COLLEGE NAME & CODE : Periyar Arts College, Cuddalore-01 & 105
COURSE NAME & CODE : II B.Sc., Microbiology & U26
SEMESTER : III
SUBJECT TITLE & CODE : HEMATOLOGY AND BLOOD BANKING & BSMB 33

UNIT-V

ABO Grouping: History / Discovery
Slide / Tube technique
Rh Typing: Slide / Tube technique
Bovine replacement technique - Coombs test: Direct /Indirect
Donor screening: Cross matching: Major / Minor
Collection of blood - Preservation / Storage.

ABO GROUPING

- HISTORY & DISCOVERY

Blood group system

- A series of antigens exhibiting similar serological and physiological characteristics, and inherited according to a specific pattern.

History:

- The ABO blood types were first discovered by an Austrian Physician, Karl Landsteiner working at the Pathological-Anatomical Institute of the University of Vienna (now Medical University of Vienna).
- Adriano Srurli & Alfred von Decastello who were working under Landsteiner discovered type AB a year later in 1902.
- Jansky is credited with the first classification of blood into the 4 types (A, B, AB, O) in 1907, which remains in use today.
- Reuben Ottenberg successfully transfused blood between two people at Mount Sinai Hospital in New York. He was the first person to record pre-transfusion testing for blood compatibility in a clinical setting.

Discovery of the ABO system:

- In 1900, Austrian Physician, Karl Landsteiner reported a series of tests, which identified the ABO Blood Group System.
- In 1910 he won Nobel prize in Physiology or Medicine in 1930 for this discovery. He mixed the serum and cells of all the researchers in his lab and found four different patterns of agglutination.
- From those studies he developed what we now know as **Landsteiner's rules** for the ABO Blood Group
    - A person does not have antibody to his own antigens
    - Each person has antibody to the antigen he lacks (only in the ABO system)
- Below are the four blood groups and the antigens and the expected, naturally-occurring antibodies present.

BLOOD GROUP	ANTIGEN on RBCs	ANTIBODY in Plasma / Serum
A	A	anti-B
B	B	anti-A
AB	A and B	neither
O	neither anti-A or anti-B	anti-A,B

- The ABO blood group system is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes.
- In human blood transfusions it is the most important of the 38 different blood type (or group) classification systems currently recognized.
- A mismatch (very rare in modern medicine) in this, or any other serotype, can cause a potentially fatal adverse reaction after a transfusion, or an unwanted immune response to an organ transplant.
- The associated anti-A and anti-B antibodies are usually IgM antibodies, produced in the first years of life by sensitization to environmental substances such as food, bacteria, and viruses.
- ABO blood types are also present in other primates such as apes and Old World monkeys.

https://www.slideshare.net/JananiMathialagan1/blood-grouping-73716192

ABO Grouping
-Slide & Tube Technique

❖ **Importance of 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 & antibodies

BLOOD GROUP	ANTIGEN on RBCs	ANTIBODY in Plasma / Serum
A	A	anti-B
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AB	A and B	neither
O	neither anti-A or anti-B	anti-A, anti-B

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❖ **UNIVERSAL DONOR & RECEIPIENT**

- ***Universal Donor***

  Group O - neither A or B antigens

- ***Universal Receipient***

  Group AB - patient has no anti-A or anti-B present

  - cannot lyse any transfused cell

❖ **ABO TYPING TECHNIQUES**

1. Slide test or technique
2. Tube technique
3. Microplate
4. Gel system

1. SLIDE METHOD

- Test should be done at room temperature or lower
- Tubes, slides should be dry and labelled properly

Advantages

– Preliminary typing tests

– Use during camps

Disadvantages

– Not routine test

– Less sensitive

– Drying of reaction giving to false positive results

1. TUBE METHOD

Recommended method

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

A. <u>CELL GROUPING</u> (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 2:1)

B. <u>SERUM GROUPING</u> (Reverse Grouping)

- Prepare 2-5% suspension of pooled cells A, B, O
- Label three tubes A cells, B celss, and O cells
- Place two drops pd 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

http://nbtc.naco.gov.in/assets/resources/training/8.pdf

Rh BLOOD GROUP SYSTEM

Rh (D) Antigen

Of next importance is the Rh type.

- Rh is a blood group system with many antigens, one of which is D.
- Rh refers to the presence or absence of the **D antigen** on the red blood cell.
- Unlike the ABO system, individuals who lack the D antigen do not naturally produce anti-D.
- Production of antibody to D requires exposure to the antigen.
- The D antigen is very immunogenic, i.e., individuals exposed to it will very likely make an antibody to it.

