect is minimized.

Types of blood donors

- Voluntary Donors –Donate Blood on their own.

- Replacement Donors : from within the patient's own family or community.

Registration of Donor Selection has Four Major Components:

1. Consent for blood donation
2. Questionnaire
3. Physical examination
4. Simple laboratory tests

Donor registration should include

- Donation date and time.
- Last and first name (middle initial if available).
- Address.
- Telephone number.
- Gender.
- Age (or DOB).
- Previous deferral record must be consulted.

Medical history

•Medical history should be taken by trained health care professional person

•It must be assured that the confidentiality of the donor should be maintained

•Direct questions or leading questions are allowed in the interview

Medical history

- The standard medical information questionnaire which helps to collect same information systematically from each donor.

- This questionnaire can give information, which make quick assessment whether to accept, Temporary defer or permanently reject the donor.

Major conditions for donor deferral

Conditions for deferral	Period of deferral
Abortion	6 Months
H/O blood transfusion	6 Months
Alcoholism	Till intoxicated
Minor surgery	3 Months
Major surgery	6 Months
Typhoid	6 Months after recovery
H/O Malaria	3 Months
Tattoo	6 Months
Breast feeding	6 Months after delivary
Rabies vaccination	1 year

Conditions for donor rejection

- Cancer
- Heart disease
- Abnormal bleeding tendencies
- Unexplained weight loss
- Diabetes-controlled on insulin & oral drugs
- Hepatitis B infection
- Chronic nephritis
- Signs & symptoms suggestive of AIDS
- Liver disease

Physical Examination

- General appearance of donor: Donor should be fit n healthy.
- Pulse:- 60-120 beats per minute.
- Blood pressure
  - Diastolic 60-100 mm Hg
  - Systolic 100-160 mm Hg

Temperature:

Maximum 37.5 ^{0}C

Donor weight:

Minimum 45 Kgs

Amount of blood to be drawn

- ≥ 55 Kg - 450ml
- 45- 54 Kg - 350ml

Lab investigation

Determination of hemoglobin and hematocrit:-

- Donors:-

  Hb 12.5-18 g/dl

  Hct 38-52

•Platelet count

>1.5 lakh (plateletpheresis)

•Complete blood count

Donation interval

1.The minimum time gap between the blood donations should be**3 months**

2. Interval between two Plateletphersis is 48-72hrs.

3. Not more than two procedures to be done in a week

4.Whole blood donation must be take for at least **1 weak** after can be donate the plateletpheresis

5. In case of re-infusion **failure** after pheresis procedure, donor should not donate whole blood **for 3 months**

Informed consent

- **That the donor has under stood all the donor information presented, and have all his questions been answered?**
- **That his blood would be tested for Transfusion Transmitted Infections(TTI).**
- **That if the screening tests are reactive, he/she may transmit TTI.**
- **Whether the donor wants to be informed about abnormal test results?**

SIGNATURE _____

DATED_____

Donor Record Register

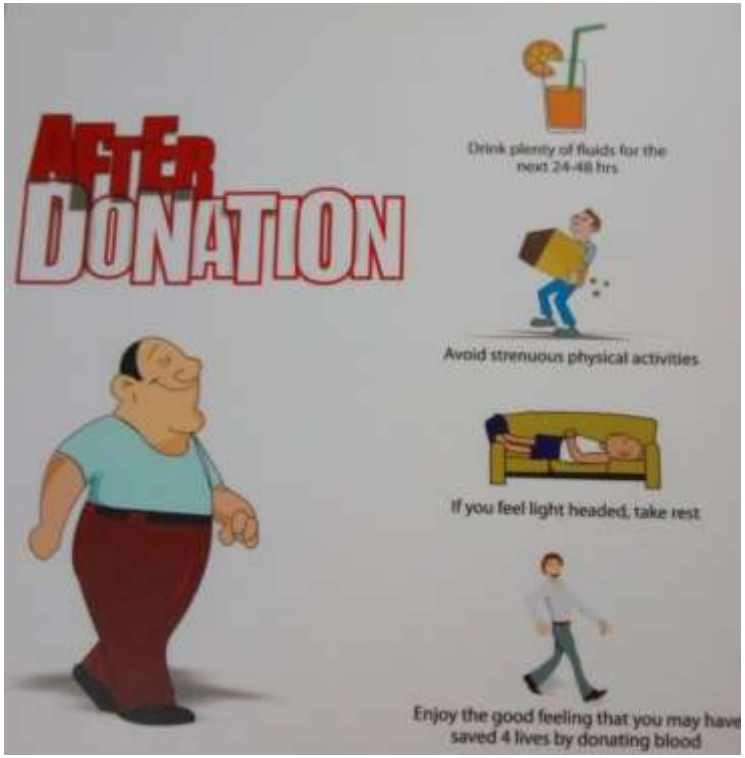
- Registration Number
- Tube segment Number
- Name of Donor
- Father's Name
- Age/Sex
- Address
- Date of Collection
- Date of Expiry
- Blood Group
- Signature of Doctor
- Signature of Phlebotomist

- Weight
- Blood Pressure
- Hemoglobin
- Type of Donation
  VD/RD
- Type of Bag
- Volume of collection
- Time of collection
- Duration of collection

After blood donation

Post-Donation care

- Drink more fluid in next 24 hours.
- Do not smoke for ½ hour after donation.
- Avoid strenuous exercise eg:- weight lifting for 24 hours.
- Do not drive for at least half an hour.
- Report to blood bank in case of any adverse reaction occur.



Donor satisfaction

- Congenial atmosphere
- Trained staff
- Care & comfort
- Confidentiality
- Constant feed back

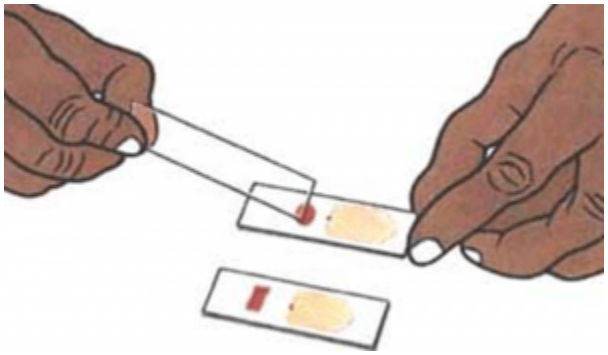
Malaria Test by Thick and Thin smear Preparation

Thick Blood smear

Thick blood film samples a relatively large volume of blood thus allowing more efficient detection of parasites (increased sensitivity).

Thick smears consist of a thick layer of dehemoglobinized (lysed) red blood cells (RBCs) which provides better opportunity to detect parasitic forms against a more transparent background.

However, they do not permit an optimal review of parasite morphology.



Making Thick Blood Smear

- Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm).
- Allow the smear to dry thoroughly. Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. You can accelerate the drying by using a fan or hair dryer.
- If there will be a delay in staining smears, dip the thick smear briefly in water to hemolyse the RBCs.

Thin Blood Smear:

Thin smears consist of blood spread in a layer such that the thickness decreases progressively toward monolayer. It allows optimal assessment of the morphology of any parasitic forms that may be present. Thin blood film is prepared similarly to that of the differential white-cell count.

Making Thin Blood Smear:

- Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide.
- Wait until the blood spreads along the entire width of the spreader slide.
- While holding the spreader slide at the same angle, push it forward rapidly and smoothly.
- Wait until the thin films are completely dry before staining.
- Fix the thin film with methanol (100% or absolute) for 15-30 second and let it dry completely before staining.

VDRL TEST.

Venereal disease research laboratory (VDRL) test is a nonspecific flocculation test which is used for the screening of syphilis. VDRL test is easy to perform and inexpensive, so it is commonly used in the screening of population for syphilis. Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm *Treponemapallidum* infection.

Sample:

1. Serum (plasma can not be used)
2. Cerebrospinal fluid (CSF)

Acceptable CSF and serum specimen should not contain particulate matter that would interfere with reading test results.

Principle of VDRL Test:

Non-treponemal antigen (Cardiolipin-Cholesterol-Lecithin) is used to detect the presence of "reagin antibodies" (IgM and IgGantibodies to lipoidal material released from damaged host cells as well as to lipoprotein like material, and possibly cardiolipin released from the treponemes) in patient's serum.
When the heat inactivated (to destroy complement) serum of patient is reacted with freshly prepared non-treponemal antigen, flocculation reaction (antigen and antibody complex are suspended) occurs. The flocculation can be observed by using microscope with 10x objective and 10x eye piece.

Reactive VDRL test serum can be quantitated to obtain the titre of "reagin antibodies" by using serial double dilution method.

Result and Interpretation of VDRL test

VDRL test is positive in most cases of primary syphilis and are almost always positive in secondary syphilis. **The titer of reagin antibodies decreases with effective treatment**, so VDRL test can be used to determine the treatment response of syphilis.

Limiation of the Test

A. False positive VDRL test result

1. Reagin antibodies may be produced in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs such as:
   - Leprosy
   - Hepatitis B
   - Infectious Mononucleosis
   - Various autoimmune diseases
2. VDRL may be reactive in persons from areas where yaws is endemic. As a rule, residual titers from these infections will be <1:8.
3. Nontreponemal test titers of persons treated in latent or late stages of syphilis or who have become reinfected do not decrease as rapidly as do those from persons in the early stages of their first infection. In fact, these persons may remain "**serofast,"** retaining a low -level reactive titer for life.

B. False negative VDRL test

It can be seen because of prozone phenomenon (no flocculation due to antibody excess). In that case test serum has to be diluted further to obtain zone of equivalence (where maximum flocculation of Ag-Ab occurs).

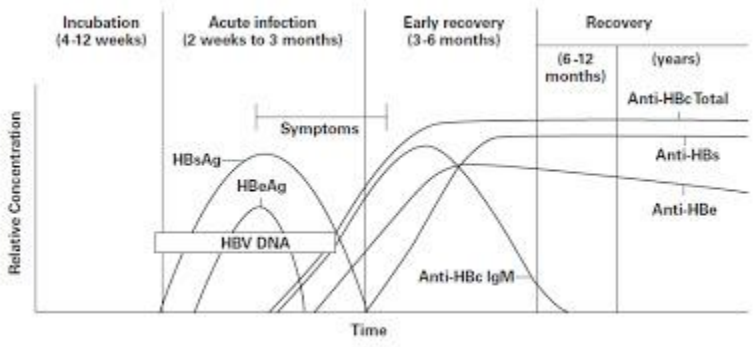
HbsAg Test.

