

ABO Grouping Techniques

- ✓ ABO cell grouping is the detection of the presence or absence of A and B of the red cell surface using known anti-A and anti-B.
- ✓ The surface has several antigenic sites for the blood groups of an individuals which can only be detected by treated the cell to known anti sera.
- ✓ The antigen found on the Rbc is therefore the blood group of the individual.
- ✓ The absence of antigen A and B from the surface of red cell. Blood group O individuals has been used to denote the absence hence blood group O.

Principles of the test :- When red cells are mixed with known anti -A and anti -B and left to react, a visible reaction (agglutination) will be observed only in the mixture where a corresponding antigen is present of the red cell surface.

Purpose of the test: - To determine the blood group of the donor of the cell.

- ✓ There are two major ABO grouping techniques.
 - (i) **Forward grouping** - where known antibodies are used to detect antigen on red blood cells.
 - (ii) **Reverse grouping** - where known cells are used o detect the antibodies in the serum.

Forward grouping technique

- ✓ Also known as direct or front grouping.
- ✓ It involves detection of presence or absence of antigen A & B on red cells surface using known antisera A&B
- ✓ This technique can also be divided into two depending on the apparatus used: -
 - **Slide grouping method** where mixture of cells and antisera is done and observed on a slide or tile.
 - **Tube grouping method** where mixture of cells and antisera is done and observed in a test tube.

Slide grouping method

Reagents & apparatus

- Anti A & anti B grouping sera
- Microscope slides & grouping tile.
- Applicator stick
- Gauze & marking pencil
- Blood specimen

Procedure

- Divide a slide into two halves and labeled 'A' & 'B'
- Put a drop of blood on each half
- Add a drop of anti-A to side 'A' and anti -B to side 'B' and mix well using applicator stick.
- Rock side to side as you look for presence or absence of agglutination in the mixture

- Tabulate the results

	Anti A	Anti B	Blood group
Sample I	+	-	A
Sample II	-	+	B
Sample III	+	+	AB
Sample IV	-	-	O

Interpretation

- Agglutination in 'A' only – presence of antigen A on red cell surface therefore, blood group A
- Agglutination in 'B' only – presence of antigen B on red cell surface therefore, blood group B
- Agglutination in 'A' & 'B' – presence of antigen A & B on red cell surface therefore, blood group AB
- No Agglutination in 'A' & 'B' – Absence of antigen A & B on red cell surface therefore, blood group O

Tube grouping method.

Reagents & apparatus

- Anti A & anti B grouping sera
- Test tube
- Gauze & marking pencil
- Blood specimen
- Centrifuge
- Pasteur pipette
- Normal saline

Procedure

- Label two test tubes 'A' and 'B'
- Make 4% cell suspension of the specimen in saline
- Put two drops in each tube, add 2 drops of anti A to 'A' & anti B to 'B' tubes.
- Shake to mix, centrifuge at 2000rpm for 1 min. and observe for strong macroscopic agglutination

Interpretation

- Agglutination in 'A' only – presence of antigen A on red cell surface therefore, blood group A
- Agglutination in 'B' only – presence of antigen B on red cell surface therefore, blood group B
- Agglutination in 'A' & 'B' – presence of antigen A & B on red cell surface therefore, blood group AB
- No Agglutination in 'A' & 'B' – Absence of antigen A & B on red cell surface therefore, blood group O

NB

- ✓ Where rhesus typing is also required, include a third reagent:- anti -D and a third slide or a tube marked 'D'

Reverse grouping technique

- ✓ Also known as ABO serum grouping, backward grouping or back typing technique.
- ✓ It involves detection of the presence or absence of antibody A & B in the serum using known antigens A & B.

Principles of the test :- When serum is mixed with known group A and group B cells and left to react, a visible reaction (agglutination) will be observed only in the mixture where a corresponding antibody is present in the serum.

Purpose of the test: - To detect the ABO antibodies present in serum

Reagents & apparatus

- Known A and B cells (4%)
- Test tubes
- Centrifuge
- Pasteur pipettes
- Serum specimen
- Marking pencil

Procedure

- Label two test tubes 'A' and 'B'
- Add 2 drops of A cells to 'A' and B cells to 'B'.
- Add 2 drops of serum in each tube & mix A & B
- Centrifuge at 2000rpm for 1 min & observe for strong macroscopic reaction.
- Tabulate the results.