Frequency in Indian population

- 92-95% Rh positive
- The most important patient population to consider is females of child-bearing age.
- If immunized to Rh (D) antigen the antibody can cross the placenta and destroy Rh (D) positive fetal cells resulting in death.
- This is why Rh negative women are given injection anti-D after birth of Rh positive baby.

Rh Antibodies

- All Rh antibodies are immune in nature, developed after immunizing event
- React at 37ºC and require antiglobulin test to demonstrate the reaction
- Generally do not react at room temperature in saline
- Most are IgG in nature and therefore can cross the placenta
- Generally, do not fix complement and cause extra vascular hemolysis
- All are important in HDN and delayed HTR

Rh typing

- Normal typing for Rh antigens only includes typing for Rh (D).
- The result of this typing determines the Rh status of the cells (Rh-positive or Rh-negative).
- Some Rh typing sera is diluted in high protein solutions and may require a negative control.
- It is recommended to use two monoclonal anti-D sera from two different manufacturers labelled as D1 and D2, especially to confirm all Rh negatives

Monoclonal Anti-D

- Three types

1. IgM anti-D monoclonal reagent
2. Blend of IgM and IgG monoclonal antibodies reagent
3. Monoclonal IgG anti-D

TUBE TECHNIQUE for Rh Typing

- Prepare 5% washed red cell suspension of test sample.
- Take three clean test tubes and label tubes 1 & 2 as "test" and tube 3 as "control".
- Place 1 drop of anti-D (D1) in tube 1 and 1 drop of anti-D (D2) in tube 2.
- Place 1 drop of 22% **bovine albumin** / control in tube 3.
- Add 1 drop of 5% test cell suspension to each tube.
- Mix well, centrifuge at 1000 rpm for 1 min.

Results:

- If there is agglutination D^u Positive.
- If there is no agglutination D^u Negative.

COOMB'S TEST

-Direct / Indirect Test

Direct and Indirect Coomb's Test

- Coomb's test is a direct **agglutination** reaction, more commonly known as **antiglobulin test**.
- It was discovered by Coombs, Mourant and Race in 1945 originally for the detection of incomplete anti-Rh antibodies.
- In the test, incomplete **antibodies** do not agglutinate erythrocytes. Incomplete antibody antiglobulin coats the surface of erythrocytes but does not cause any agglutination.
- When such erythrocytes are treated with antiglobulin or Coombs' serum then the cells are agglutinated.

Objectives of Coomb's Test

- To detect red blood cells sensitized with IgG alloantibodies, IgG autoantibodies or complement components.

Types of Coomb's Test

There are two types of Coombs tests: the direct Coomb's test and the Indirect Coomb's test.

Direct Coomb's Test (Direct Antiglobulin Test)

- The direct test is more common and checks for antibodies that are attached to the surface of red blood cells.
- In this test, the sensitization of red blood cells (RBCs) with incomplete antibodies takes place in vivo.
- The cell-bound antibodies can be detected by this test in which antiserum against human immunoglobulin is used to agglutinate patient's red cells.

Indirect Coomb's Test (Indirect Antiglobulin Test)

- The indirect test checks for unattached antibodies that are floating in the bloodstream.
- In this test, the sensitization of RBCs with incomplete antibodies takes place in vitro.
- The patient's serum is mixed with normal red cells and antiserum to human immunoglobulin is added. Agglutination occurs if antibodies are present in the patient's serum.

Procedure of Coomb's Test

– The use of antihuman globulin serum to detect sensitization of red cells in vitro is a two stage technique constitute indirect antiglobulin test (IAT).

– On the other hand, sensitization of red cells in vivo is detected by one stage technique – the direct antiglobulin test (DAT).

Direct Coomb's Test

i. Red cells suspected of being sensitized is washed 3 to 4 times in large volume of saline.
ii. Two drops of anti-human globulin serum is added to the sedimented cells.
iii. It is mixed well and centrifuged at 1500 rpm for one minute.
iv. Agglutination is examined by holding against a lighted background and tapping the bottom of the tube.

Indirect Coomb's Test

i. 4% saline suspension of the test cells is prepared.
ii. Two drops of cell suspension is added to a small test tube.
iii. Two drops of antiserum is added to the cell suspension.
iv. It is incubated in a water bath at 37°C for 30 min.
v. The tube from the water bath is removed and washed 3 to 4 times with large volume of saline. It is completely decanted after last washing.