Serological Diagnosis of Hepatitis B virus Infection

HBsAg and hepatitis B e antigen (HBeAg) are the first markers identified in the serum of patients acutely infected with HBV.

Detection of Antigen:

1. HBsAg (also called Australia antigen or hepatitis-associated antigen):



Timeline for Acute Hepatitis B Virus Infection

HBsAg generally appears before symptom onset and peaks during overt disease. In patients who successfully clear the HBV infection and do not progress to the chronic carrier stage, HBsAg typically is undetectable 4 to 6 months after infection

2. HBeAg:

HBeAg, a soluble protein which is contained in the core of Hepatitis B Virus. HBeAg is generally considered to be a marker of HBV replication and infectivity.

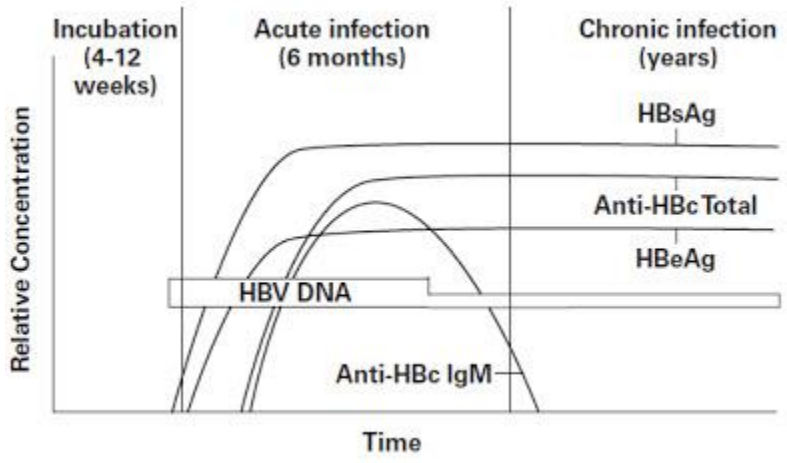
Detection of Antibody

1. IgM anti-HBc (IgM class antibody to HBcAg)

The first antibody to appear is IgM antibody to hepatitis B core antigen (IgM anti-HBc). The presence of the IgM anti-HBc antibody is diagnostic of acute HBV infection. IgM anti-HBc is first detectable in the serum shortly before the onset of symptoms at a time generally concurrent with the onset of elevated transaminase levels.

2. IgG anti-HBc (IgG class antibody to HBcAg)

The presence of IgG anti-HBc indicates that the patient has a history of infection with HBV. Within several weeks, the IgM anti-HBc disappears, and IgG anti-HBc is detected. IgG anti-HBc may remain present for life.



Timeline for Chronic Hepatitis B Virus Infection

3. Anti-HBs

The presence of anti-HBs is likely to be protective against repeated HBV exposure, and the anti-HBs may persist for life.

4. Anti-HBe

When viral replication slows and infectivity declines, the HBeAg disappears and antibodies to hepatitis B e antigen (anti-HBe) may be detected. HBeAg to anti-

HBeseroconversion occurs early in patients with acute infection, before HBsAg to anti-HBs seroconversion. Anti-HBe may persist for years.

HCV Test.

- **Introduction**

Laboratory tests for hepatitis C are divided into four general categories:

- **Screening:** Screening for hepatitis C virus (HCV) is done with a serologic test for the HCV antibody (Ab).
- **Confirmatory:** Diagnosis of chronic hepatitis C requires the presence of HCV RNA, commonly called hepatitis C viral load.
- **Genotype:** Once it is determined that HCV RNA is present, the specific genotype and subtype of the virus can be determined with a genotype test.
- **Drug resistance:** Mutations of some proteins in HCV can allow the virus to have resistance to direct-acting antivirals (DAAs), commonly referred to as resistance-associated variants (RAVs) or resistance-associated polymorphisms (RAPs).

➢ HCV Serologic Testing (HCV Ab)

Enzyme immunoassays for Detection of Hepatitis C Antibody

The HCV Ab test is used for initial screening for hepatitis C. The test is performed by enzyme immunoassays (EIAs), which detect the presence of hepatitis C antibodies in serum. The result of the test is reported as positive or negative. Third-generation EIAs have a sensitivity/specificity of approximately 99%. However, the presence of HCV Ab does not indicate whether the infection is acute, chronic, or resolved. A positive antibody test result should be followed up with an HCV RNA test to confirm that viremia is present.

➢ HCV RNA Testing

The presence of HCV RNA is required to confirm chronic HCV infection. Therefore, a positive HCV Ab screening result must be followed by a test for the HCV RNA. The HCV RNA tests can detect virus within 1-2 weeks following exposure.

Appropriate Uses of the HCV RNA Test

There are 4 major reasons that HCV RNA tests are used:

1. To confirm a positive HCV Ab result and make the diagnosis of current HCV infection
2. To measure a patient's baseline viral load prior to starting HCV therapy
3. To monitor a patient's response to therapy
4. To determine whether a patient has achieved a sustained virologic response (SVR)

More rarely, HCV RNA is used when either very acute HCV infection is suspected or a false HCV Ab is suspected.

It would not be appropriate to repeatedly order HCV RNA viral load screening for a patient who is not on or was recently on HCV treatment, or to use the HCV viral load to determine the severity of the patient's infection or the patient's risk of developing significant liver disease.

➢ HCV Reflex Testing

Per VA policy, an HCV RNA test is automatically performed on all positive HCV antibody specimens (reflex testing). The reflex process helps in the clinical management of the patient by avoiding the need for a patient to return for a second blood draw if the antibody result is positive.

HCV Genotype Testing

There are at least six HCV genotypes. These are classified as genotypes 1-6. There are also 30 subtypes of HCV, which are referred to as genotypes 1a, 1b, 2a, etc.

Identifying HCV genotypes is essential for selecting treatment regimens and predicting treatment response. Within genotype 1, it is also important to determine whether the patient is subtype 1a or 1b, as this determines treatment duration and the need for ribavirin in the treatment regimen. Patients only need to be genotype tested once in their lifetime, as the genotype remains the same throughout the course of infection. Repeating a genotype test is warranted only if there is suspicion that a patient may have been reinfected with a different genotype after achieving an SVR.

Genotype testing is performed by analyzing the sequences of various regions of the HCV genome. Most genotype assays rely on the amplification of short HCV RNA regions from clinical specimens, followed by a type-specific assay, such as restriction fragment length polymorphism (RFLP) analysis, line probe reverse hybridization, or sequence analysis. Most assays target the 5' untranslated region (5' UTR), as it is the most conserved region throughout the HCV genome and is most suitable for reverse transcription polymerase chain reaction (RT-PCR) amplification.

- **HCV Resistance Testing (RAV testing)**

DAAs are drugs that target specific steps in the life cycle of the hepatitis C virus. When these steps are disrupted, replication of HCV is stopped. DAA drug classes include NS5A inhibitors, NS5B polymerase inhibitors, and NS3/4A protease inhibitors. Resistance Associated Variants (RAVs) refer to mutations that occur in the target enzymes that confer resistance to DAAs. RAV testing is done in most patients who have failed a prior DAA-containing regimen before they initiate re-treatment with another DAA regimen. For example, genotype 3 patients are recommended to have RAV testing if they are treatment experienced before starting re-treatment with sofosbuvir/velpatasvir and to determine whether ribavirin is needed. RAV testing is occasionally done in treatment-naive patients if it may change the regimen or the duration of treatment. For example, genotype 1a patients who are treatment naive should be RAV tested before starting treatment with elbasvir/grazoprevir to determine whether ribavirin is needed or whether an extended duration of treatment is needed. Genotyping of the NS5A, NS5B, and NS3/4A genes to identify RAVs can now be accomplished by RT-PCR and

population-based sequencing methods at the VA Palo Alto Public Health Reference Laboratory (PHRL) and at commercial laboratories including Monogram Biosciences (LabCorp) and Quest Diagnostics. HCV drug resistance testing should be ordered only by experienced HCV clinicians.

HIV TEST.

An HIV test shows whether you are infected with HIV (human immunodeficiency virus). HIV is a virus that attacks and destroys cells in the immune system. These cells protect your body against disease-causing germs, such as bacteria and viruses. If you lose too many immune cells, your body will have trouble fighting off infections and other diseases.

There are three main types of HIV tests:

Antibody Test. This test looks for HIV antibodies in your blood or saliva. Your immune system makes antibodies when you are exposed to bacteria or viruses, like HIV. An HIV antibody test can determine if you have HIV from 3–12 weeks after infection. That's because it can take a few weeks or longer for your immune system to make antibodies to HIV. You may be able to do an HIV antibody test in the privacy of your home. Ask your health care provider about at-home HIV test kits.

HIV Antibody/Antigen Test. This test looks for HIV antibodies *and* antigens in the blood. An antigen is a part of a virus that triggers an immune response. If you've been exposed to HIV, antigens will show up in your blood before HIV antibodies are made. This test can usually find HIV within 2–6 weeks of infection. The HIV antibody/antigen test is one of the most common types of HIV tests.

HIV Viral Load. This test measures the amount of the HIV virus in the blood. It can find HIV faster than antibody and antibody/antigen tests, but it is very expensive. It is mostly used for monitoring HIV infections.

CROSS-MATCHING

INTRODUCTION

- **Cross-matching is one of the most important serological procedure pertaining to blood group serology and is the fundamental procedure responsible for safe blood transfusion.**

- **Basically Cross-matching is an antigen-antibody reaction, a correct interpretation of which is the most essential preliminary step in the practice of safe transfusion of blood.**

- **By cross-matching we are able to detect the atypical and clinically significant antibody mostly IgM and IgG present in recipient serum or in donor serum, also by autocontrol we are able to detect auto-antibody in patient himself.**

CONTINUE...

- **Cross match test is carried out to ensure that there are no antibodies present in patients serum that will react with donor cells when transfused.**

- **Unless there is an urgent need for blood, a cross-match must be preformed for red cell transfusion.**

FUNCTIONS OF CROSS-MATCH

- It is final check of ABO compatibility between the donor and patient.
- It may detect the presence of an antibody in the patient's serum which will react with an antigen on donor red cells
- To ensure that patient/ recipient is supplied with a compitable unit of antigen negative blood.
- To prevent hemolytic transfusion reaction.
- To detect immunologic auto antigen and auto antibody, and blood to be issued has to be processed accordingly.

CROSS MATCH TESTING PROCEDURES HAD BEEN DIVIDED INTO TWO PARTS.....

