Notes

7

ABO BLOOD GROUPING

7.1 INTRODUCTION

Several blood group systems have been described in humans. Of these, the ABO blood group system is most significant.

OBJECTIVE

After reading this lesson, you will be able to:

- explain commonly used terms in ABO grouping
- describe antigen antibody reactions
- describe the basis of ABO grouping.
- explain the techniques of ABO grouping.

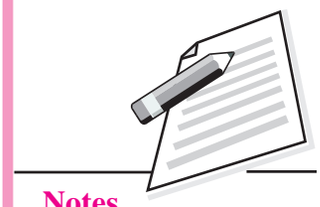
7.2 SOME COMMON ASPECTS OF IMMUNO-HEMATOLOGY

We will learn about some commonly used terms before beginning ABO grouping. This will help in better understanding of the subject.

Antigen: An antigen is a substance usually a protein which when introduced into an individual who recognizes it as foreign, leads to the production of antibody. This antibody specifically reacts with the antigen.

On the **red cell surface** there is presence of glycoproteins and glycolipids which act **as antigens**. They are called **blood group antigens**. These antigens can be on the surface, below or protrude from the red cell membrane. If introduced into the body of an individual who lacks the antigen, an immune reaction can occur.

Antibodies: These are immunoglobulins present in the serum and can be of 5 types: IgG, IgM, IgD, IgA and IgE.



If red cells carrying an antigen are introduced into the circulation of an individual who lacks that antigen, antibodies will form and cause destruction of the introduced red cells. These are **immune** or acquired antibodies and are **IgG** in nature. They react best at 37°C.

Certain antibodies occur without antigenic stimulus and are called **naturally occurring antibodies** e.g. **ABO antibodies**. They are IgM in nature and react at room temperature.

INTEXT QUESTIONS 7.1

1. Substance leading to production of antibody is .....
2. ..... & ..... acts as antigens.
3. Immunoglobulin of sera are .....
4. Antibodies are ..... present in sera.
5. Antibodies without antigenic stimulus is called as .....

7.3 ANTIGEN ANTIBODY REACTIONS

The antigen antibody reactions relevant to blood banking are:

- sensitization
- agglutination
- hemolysis
- neutralization

Sensitization is the combination of antigen and antibody. This is a reversible reaction.

Agglutination is the clumping of red cells. It occurs when sensitized cells come into contact with each other resulting in formation of bridges between them and formation of aggregates. It is the most common procedure in blood banking.

Hemolysis as the name suggests is destruction of red cells resulting in the release of hemoglobin from the cells due to the action of complement. This is used in antibody screening tests.

Neutralization: Blood group antigens when added to serum containing antibody can neutralize it. This is used in determining secretor status. If the strength of the antibody reduces, the antigen antibody reaction is assumed to have occurred.

Genotype: This refers to the genes present on the chromosome inherited from each parent irrespective of whether they produce any product which is detectable.

Phenotype: This is used to describe the observable effect of the inherited genes or expression of the genes i.e. the blood group.

INTEXT QUESTIONS 7.2

Match the following

1. Sensitization	(a) Observable effect of the inherited genes
2. Agglutination	(b) Destruction of red cells
3. Hemolysis	(c) Genes on chromosome
4. Genotype	(d) Combination of antigen & antibody
5. Phenotype	(e) Clumping of red cells

7.4 ABO BLOOD GROUP SYSTEM

The ABO grouping system is subdivided into 4 types based on the presence or absence of **antigens A and B** on the red cell surface as shown below. Red cells that only have antigen A are called group A. Those that only have **B antigen** are called **group B**. Cells that have **both A and B antigens** are group **AB**. Cells that **lack both antigens are O.**

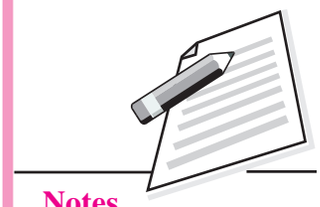
7.5 ANTIBODIES

The ABO antibodies ; anti-A and anti-B are **naturally occurring** antibodies and are present in the sera of individuals who lack the corresponding antigen. Cells with **A antigen** will have **anti-B in the serum**. Cells with **B antigen** will have **anti-A in the serum** and cells with **AB antigens** will **not have any antibody**. Group O individuals will have both anti-A and anti-B antibodies. These antibodies are IgM in nature.