	Anti A	Anti B	Antibody in serum	Blood group
Sample I	+	-	Anti A	B
Sample II	-	+	Anti B	A
Sample III	-	-	None	AB
Sample IV	+	+	Anti A & B	O

Interpretation

- Agglutination in tube 'A' only - presence of antibody A in serum therefore, blood group B
- Agglutination in tube 'B' only - presence of antibody B in serum therefore, blood group A
- Agglutination in tubes 'A' & 'B' - presence of antibodies A & B in serum therefore, blood group O

- No Agglutination in 'A' & 'B' – Absence of antibodies A & B in serum therefore, blood group AB

NB

- ✓ Though not commonly employed, slide method can also be performed from reverse grouping.
- ✓ It is imperative to note that Rhesus grouping is impracticable by reverse technique.
- ✓ Infants of less than 3 months who have not developed antibodies will not be grouped by reverse technique.

Sources of error in ABO grouping

False positive

- Dirty glassware or cloudy saline.
- Over centrifugation making cell bottom different to respond a smooth mixture.
- Presence of atypical antibody.
- Bacteria contamination of blood specimen.
- Rouleaux formation (forms like a stack of coins)
- Cold agglutination of other blood groups.

False negative

- Impotent/ expired reagent
- Misinterpretation of results
- Under centrifugation
- Use of non-viable reagents e.g. low titer
- Failure to add reagents to tubes
- Failure to follow manufacturer's instructions.
- Too heavy cell suspension in the tube (4.5% cell suspension) can bring about prozone effect.
- Less cell suspension leading to prozone.

Factors affecting Antigen -Antibody reactions

- 1) Temperature :** - Some antibodies react best at warm temperature e.g. Immune antibodies while others react at cold temperature e.g. natural antibodies (IgM)
- 2) Time of incubation:** - Some reactions require longer incubation time while others shorter (IgG). The numbers Of antibodies attached to e.g. after 15min is considered & gives strong reaction.
- 3) Concentration of reactants :-** Correct concentration of antigen and antibody without biased excesses influences The end results (5% cell suspension)
- 4) PH :-** Antigen and antibody reaction is favored best at PH 6.5 – 7.5
- 5) Avidity:** - This is the speed at which antibody agglutinates red cells. An antibody with high avidity takes shorter time to react while that with low avidity takes longer.

- 6) **Medium of suspension:** - Some reactions are best in saline medium(IgM) while some require enzyme or albumin medium i.e. IgG
- 7) **Potency of antigen :-** Some antigens reacts weakly due to age of storage.
- 8) **High protein medium:-** e.g. albumin technique, Antiglobulin test (AHG),enzyme test used in suspension medium

ABO Anomalies

(1) **Pan Agglutination (Reuben - Friederich)**

- ✓ This is spontaneous clumping of all red blood cells by human sera. This is due to:-
 - (a) Bacterial or viral action on red cells has the power to remove T-receptor exposing T-antigenic site.
Since all human sera have anti -T, it will attach to T- antigenic site causing agglutination. Example
Of such bacteria are clostridium welchi, Vibrio cholera & staphylococcus.
 - (b) Bacteria action on serum, in vivo has the ability to produce H antibodies. Since all human red cells have the H antigen, It will react with the antibody in such serum causing agglutination. Such bacteria include Corynebacterium species.

NB

The only solution to Pan Agglutination is to have cells washed in warm saline at 37°C and grouping done at 37°C.

(2) **Auto - Agglutination**

- ✓ This is agglutination of patients cells by his own antibodies which may be warm or cold

<u>Warm/ immune antibodies (IgG)</u>	<u>Cold/ natural antibodies (IgM)</u>
They react at 37oC	They react at room temperature
Produced by antigenic stimulus.	Occurs naturally.
They fix complement and are hemolytic.	Molecular weight is 900,000 - 1000,000 dalton e.g. anti H
Molecular weight s 150,000 dalton e.g. anti A	Occurs in condition e.g. PNH, viral pneumonia, congenital syphilis etc.

NB : -The solution here is to wash the cells at 37°C

(3) **Poly agglutination**

- ✓ This is agglutination of red cells by a portion of normal human sera.
- ✓ Poly agglutinable cells are agglutinated by many sera.

(4) **Rouleaux formation**

- ✓ This is pseudo-agglutination where there is clumping of red cells by other means other than antigen - antibody reaction. It shows a characteristic appearance of a stack of coins piling together. Caused by: -
 - Infusion of high molecular weight drugs
 - Infusion of dextra 40

- Raised fibrinogen concentration(hyperfibrinogenaemia)
- Altered albumin -albumin ratio in serum especially in presence of abnormal protein such as macroglobulin in multiple myeloma, kalazar and trypanosomiasis.

Solution

- Addition of drop of saline to a cell -serum mixture on glass slide or washing cell in saline disperse rouleaux
- Gently rocking of cell - serum mixture on the slide will disperse rouleaux reaction but not agglutination.
- Centrifuging cell -serum mixture, removing serum and replacing with normal saline disperses rouleaux.