- Major cross match
- Minor cross

MAJOR CROSS MATCH

DONOR RED CELLS + PATIENT SERUM

MINOR CROSS MATCH

PATIENT CELLS + DONOR SERUM

PRINCIPLE OF CROSS MATCH

- **Major cross match is done to detect any serological incompatibility b/w donor's cells and patients serum.**
- **Minor cross-match is done to detect any serological incompatibility b/w patient cells and donor serum.**
- **Cross-match is verified with a coombs reaction to detect even the incomplete antibodies.**

CROSS-MATCH TECHNIQUES

- Immediate spin method
- Saline room temperature technique
- Indirect Antiglobulin technique
- Albumin addition technique

IMMEDIATE SPIN TECHNIQUE/SALINE ROOM TEMPERATURE TECHNIQUE

- **Immediate spin technique or saline room temperature technique is enough to rule out any ABO grouping error.**
- **But this technique is inadequate for identification of clinically significant IgG type of antibodies.**
- **Both these techniques are not good, because antibody screening has not been carried out in our country.**

PROCEDURE

- label the tube as major/minor cross-match with donor number & patient ID.
- Using a micropipette add 1 volume of 2-5 % red cell suspension to the labeled tube and 2 volume of serum to the same tube.
- Mix the tube well and centrifuge at 1000RPM for 1 min.

CONTINUE..

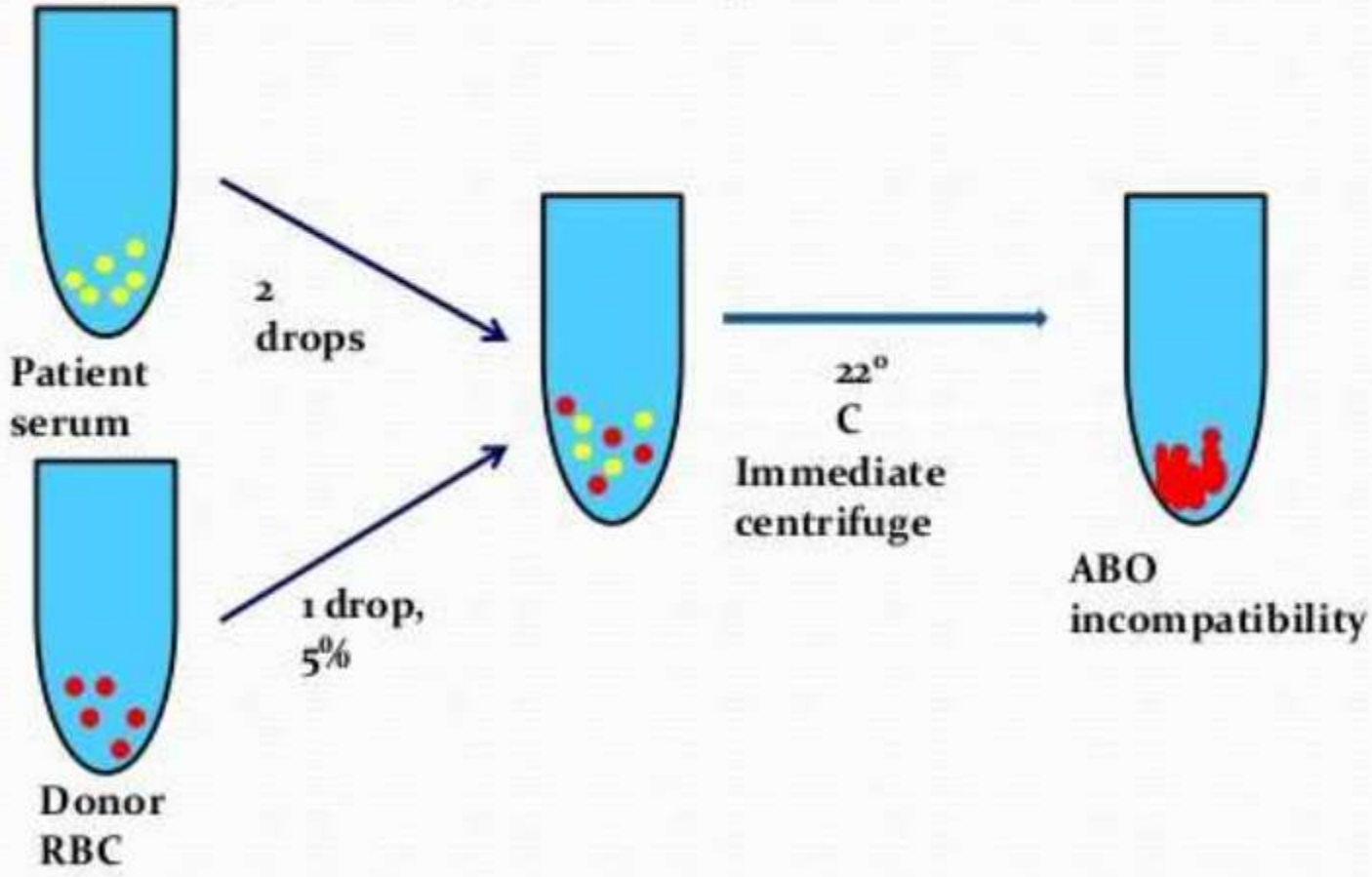
- **Gently shake and observe for agglutination or hemolysis**

NO agglutination or hemolysis: Compatible

Agglutination or hemolysis seen : Incompatible

Immediate spin technique

- ✓ Detects only IgM antibody, reactive at 22°C.
- ✓ Clinically significant IgG antibody reactive at 37°C not detected



INDIRECT ANTI GLOBULIN TECHNIQUE (IAT)

- **IAT test is widely used in cross matching as it detects majority of incomplete antibodies.**

PROCEDURE

- Put 2 drops of serum in prelabelled test tube, & one drop of 2 – 5% suspension of red cells
- Incubate 45 to 60 min at 37°c
- Gently shake and observe for agglutination or hemolysis
- If test tube show any agglutination or hemolysis that means incompatible at 37°C

CONTINUE...

- If no agglutination or hemolysis present, wash the cells three to four times with saline and decant the last wash completely.
- Add 2 drops of AHG reagent to the test tube
- Gently shake and read the result immediately, if no reaction, Wait for 5 min
- Centrifuge at 1000rpm for 1 min

CONTINUE...

- **Shake gently and observe for agglutination or hemolysis with optical aid**

NO agglutination or hemolysis: Compatible

Agglutination or hemolysis seen : Incompatible

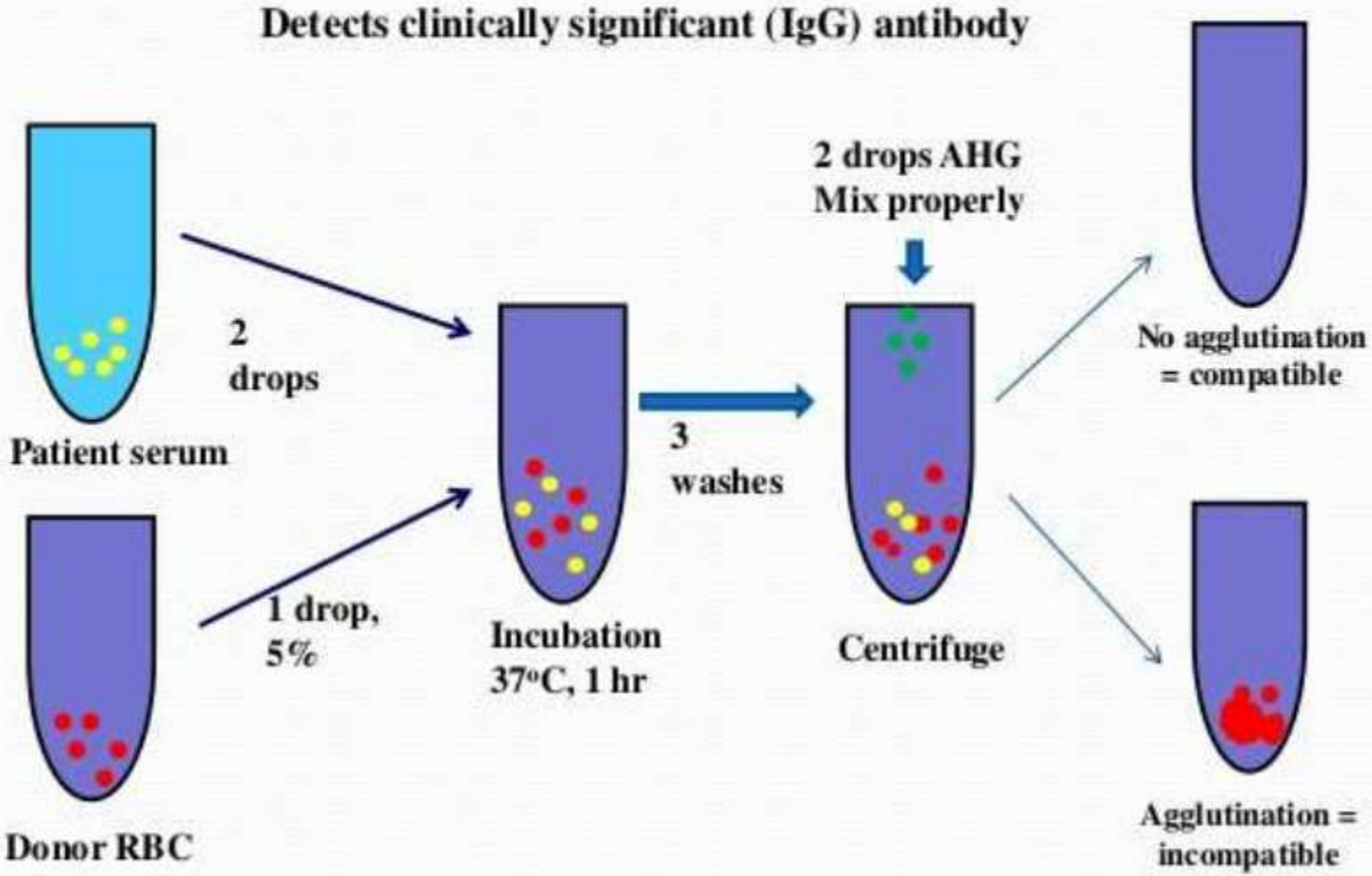
CONTINUE..