The antigens and the corresponding antibodies in each blood group are shown below.

Table 7.1

Group	Antigen	Antibody
A	A	Anti-B
B	B	Anti-A
AB	A and B	None
O	None	Anti-A, Anti-B



Genetics: All features in humans are controlled by **genes** present on **chromosomes**. Each cell has 23 pairs of chromosomes. There is one locus on chromosome 9 occupied by one of the three alleles A, B, O. The genes of the ABO system are inherited as mendelian codominant. Each individual inherits one gene from each parent. The chromosome from the mother carries one of A,B or O gene. Similarly the chromosome from the father also has one of A, B or O gene. The gene on each chromosome determines the blood group as shown below. The A and B genes are dominant over the O gene.

Table 7.2

	Father	Mother	
	OO	AA	Genotype
Children	AO AO	AO AO	Genotype
Blood group of children	A A	A A	Phenotype

Mother group A, father group O and all children are group A.

INTEXT QUESTIONS 7.3

Match the following

Blood Group	Antigen
1. Group A	(a) Has both A & B antigen
2. Group B	(b) Antigen A
3. Group C	(c) Lack of A & B antigen
4. Group D	(d) Antigen B

Technique of ABO grouping: Various techniques are available for ABO grouping in the laboratory. These are

1. Slide technique
2. Tube technique
3. Microplate technique
4. Gel card technique

Slide technique

This can be performed in emergency or outdoor camps but **must not be performed as a routine test.**

Notes

Material required

1. Glass slides/white tile
2. Monoclonal Antisera A and Antisera B
3. Glass rod for mixing
4. Marker pen

Sample: Blood collected in a plain vial. Sample must be tested within 48hours. It should be kept in the refrigerator till processed. There should be no evidence of hemolysis in the sample.

Method

1. Mark one side of the glass slide as A and the other side as B.
2. Put one drop of antisera A on the side marked as A and one drop of antisera B on the side marked as B.
3. Add one drop of test blood sample/20% cell suspension to each antisera.
4. Mix the blood with the reagent using a clean stick. Spread the mixture over an area of 15mm diameter.
5. Gently rock the slide to and fro and look for agglutination.
6. Record the result.

Interpretation

Agglutination if present indicates a positive result

Advantages

1. Can be used in emergency and blood camps for preliminary grouping.
2. Easy to perform
3. Quick

Disadvantages

1. Not reliable for weak reactions as negative results cannot be checked microscopically.
2. Serum testing cannot be performed.
3. The test mixture tends to dry fast.
4. Drying causes aggregation of cells which can be interpreted as agglutination.
5. Less sensitive than tube technique.

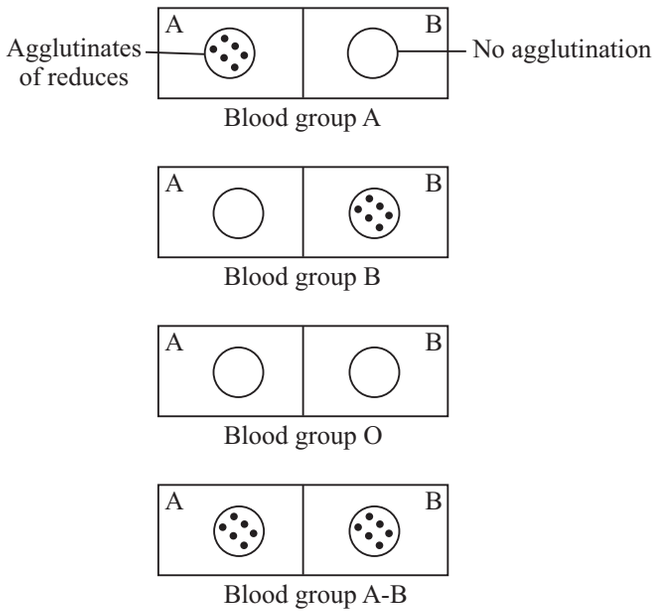


Fig. 7.1

Tube technique

This is the recommended method for grouping. It involves

- cell grouping or forward grouping: testing test red cells with known antisera.
- reverse or serum grouping: testing serum of donor/patient with known cells.