Result Interpretation of Coomb's Test

- **Positive:** A clumping of the red blood cells (agglutination) during the test.
- Agglutination of blood cells during a direct Coomb's test suggests that antibodies may be present on red blood cells of the patient and that the condition of hemolysis may persist.

Applications of Coomb's Test

a. Coomb's test is one of the blood tests employed to help find out the kind of anemia an anemic patient is suffering from.
b. Indirect test is administered to determine if there was a potential bad reaction to a blood transfusion.
c. Blood banks use the indirect Coombs test to determine whether there is likely to be an adverse reaction to blood to be transfused.

Limitations of Coomb's Test

- Sometimes, especially in older adults, a Coomb's test will have an abnormal result even without any other disease or risk factors.
- The test can only be rarely used to diagnose a medical condition.

https://microbenotes.com/coombs-test-direct-and-indirect-coombs-test/

CROSS-MATCHING

- A pre-requisite for blood transfusion
- Purpose: to avoid reactions of mismatched transfusion

Procedure

- In test tube place 2 drops of recipient's serum
- Add washed donor red cell suspension
- Mix and incubate at 37 ºC for 30 min
- Centrifuge at 300 rpm for 1 minute
- Examine for agglutination and hemolysis

Interpretation

- Matched – no agglutination and hemolysis
- Mismatched – either agglutination or hemolysis

PRESERVATION & STORAGE OF BLOOD

COLLECTION OF BLOOD

Introduction

- The collection of blood from donors may take place within the blood transfusion centre or hospital blood bank.
- It is also often collected from donors during mobile blood collection sessions.
- The blood is then taken to a laboratory for testing and processing into components and for storage and distribution as the need arises.
- Blood is collected at body temperature, i.e. +37°C. But in order to maintain its vital properties, it must be cooled to below +10°C to be transported, and stored at refrigeration temperatures of around +4°C until use.

- Anticoagulants and preservatives initially prevent clotting and thereafter maintain cell viability and function during storage.
- Storage conditions relate largely to the maintenance of temperature from the time of collection, through processing, testing and labelling and transportation, up to the point of issue for transfusion into a patient. This is known as **'cold chain management'.**

Harmful effects of Improper Storage

- If blood is stored or transported outside of these temperatures for long, it loses its ability to transport oxygen or carbon-dioxide to and from tissues respectively upon transfusion.
- Other factors of serious concern are the risk of bacterial contamination if blood is exposed to warm temperatures.

STORING BLOOD

- Blood is collected from the donor
- A small sample is collected for testing
- The donation is stored in a plastic bag
- Blood needs to be stored in the correct conditions

Safe storage of blood

Why is blood stored at 4 ºC?

- Blood must be stored at temperatures low enough to prevent enzyme activity
- Blood proteins (e.g., Hemoglobin) must not be allowed to denature
- If we freeze blood ice crystals would form inside the RBC, these would damage the cell membranes so the cells would be destroyed when the blood thawed out.

A.Whole blood:

- Whole blood and red cells must always be stored at a temperature between +2°C and +6°C.
- If blood is not stored at between +2°C and +6°C, its oxygen-carrying ability is greatly reduced.
- The anticoagulant/ preservative solution in the blood bag contains nutrients for the blood during storage and stops the blood from clotting.
- The red cells can carry and deliver oxygen only if they remain viable.

B. Fresh frozen plasma

- Fresh frozen plasma (FFP) is plasma which is separated from a unit of whole blood within 6 hours of collection, and has been rapidly frozen and maintained at all times at a temperature of minus –30°C or lower.
- FFP, once thawed has a shelf life of 24 hours at 10ºC to 60ºC.
- Plasma contains water, electrolytes, clotting factors and other proteins (mostly albumin), most of which are stable at refrigerator temperature, i.e. +2°Cto +6°C.

C. Platelet concentrates

- Platelet-rich plasma (PRP) / Platelet concentrate (PC) must be separated from whole blood by centrifugation within 6 hours of collection.
- Whole blood should be kept at between +20°C and +24°C until it is processed into platelet concentrates and other blood components.
- Platelet concentrates should be stored at a temperature of between +20°C and +24°C i.e., 22±2°C with continuous gentle agitation. This is essential to prevent platelet aggregation which results in loss of viability.

D. Plasma derivatives

- Unlike blood components, plasma derivatives such as albumin or immunoglobulin are concentrated, sterile specific proteins, obtained from large pools of donor plasma through a complex pharmaceutical process called plasma fractionation
- It is essential to store all plasma derivatives according to the manufacturer’s instructions