- If the test is negative add one drop control IgG coated red cells. Centrifuge again at 1000 rpm for 1 min
- look for hemolysis or agglutination
- if no agglutination or hemolysis, the test is invalid repeat the procedure

INTERPRETATION

- **Haemolysis or agglutination at any stage of the test procedure except after adding control IgG coated red cells indicates incompatible**

Indirect Anti globulin Technique



FACTORS AFFECTING IAT

- **Temperature (37°C)**
- **Serum : Cell ratio**
- **Incubation time**
- **Suspending Medium**

(sensitivity of IAT increased with addition of 22 % bovine albumin , enzyme or LISS)

ALBUMIN ADDITION TECHNIQUE

- Put 2drops of serum of serum 1drop of 2-5% red cell suspension in to a pre labeled test tube, Gently shake the tube
- Add 2 drops of bovine serum albumin & Gently shake the tube
- Incubate the test tube 30min at 37°c
- Centrifuge 1000rpm for 1 min

CONTINUE...

- Wash 3 times(minute amount of human protein can neutralize AHG)ensure that the saline is completely decanted after each wash
- Add AHG 2 drops to the dry cell button
- Gently shake and read the result immediately, if no reaction, Wait for 5 min
- Centrifuge at 1000rpm for 1 min

CONTINUE...

- **Shake gently and observe for agglutination or hemolysis with optical aid**

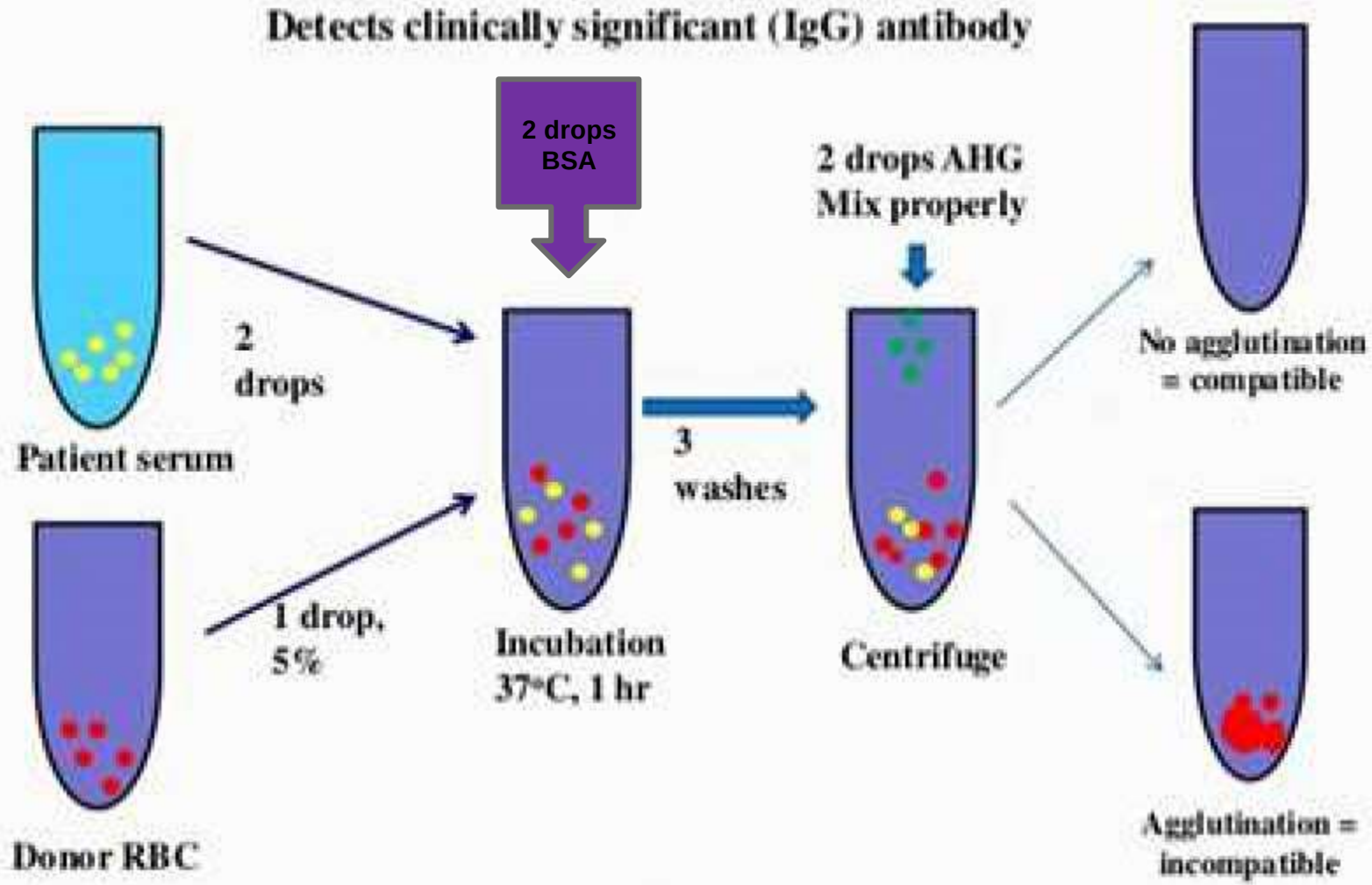
NO agglutination or hemolysis: Compatible

Agglutination or hemolysis seen : Incompatible

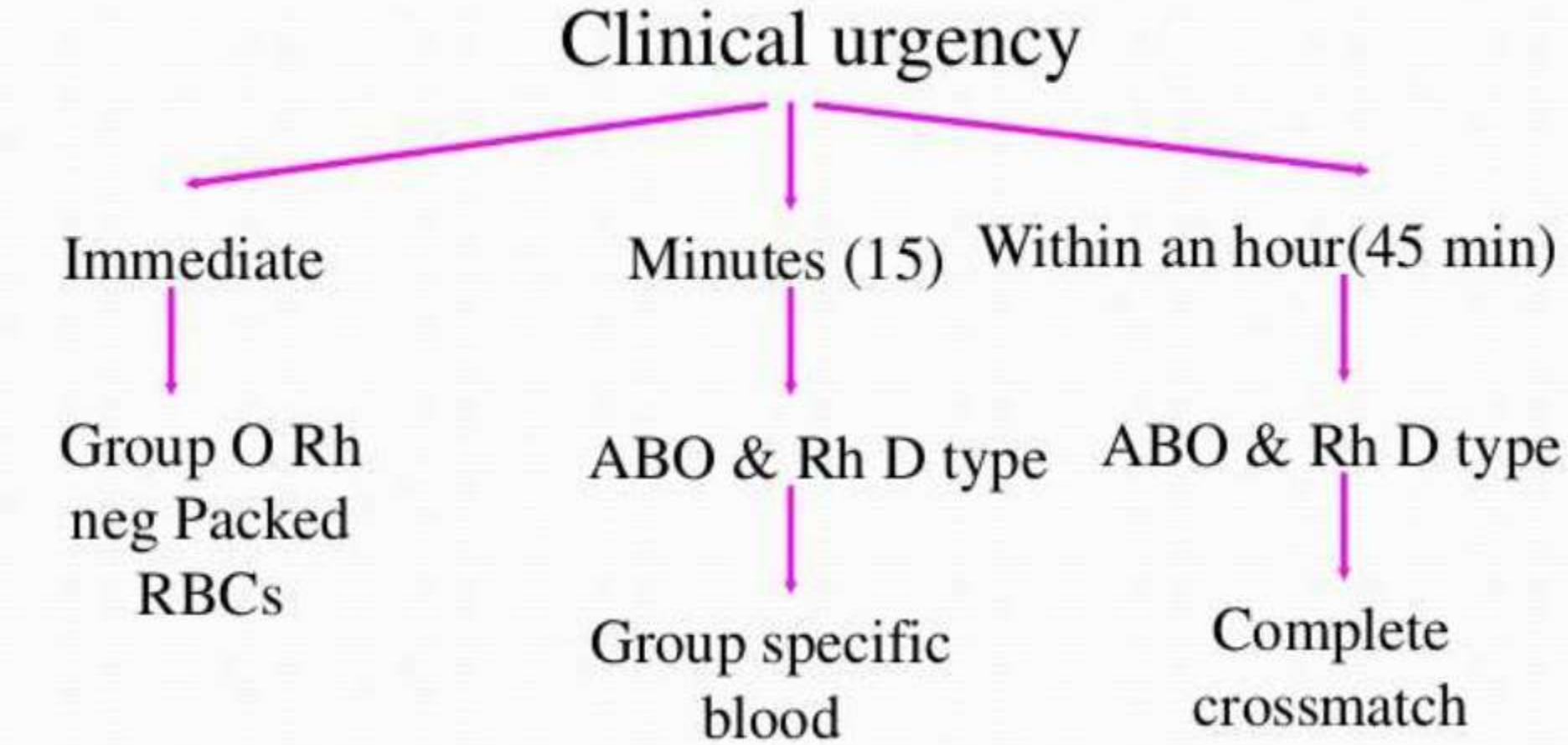
CONTINUE..

- If the test is negative add one drop control IgG coated red cells. Centrifuge again at 1000 rpm for 1 min
- look for hemolysis or agglutination
- if no agglutination or hemolysis, the test is invalid repeat the procedure

Albumin Addition Technique



Cross matching: Special Circumstances



CAUSES OF POSITIVE RESULTS IN CROSS-MATCH

- Incorrect ABO grouping of patient or donor.
- An allo-antibody in the patient serum reacting with the corresponding antigens on donor cells.
- An auto-antibody in patients serum reacting with corresponding antigen on donor red cells. This can be solved by putting auto control which will be positive.
- Donor red cells with a positive DAT.
- Problems in patients serum E.g. Multiple myeloma.
- Dirty glass ware.