The procedure for cell and serum grouping is described separately but in all samples both the procedures should be done simultaneously and the results crosschecked.

Reagents required

1. Monoclonal Antisera-A and Antisera-B. Antisera-A, B is optional.
2. Normal saline
3. Known cells of group A, B, O

Equipment required

1. Centrifuge
2. Glass tubes 12 × 100 mm
3. Glass tubes 75 × 10 mm

Notes

Cell or forward grouping

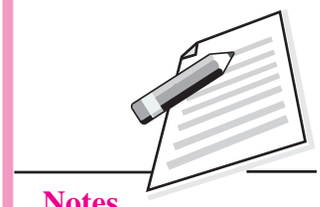
In this the donor/patient red cell are tested with known antisera.

Method

1. Check that the name and number of the donor/patient on the vial matches with the form. Write the same donor number on each tube in which grouping will be performed.
2. Centrifuge the sample to separate the cells and serum.
3. Prepare a 2-5% cell suspension of test red cells in normal saline as follows
   - Add the cells to a pre labeled tube (75 × 10mm) filled three fourth with normal saline.
   - Mix and centrifuge at 1000- 2000 rpm for 1-2 minutes. Decant the supernatant completely.
   - Add saline and repeat the procedure till the supernatant is absolutely clear.
   - After three washes, decant the supernatant and to the cell button add saline by counting the drops to make a 2-5% cell suspension.(10ml of normal saline and 0.2 ml/0.5 ml for 2 and 5% respectively).
   - Invert gently several times to make an even suspension.
4. Label three tubes as Anti-A, Anti-B and Anti-A,B.
5. Add one drop of Anti-A to tube marked A, one drop of Anti-B to tube marked B and one drop of Anti-A,B to tube marked A,B.
6. Add one drop of 2-5% red cell suspension of donor/patient to each tube and mix gently. Leave at room temperature for15- 30min or centrifuge at 1000rpm for 1 minute after 5-10 min incubation at room temperature.
7. Resuspend the cell button and check for agglutination. Also look for any evidence of hemolysis in the supernatant which is read as a positive result.
8. If no agglutination is seen, the contents of the tube must be examined microscopically.
9. Record the results.
10. An autocontrol (patient's serum and cells) can be set up in grouping. No agglutination should be seen in this tube.

Serum grouping

In this the serum of the donor/patient is tested with known cells. The Acells, Bcells and O cells are obtained by pooling fresh group A, B and O cells from



at least 3 individuals of these known groups and a 5% cell suspension (1ml of normal saline and 50µl of washed red cells)is prepared in a similar manner as the cell suspension in cell grouping by washing in saline. The cell suspensions must be prepared fresh everyday and may be tested using the corresponding antisera before use. The unit number from which the pooled red cells are prepared must be entered in the blood grouping register.

Method

1. Label three tubes as A cell, B cell and O cell.
2. Place two drops of the donor/patient serum in each tube.
3. Add one drop of A cells to tube marked A, one drop of B cells to tube marked B and one drop of O cells to tube marked as O.
4. Mix the contents by gentle shaking and leave undisturbed at room temperature for 30-60min or centrifuge at 1000rpm for 1minute.
5. Look for agglutination. Also look for any evidence of hemolysis in the supernatant which is read as a positive result.
6. If no agglutination is seen, the contents of the tube must be examined microscopically.
7. Record the results immediately.

Interpretation

Presence of agglutination or hemolysis is a positive result.

A smooth cell suspension after the button is resuspended is a negative result.

Grading agglutination reactions

The reaction result obtained in grouping is graded as follows:

H Hemolysis. This is a positive result

0 No agglutination, only a smooth suspension

1+ Many small clumps, supernatant has free cells

2+ Many small clumps with clear supernatant

3+ 2-3 clumps, no free cells

4+ One big clump, no free cells

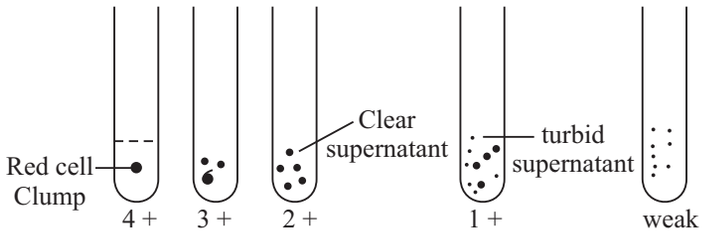


Fig. 7.2: Grading agglutination reactions for blood grouping by tube technique

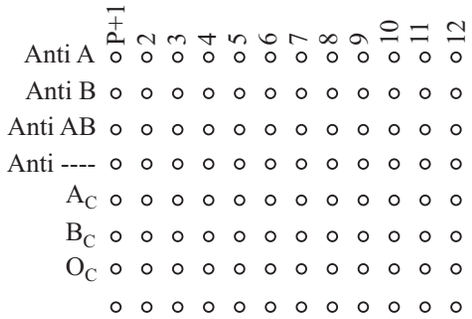


Fig. 7.3: Blood grouping by microplate technique

Advantages

1. Easy to perform
2. Accurate
3. The cell mixture can be incubated for a long time without drying.
4. The centrifugation used enhances the reaction and hence even weak antigens/antibodies can be detected.
5. Uses smaller quantity of reagents.

Interpretation of result

The results seen in different blood groups are shown below

Table 7.3

	Cell grouping			Serum grouping		
Group	**Anti-A**	**B**	**AB**	**Ac**	**Bc**	**Oc**
A	4+	-	4+	-	2+	-
B	-	4+	4+	2+	-	-
O	-	-	-	2+	2+	-
AB	4+	4+	4+	-	-	-

Recording results of ABO grouping

Each blood transfusion centre must have a record sheet in which blood grouping results are recorded.

All reactions must be graded.

Results must be recorded immediately after the test is performed.

Tallying results of forward and reverse grouping

The results of forward and reverse grouping must tally with each other. If a disparity is noted between the cell and serum grouping, inform the Blood Bank incharge and follow the necessary instructions on how to resolve it. Do not release the unit for transfusion till the discrepancy is resolved.

Microplate technique

The microplate consists of a plastic tray with 96 wells as shown in the fig below.

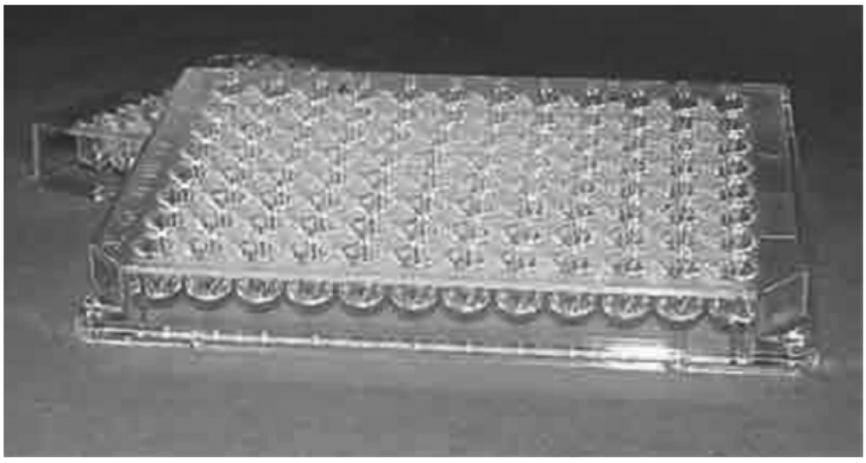


Fig. 7.4: A microplate with wells

Materials required

1. Microplate: U bottom
2. Plate centrifuge
3. Plate shaker

Reagents

1. Antisera A,B,AB. A working dilution of these antisera is prepared in saline.

Notes

Method

1. Arrange the blood samples in serial order.
2. Seperate the cells and serum. Make a 2-3% cell suspension of patient/donor cells as described in tube technique.
3. Each microplate has 8 wells vertically. In the first three wells, put one drop of diluted Anti-A, Anti-B and Anti-A,B respectively. In the next three wells put one drop of Ac, Bc and Oc. The remaining two wells can be used for Rh typing.
4. Add one drop of 2%cell suspension in the first three wells.
5. Put one drop of patient/donor serum in the next three wells.
6. Gently tap the plate and incubate at room temperature for 1hour. If a microplate centrifuge is available, centrifuge at 200g for 1min after 15min incubation.
7. Resuspend the red cells using a microplate shaker/manually briefly.
8. Record results.