DRAWBACK IN CROSS-MATCHING

- **Rh typing errors cannot be detected by cross-matching.**
- **It can detect only antibody specific for red cell antigen of the donor. A donor unit without appropriate antigen or with very weak antigen may thus fail to detect the corresponding antibody even if it present in serum of the patient**
- **Present method of compatibility testing cannot detect any antibodies to either leucocyte antigen or platelet antigen**

COOMBS TEST

The Antiglobulin Test

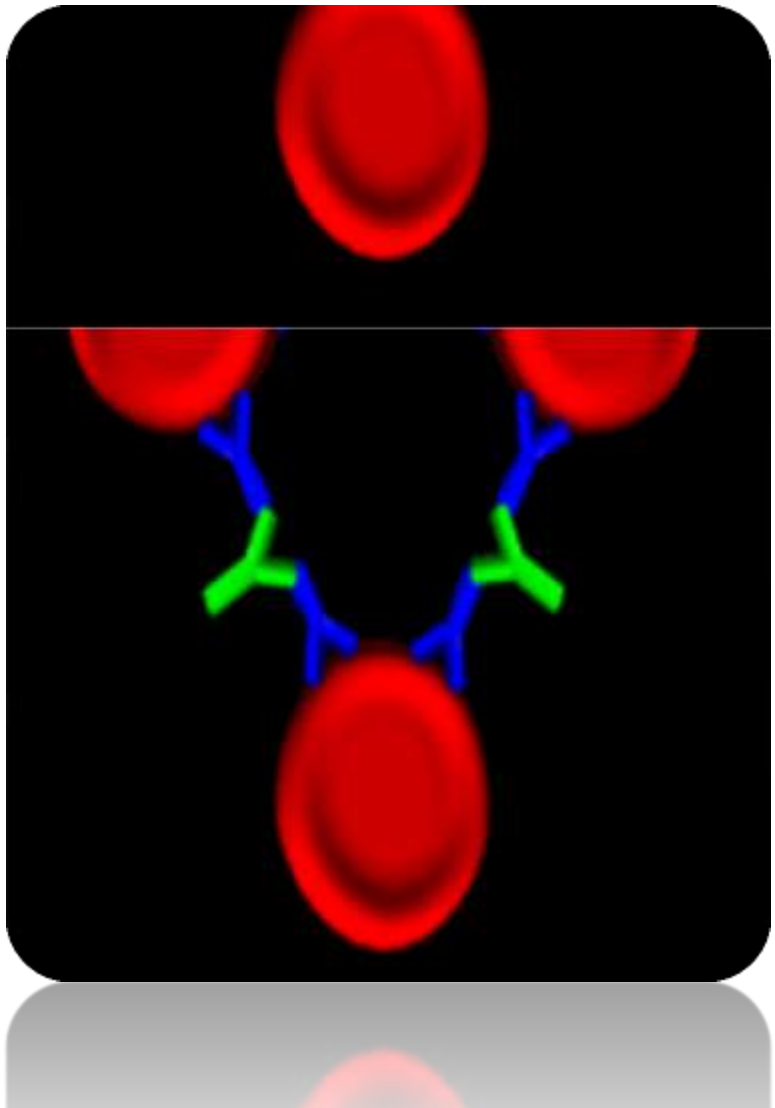
- **Antiglobulin serum** (Coombs'Serum) was discovered by Coombs etal in 1945.
- The antiglobulin test can be used to detect red cells sensitized with **IgG alloantibodies**, IgG autoantibodies or complement components.
- Sensitization of red cells can occur in vivo or vitro. The use of AHG serum to detect sensitization of red cells in vitro is a two stage technique known as indirect antiglobulin test (IAT). The sensitization of red cells in vivo is detected by one stage technique the direct antiglobulin test (DAT).

Principle of Antiglobulin Test

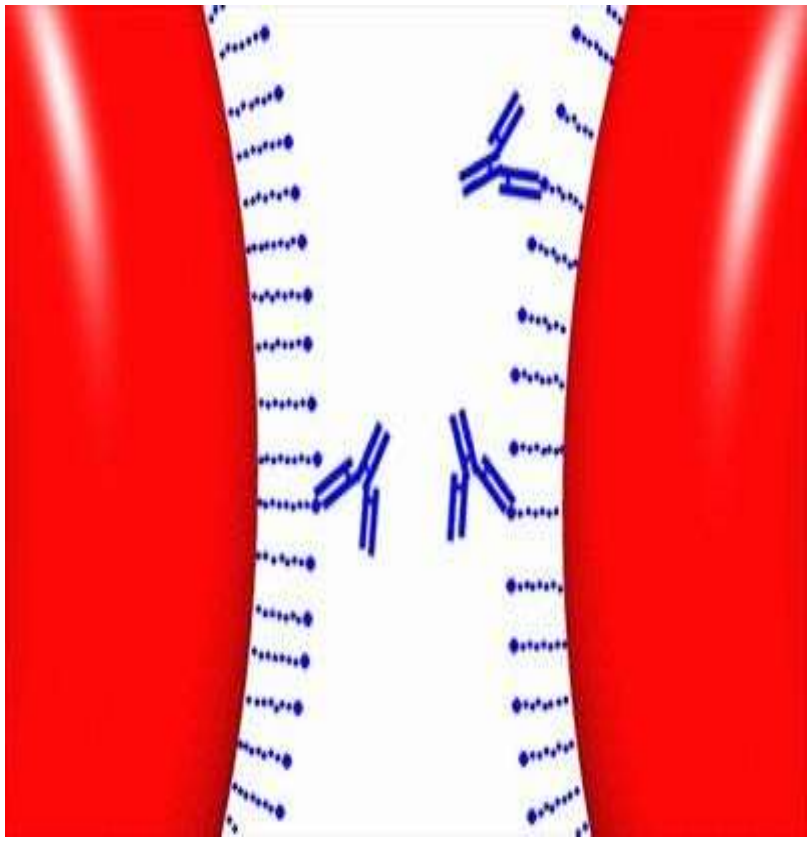
- The incomplete antibodies (IgG) attach to red cell membrane by the Fab portion of the immunoglobulin molecule (IgG).
- The IgG molecules attached to the red cells are unable to bridge the gap between sensitized red cells which are separated from each other by the negative charge on their surface and the sensitized red cells do not agglutinate.

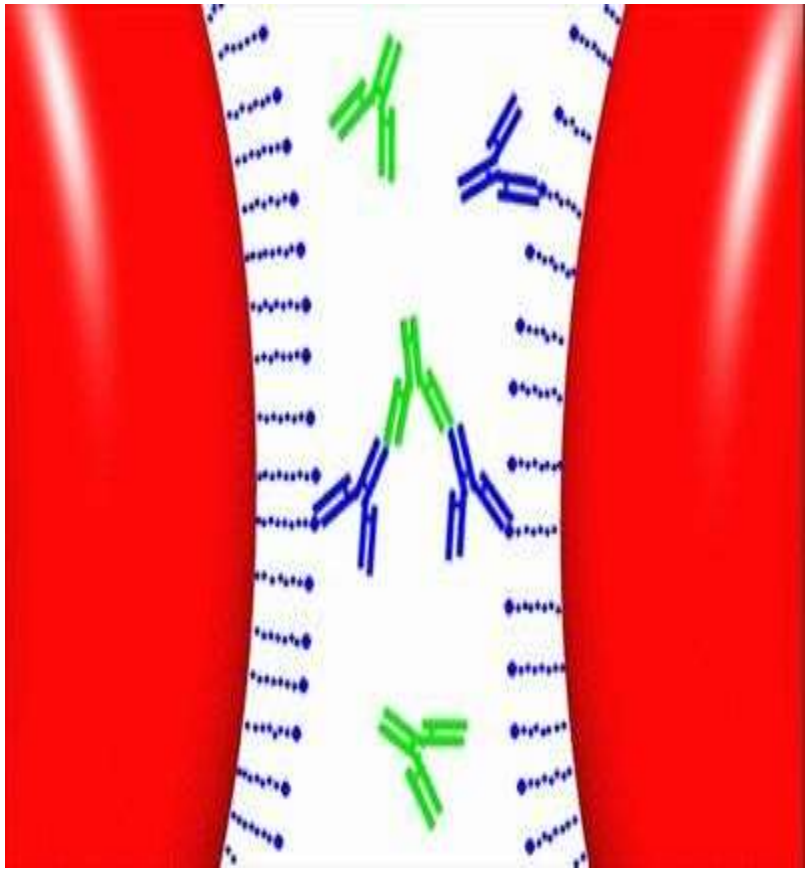
What is Coombs' Serum

- Serum from a rabbit or other animal previously immunized with purified human globulin to prepare antibodies directed against IgG and complement, used in the direct and indirect Coombs' tests. Also called *antihuman globulin*.



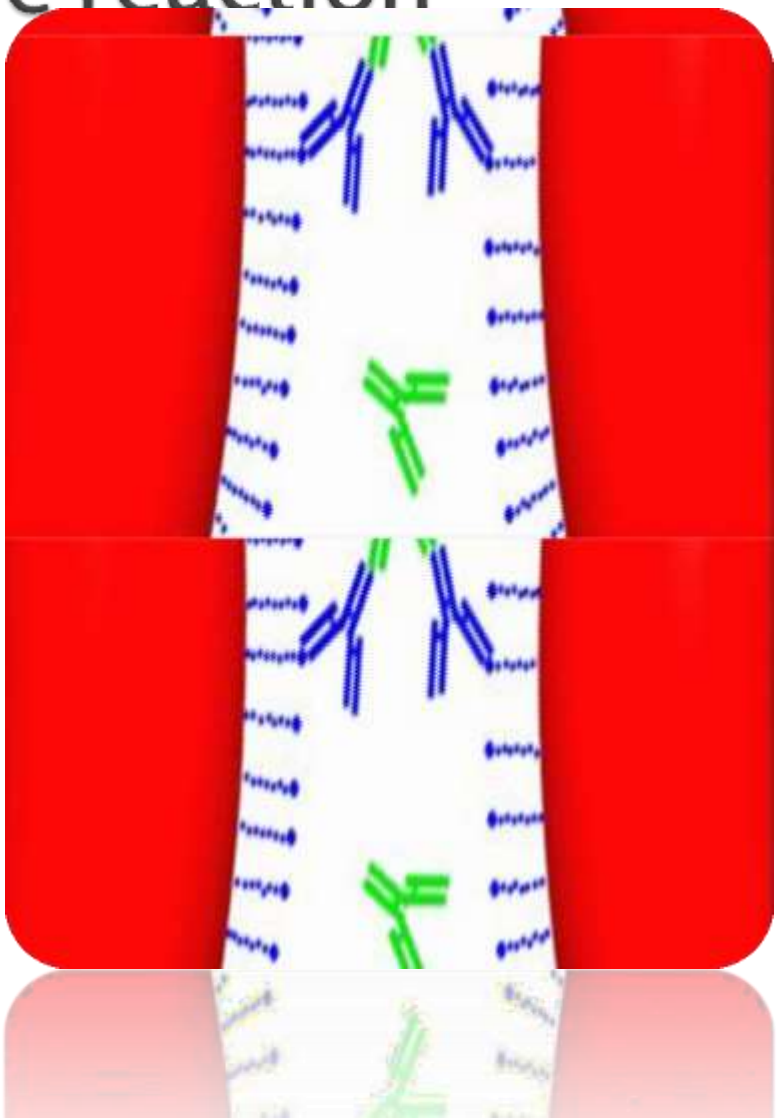
Showing incomplete and complete Agglutination Reactions



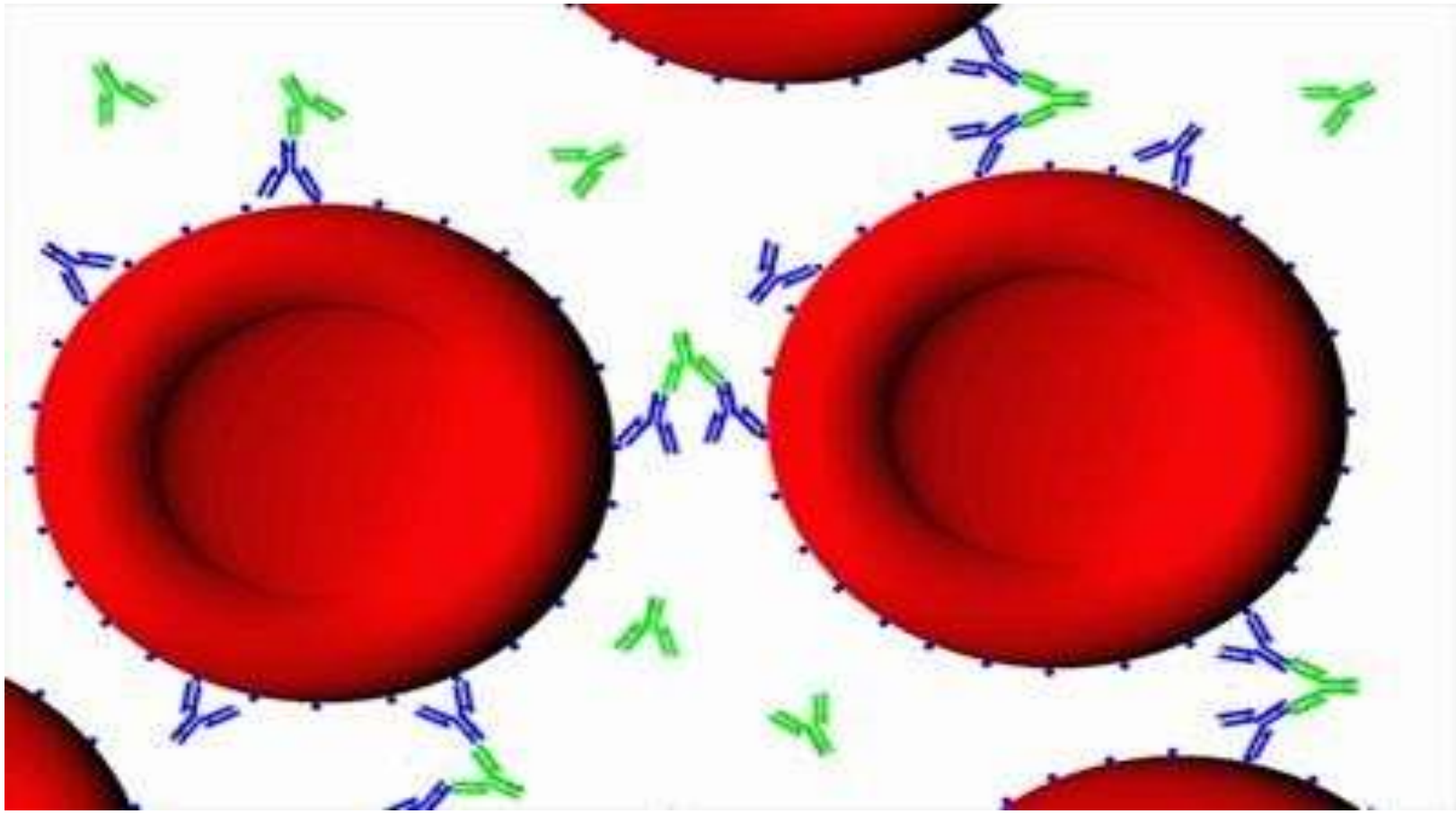


Adding of Antiglobulin serum completes the reaction

- When AHG serum is added to the washed sensitized cells, the Fab portion of the AHG molecule (anti-IgG) reacts with the Fc portions of two adjacent IgG molecules attached to red cells thereby bridge the gap between sensitized red cells and cause agglutination



Showing a Complete Reaction with Coombs Serum



How and Why to prepare for the test

- No special preparation is necessary.
- Infants or young children:
- The preparation you can provide for this test depends on your child's age, previous experiences, and level of trust.
- Why the test is performed
- The indirect Coombs' test detects circulating antibodies against red blood cells (RBCs). The major use of this test is to determine if the patient has antibodies in the blood capable of attaching to RBCs. These antibodies are other than the major ABO system or the Rh type.

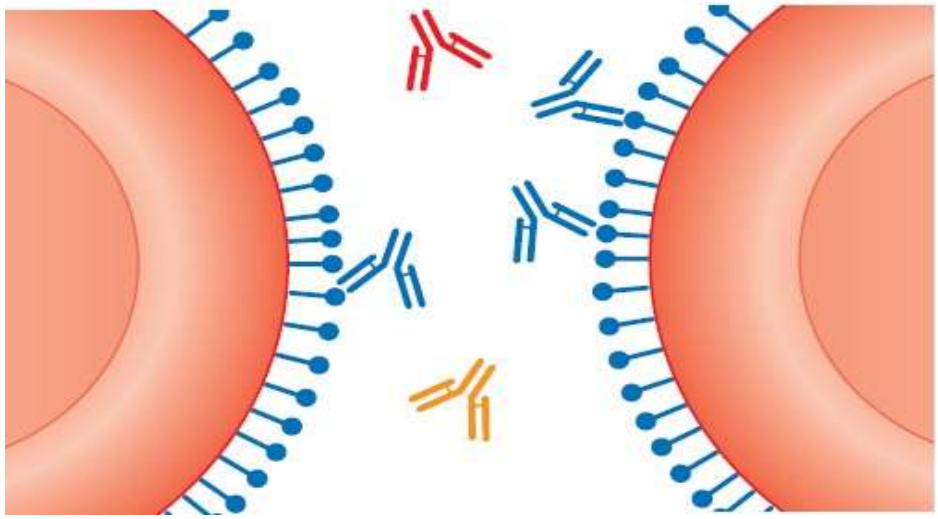
Indirect Coombs test (Indirect Antiglobulin test):

- This test is performed to detect presence of Rh-antibodies or other antibodies in patients serum in case of the following:

1. To check whether an Rh-negative women (married to Rh-positive husband) has developed Anti Rh-antibodies

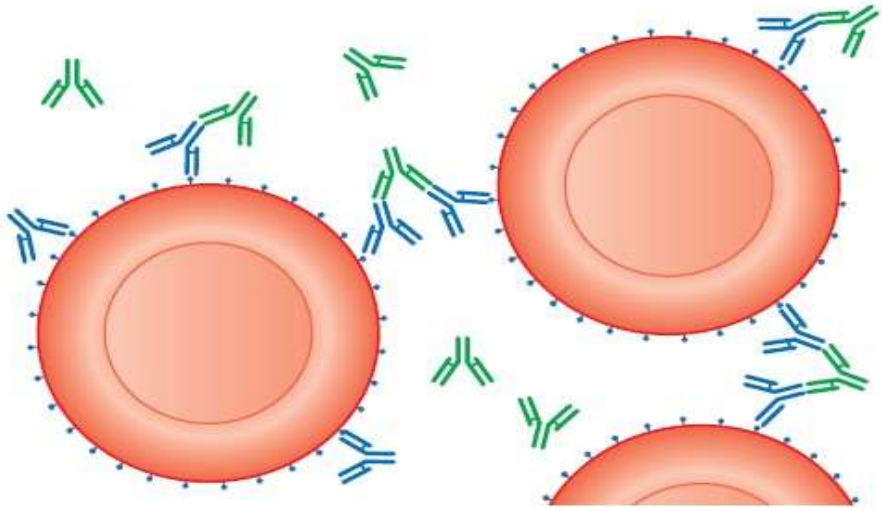
2. Anti D may be produced in the blood of any Rh-negative person by exposure to D antigen by-
    - **Transfusion of Rh positive blood**
    - **Pregnancy, if infant is Rh positive (if father is Rh-positive)**
    - **Abortion of Rh-positive fetus.**