Interpretation

Positive result : carpet of red cells which line the bottom of the well.

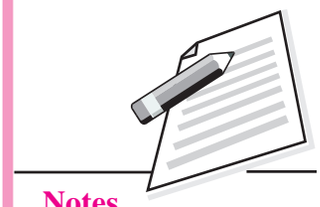
Negative result : compact button with smooth edges which streams when the plate is tilted.

Advantages

1. Uses small volume and low concentration of sera and red cells making it cost effective.
2. Easily handled microplate in place of 96 tubes
3. Large number of samples can be processed at the same time. There is economy of space and time.
4. The technique can be automated which helps in reduction in reading and transcription errors.

Gel card method for blood grouping

Microtubes containing sephadex gel prepared in a buffer such as LISS (low ionic strength saline) or saline are available. In the cards used for blood grouping, red cell specific antisera and a preservative are also added to the gel at the time of manufacture.each card contains six such microtubes.



Materials required

1. ID-Card "Diaclon ABO/D + reverse typing cards" containing monoclonal anti-A_1, anti-B and anti-D within the gel matrix. The microtube control is the negative control. Two microtubes with neutral gel serve for reverse grouping with A & B cells.
2. ID-Diluent 2(modified low ionic strength saline)
3. Test cell reagents- ID Diacell A_1 & B, O in a 0.8% ±0.1% suspension. This is available in 10ml vials which are ready to use.
4. 1D-Dispenser
5. ID-Pipetor and tips
6. ID-Working Table
7. ID-Centrifuge

Sample

A 5% red cell suspension is prepared by adding 0.5ml of Diluent 2 to 50µl of whole blood or 25 µl of packed cells and mixing gently.

Procedure

1. Identify the ID-Card with unique patient/donor no.
2. Remove the aluminum foil from as many microtubes as required by holding the ID card in upright position.
3. Pipette 50 µl ID Diacell A_1 to microtube 5(A1).
4. Pipette 50 µl ID Diacell B to microtube 6(B).
5. Pipette 50 µl of patient's serum to both microtubes 5&6.
6. Pipette 10 or 12.5 µl of patient's red cell suspension to microtubes 1-4 (A,B,D,ctrl)
7. Incubate at room temp for 10 min
8. Centrifuge the ID Cards for 10 mins in the ID Centrifuge.
9. Read and record the results.

Interpretation

Positive: agglutinated cells forming a red cell line on the surface of gel or agglutinates dispersed in gel

Negative: compact button of cells on the bottom of the microtube

Precautions in blood grouping

1. Perform the grouping at room temperature(20-24°C).
2. Include both cell and serum grouping as this serves as a check.
3. Use antisera as per manufacturer's instructions.
4. Store antisera in the refrigerator when not in use.
5. Check the antisera regularly with known cells.
6. All glassware must be dry and clean.
7. Label all tubes accurately.
8. Record results immediately.
9. Use correct speed and time of centrifugation.

WHAT HAVE YOU LEARNT

- Antigen is a substance which when introduced into an individual leads to the production of antibody
- On the red cell surface there is presence of glycoproteins and glycolipids which act as antigen, they are called group antigen
- Antibodies are Immunoglobulins present in the serum and are IgG, IgM,IgD,IgA & IgE
- Certain antibodies occur without antigenic stimulus and are called naturally occurring antibodies like ABO antibodies
- Antigen Antibody reaction may be sensitization, agglutination, hemolysis and neutralization
- ABO grouping system is subdivided into 4 types based on the presence or absence of Antigen A & B on the red cell surface
- Slide, Tube, Microplate & Gel card techniques are various techniques available for ABO grouping

TERMINAL QUESTIONS

1. Write the reaction seen with Anti-A and Anti-B with each blood group. Mark positive result as + and negative result as

Group	Anti-A	Anti-B
AB		
A		
O		
B		

2. Write the reaction with the known cells seen in the blood groups. Mark as in Q1.

Group	A cells	B cells	O cells
B			
AB			
A			
O			

ANSWERS TO INTEXT QUESTIONS

7.1

1. Antigen
2. Glycoproteins & glycolipids
3. Antibody
4. Immunoglobulin
5. Naturally occurring antibody

7.2

1. d 2. e 3. b 4. c 5. a

7.3

1. b 2. d 3. a 4. c