Indirect antiglobulin test

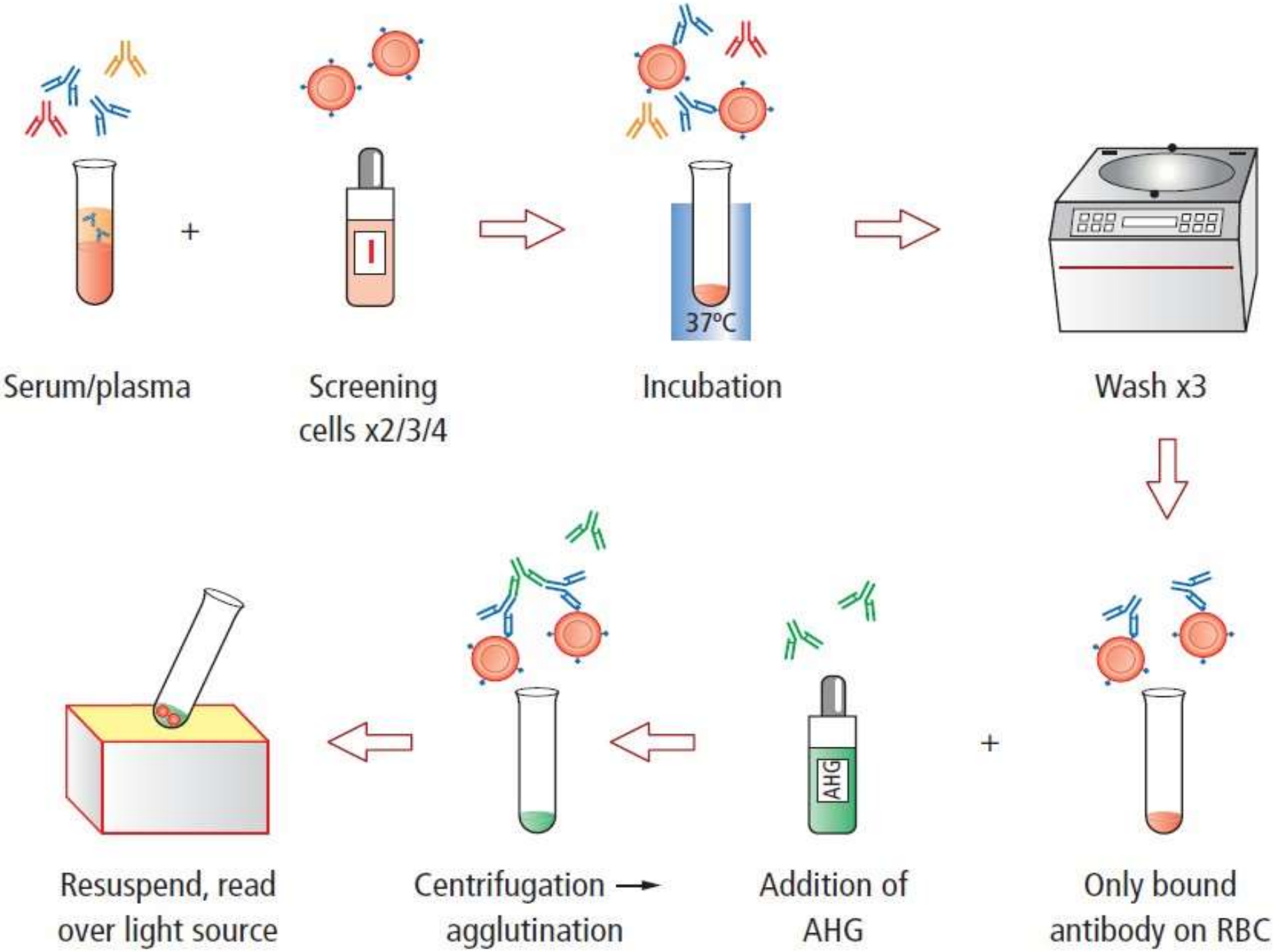


Serum with specific antibody mixed with reagent red cells

Washed x3 after incubation to remove unbound globulins



Anti-human globulin (AHG) added to promote agglutination on centrifugation



Serum/plasma

Screening cells x2/3/4

Incubation

Wash x3

Only bound antibody on RBC

Addition of AHG

Centrifugation → agglutination

Resuspend, read over light source

Performing the test

Requirements:

- Test tubes: (10x75 mm)
- Pasteur pipettes
- Incubator
- Centrifuge

Specimen: Serum (need not be fasting)

Reagents:

1. Antihuman serum
2. Anti-D serum

Additional Requirements:

- Coombs control cells
  - A. Make a pooled 'O' Rho (D) positive cells from at least three different 'O' positive blood samples.
  - B. Wash these cells three times in normal saline (these cells should be completely free from serum with no free antibodies).

Procedure

Procedure:

1. Label three test tubes as ‘T” (test serum) PC (Positive control) and NC (negative control).
2. In the tube labelled as ‘T’, add two drops of Anti-D serum
3. In the tube ‘PC’ add one drop of saline
4. Add one drop of 5 % saline suspension of the pooled O Rho (D) positive cells in each tube.
5. Incubate all the three tubes for one hour at 37°C

Procedure: (cont.)

Procedure: (cont.)

- Wash the cells three times in normal saline to remove excess serum with no free antibodies, (in the case of inadequate washings of the red cells, negative results may be obtained).
- Add two drops of Coombs serum (anti human serum) to each tube. Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.
- Resuspend the cells and examine macroscopically as well as microscopically

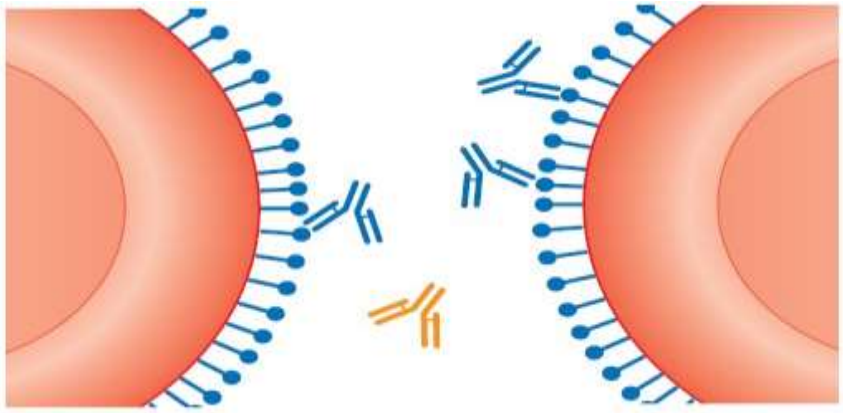
Test Interpretation:

		Observations	Conclusions
1	**Positive Control (PC)**	(A) Agglutination (B) No Agglutination	Correctly performed test procedure. Coombs serum may not be proper. Repeat the test again.
2	**Negative control (NC)**	It should show no agglutination, since saline does not contain Anti-D or any other antibodies.	
3	**Test (Serum) (T)**	(A) Agglutination (and if PC results are correct) No Agglutination	Patients serum contains Anti-D.

Direct Coombs test (direct antiglobulin test):

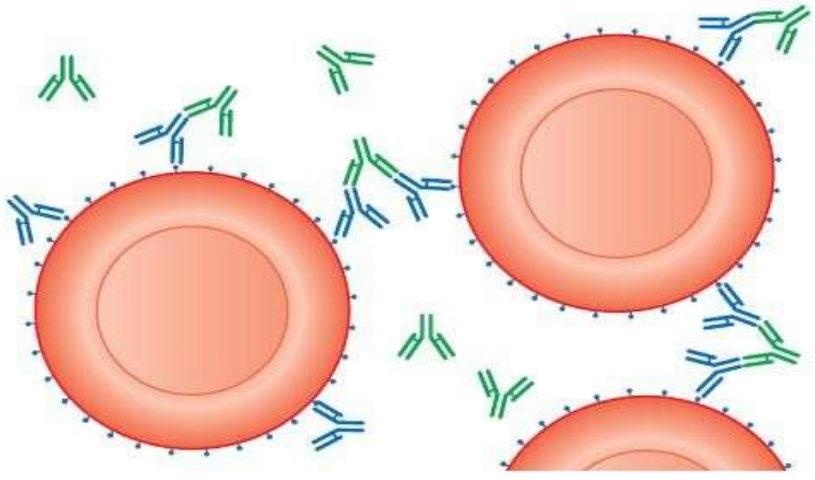
- This test is performed to detect anti-D antibody or other antibodies attached to the red cell surface within the blood stream.
- This occurs in the following circumstances:
  - **When there is a Rh-positive baby in the womb of a sensitized Rh-negative women; the antibodies produced in the mothers serum cross the placenta and after entering the baby's blood stream, these antibodies will attach to the baby's Rh-positive red blood cells. These coated (or sensitized) cells are clumped and removed from the circulation, causing hemolytic anemia (Hemolytic Disease of the Newborn: Erythroblastosis Fetalis). When the baby is born, the baby's blood is collected (or cord blood is collected from umbilical cord) and tested by the anti globulin Coombs test (direct) to detect anti D antibodies coated on red blood cells.**
  - **Transfusion reactions**
  - **Drug induced red cells sensitization**
  - **Autoimmune hemolytic anemia**

DIRECT ANTIGLOBULIN TEST (DAT)



Cells coated *in vivo*

Washed to remove unbound globulins



Addition of anti-human globulin (AHG) promotes agglutination after centrifugation

Direct antiglobulin test (DAT)

- The direct antiglobulin test (DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.
- In vivo coating of red cells with IgG and/or complement may occur in any immune mechanism is attacking the patient's own RBC's.
- **This mechanism could be autoimmunity, alloimmunity or a drug-induced immune-mediated mechanism.**

Requirements: (same as that for Indirect Coombs test)

Requirements: (same as that for Indirect Coombs test)

- Test tubes: (10x75 mm)
- Pasteur pipettes
- Incubator
- Centrifuge

Specimen: Blood drawn into EDTA is preferred but oxalated, or clotted, citrated whole blood may be used (specimen need not be fasting sample)

Procedure

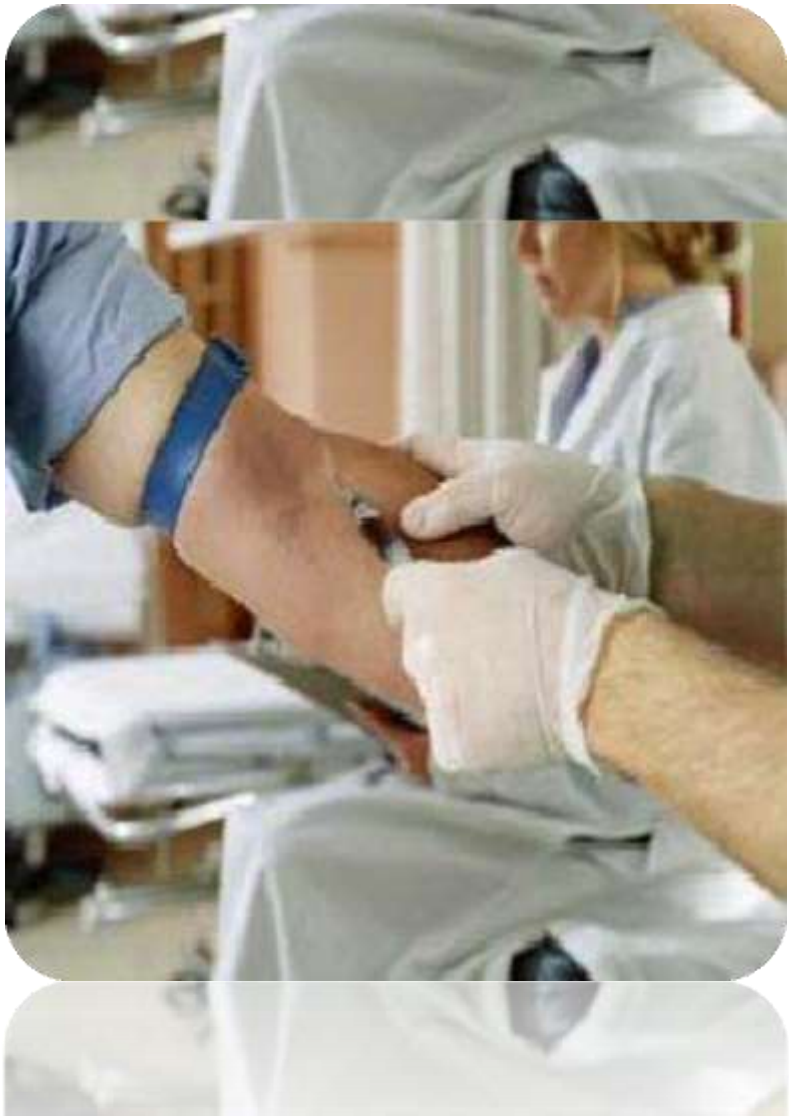
1. Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested.
2. With clean Pasture pipette add one drop of the prepared cell suspension to a small tube.
3. Wash three times with normal saline to remove all the traces of serum.
4. Decant completely after the last washing
5. Add two drops of Antihuman serum.
6. Mix well and centrifuge for one minute at 1500 RPM.
7. Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

False positive results: DAT and IAT

- In specimens containing potent cold-reactive antibodies agglutination may occur before adding the AHG reagent.
- Dirty glassware may cause clumping of cells.
- Over centrifugation
- **DAT (Direct Agglutination Test)**
- A positive DAT from a clotted sample should be repeated on an EDTA sample
- Samples collected from infusion lines may have complement present on the cells.
- **IAT Cells with a positive DAT will give a positive result in any indirect antiglobulin procedure.**

Coombs Test in Blood Banks

- The test is only rarely used to diagnose a medical condition, but is essential for use by laboratories such as blood banks. Blood banks use the indirect Coombs' test to determine whether there is likely to be an adverse reaction to blood to be transfused.



PRACTICAL-10

AIM:Preparation of platelet rich plasma and platelet poor plasma.

Platelet Rich Plasma &Platelet Poor Plasma:

Both platelet-poor plasma and platelet-rich plasma serve important functions in medical science. Both are used in the field of regenerative medicine. In areas as diverse as physical medicine, rheumatology, rehab and orthopedics, PRP and PPP therapy are used to speed up the human body's natural healing process.

Human blood contains many chemical substances, primarily platelets, red blood cells, white blood cells and plasma. Platelet-rich plasma, also called PRP, is the

part of the blood sample that includes mostly platelets and pure plasma, leaving out both the red and white blood cells.

Platelet-poor plasma (PPP), as the name indicates, is similar to PRP in one way: it contains plasma. However, PPP contains almost no platelets. When collected and prepared correctly, human blood yields both PRP and PPP.

Collecting Platelet-Rich Plasma:

- There are at least two common methods for collecting and preparing PRP.
- In the so-called "PRP method," as opposed to the buffy-coat method,
- A freshly-collected sample of whole blood from a human donor is placed in multiple tubes which are then loaded into a centrifuge.
- After the initial spin, the substance is divided into three distinct layers: the lowest layer contains red blood cells, the middle layer, also called the buffy coat, contains a high concentration of white blood cells, and the topmost layer contains a mixture of platelets and white blood cells.
- It is the topmost layer that interests researchers who are preparing PRP because that is the layer with the highest concentration of platelets.
- After collecting the topmost layers from the tubes and transferring the platelet-rich liquid to another set of test tubes, researchers perform a second spin.
- When complete, the bottom layer is collected to acquire the plasma with the highest concentration of platelets.
- The buffy-coat method of preparing PRP is very similar to the PRP method.
- In fact, there are many ways to collect PRP but researchers have not settled on one as being the most efficient. Currently, the so-called "PRP method" appears to be the most common.

Collecting Platelet-Poor Plasma:

- Collecting PPP, or platelet-poor plasma follows a process very similar to collecting PRP.
- An initial sample of whole blood is taken from a donor, centrifuged to separate into the three layers noted above, and the spun a second time.
- After the second spin, researchers can remove the topmost layer to prepare PPP.
- Note that after the second centrifuge spin, the sample is divided into PRP and PPP samples, the PPP being on top and the PRP being on the bottom.
- That's because the second centrifuge spin pulls the platelets to the bottom of the tube, leaving the platelet-poor plasma on top.

BLOOD TRANSFUSION REACTIONS

Types of Reactions

- Acute haemolytic transfustion reaction (AHTR)
- Anaphylactic transfusion reaction
- Febrile non-haemolytic transfusion reaction (FNHTR)
- Primary hypotensive reactions
- Transfusion-associated circulatory overload(TACO)
- Transfusion-associated sepsis
- Transfusion-related acute lung injury (TRALI)
- Urticarial transfusion reaction (UTR)

Frequency of reactions

- Urticaria- 1 to 3 percent
- FNHTR – 0.1 to 1 percent
- TACO - < 1 percent
- TRALI- < 0.01 percent
- Anaphylaxis – 1:20,000 to 1: 50,000
- AHTR- 1:76,000
- Sepsis – 1:50,000 for platelets and 1:5,000,000 RBCs
- Frequency too rare to calculate for primary hypotensive reactions associated with ACE inhibitors, non-immune hemolysis an Air embolism.

Definitions

- **AHTR:** life threatening reaction caused by acute intravascular hemolysis of transfused rbc
- Caused by clerical error
- Tranfusion of product not intended for the recipient

- Treatment:
- Aggressive hydration
- And diuresis

- **Anaphylactic transfusion reaction** : Any allergic reaction other than hives constitutes an anaphylactic transfusion reaction.
- Includes
- Angiodema, wheezing, and or hypotension.
- Occur in IgA deficient individuals
- management include; EPINEPHRINE, antihistamines and vasopressors depending on the degree of allergic symptoms

FNHTR

- Common
- Fever usually accompanied by chills in absence of systemic symptoms
- Diagnosis of exclusion
- Most common cause is due to release of cytokines from white blood cells in a product that has not been leukoreduced.
- Management: symptomatic .

Primary hypotensive reactions

- Rare
- Drop in BP
- Decrease by 30 mmHg systolic, diastolic or both
- Returns to normal once transfusion is stopped
- Most commonly reported with platelet transfusion
- Predisposing factors include ACE inhibitor use ,
- Rapidly reversible and generally do not require specific treatment

TACO

TACO is a form of pulmonary is a form of pulmonary edema due to volume excess or circulatory overload

Occurs in patients receiving large volume of transfused product over short period of time. Or in those with cardiovascular disease

Management:

Diuresis

Supplementary oxygen

Ventilatory support may rarely be required

Transfusion – associated sepsis

- Caused by transfusion of a product that contains a microorganism.
- Initial findings may include fever , chills and hypotension.
- Transfuson –associated sepsis may involve a large intravenous inoculum which in the case of gram negative organisms could include infusion of endotoxin.

- Treatment includes
- Broad-spectrum antibiotics and hemodynamic support.

Transfusion-related acute lung injury(TRALI)

- Life threatening form of acute lung injury that occurs when recipient neutrophils are activated by the transfused product in an appropriately primed pulmonary vasculature.
- Findings
- Fever , chills , respiratory distress
- Therapy is largely supportive and may include intubation and Mechanical ventilation

Urticarial transfusion reaction

- Associated wit hives but no other allergic findings (no wheezing, angioedema, hypotension)
- Most common cause is antigen-antibody interaction that occurs between patient and the product, commonly implicated antigens include a number of donor serum proteins

- UTR is not a contraindication for continuing blood transfusion
- Antihistamines can be given.

mortality

- Rare event
- 0.6 per million to 2.3 per million
- Greatest number of deaths were TRALI and AHTR
- Fatal in decreasing order include TRALI, TACO, AHTR,SEPSIS AND ANAPHYLAXIS.

When to suspect

- Most of the severe reactions occur within 15 minutes of transfusion
- Most common signs and symptoms are
- Fever 1 degree raise from baseline
- Chills
- Pruritus
- Urticaria
- More severe are respiratory distress, haemoglobinuria, loss of consciousness, hypertension, hypotension, flank or back pain, jaundice, abnormal bleeding or oliguria/anuria.

What to do

- Immediately **stop the transfusion**
- Save the remaining bag and tubing for potential analysis
- Maintain a **patent iv line** with normal saline
- **Confirm the correct product** was transfused to the intended patient based on product labelling and patient identification. Also assess the product for gross color changes or bubbles suggestive of bacterial contamination
- **Assess the patient** including symptoms of fever, respiratory distress, chest pain, back pain, itching, angioedema, measure vital signs, perform a limited examination guided by symptoms
- **Contact the transfusion service** to discuss the appropriated evaluation and initial management

What does Laboratory do with samples

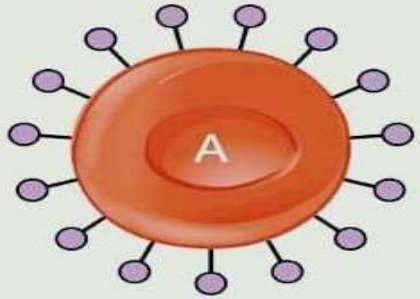
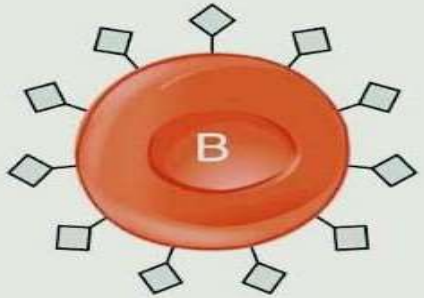
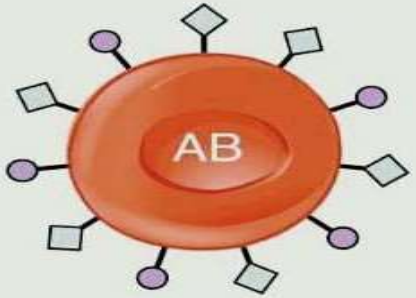
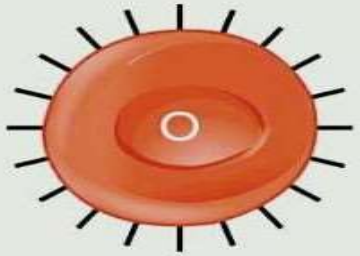
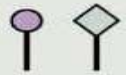
- Blood bank or laboratory will do a
- Clerical check

- Repeat ABO testing

- Visual check for hemolysis

- Direct antiglobulin (coombs) test

Blood Type

	A	B	AB	O
Red Blood Cell Type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red blood Cell	 A antigen	 B antigen	 A and B antigens	None
Blood Types Compatible in an Emergency	A, O	B, O	A, B, AB, O (AB^+ is the universal recipient)	O (O is the universal donor)