Notes

12

RHESUS BLOOD GROUP SYSTEM

12.1 INTRODUCTION

The Rhesus blood group system is the second most important system in transfusion practice. Rh typing is routinely performed along with ABO grouping in all donors and recipients.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the nature of Rh antigens and antibodies
- explain the significance of Rh grouping
- discuss the method of Rh grouping

12.2 ANTIGENS OF THE RH SYSTEM

The most significant antigen of the Rh system is D because it is highly antigenic. An individual is labeled Rh positive or negative depending on the presence or absence of the D antigen on the surface of the red cells. The D antigen has greater immunogenicity than all other red cell antigens. In India, 95 percent of the population is Rh positive and 5 percent is Rh negative.

Besides antigen D, **four other antigens** of the Rh system have been described: ***C,c, E, e.*** Unlike the ABO antigens the Rh antigens are **only present on red cells**.

Antibodies

The Rh antibodies are always **immune** in nature. They are of IgG subtype and form after exposure to red cells possessing the D antigen. This exposure may occur through **transfusion** or **during pregnancy**.

Rh antibodies are the most important cause of **hemolytic disease of new born** and can cross the placenta to cause destruction of fetal red cells. Once formed their effect persists for many years. Even if their level in the circulation falls, subsequent exposure to the antigen results in a very rapid secondary response.

Rh genes

There are two homologous genes on the short arm of chromosome 1 which code for the nonglycosylated polypeptides that express Rh antigenic activity. One gene is designated **RHD** and it confers D activity on the red cell. It is **present in D-positive** individuals while D-negative individuals have no genetic material at this site. The D antigen has no allele. D- negative individuals lack RHD .

At the other adjacent locus, is a gene called **RHCE**. This determines the presence or absence of C, c, E and e antigens.

INTEXT QUESTIONS 12.1

1. ..... in all donors and recipients are important to perform Rh typing.
2. Rh system's most significant antigen is ..... because of its high .....
3. Rh antigens are only located on .....
4. ..... & ..... are the two homologous genes on the short arm of chromosome 1 which expresses Rh antigenic activity.
5. RHD determines the ..... on the red cells and RHCE concludes the presence or absence of .....

12.3 SIGNIFICANCE OF RH TYPING

Rh antibodies are immune in nature and form after exposure to red cells containing D antigen. Exposure can occur during pregnancy or after transfusion of an Rh negative individual with Rh positive blood. More than 80 percent of D negative individuals who receive a D positive blood transfusion develop Anti-D. To prevent this, the blood of all recipients and donors is routinely tested for D antigen to ensure that D negative individuals are identified and receive D

negative blood only. As Anti-D antibodies can cause hemolytic disease of new born, Rh negative pregnant women are screened during pregnancy for the development of unexpected antibodies.

D^u phenotype

D^u is a weaker variant of D antigen. D^u red cells are agglutinated by some anti-D antisera but not by others. Those which are not agglutinated by anti-D antisera can be detected by AHG technique.

Importance of detecting D^u

1. Though D^u is less antigenic than D, these red cells may be destroyed if transfused to a patient who has anti-D. Hence these donor units must be labeled as Rh positive and du testing must be done on donors.
2. D^u testing is not done routinely on recipients as they are labeled as Rh negative and transfused with Rh negative blood.
3. If a neonate is D^u positive, he can suffer from HDN if the mother has anti-D.
4. Rh negative mothers of a D^u positive neonate must receive RhIg.

Technique of Rh grouping

In Rh grouping, testing is done only for D antigen. Tests for other antibodies are performed only when specifically indicated.

The **methods** for Rh typing are

Slide

Tube

Microplate

Gel card

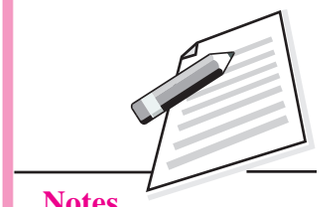
Types of Anti-D antisera

Different types of anti-D antisera are available commercially. These include

(a) Polyclonal human anti-D serum

(b) High protein antisera: This is used for slide grouping. It has macromolecular additives and gives rapid, reliable results.

(c) Saline reactive antisera: This is prepared from IgG antibodies which are modified so that they agglutinate antigen positive cells suspended in saline.



(d) Monoclonal antisera These are of three types: IgM anti-D monoclonal antisera, blend of IgM and IgG monoclonal antisera and blend of monoclonal IgM and polyclonal IgG. IgM anti-D antisera are highly specific and saline reacting. They react equally well at room temperature and at 37°C. They can be used for slide or tube test. For Du testing a blend of IgM and IgG monoclonal is used.

INTEXT QUESTIONS 12.2

1. Exposure of Rh antibodies on red cells containing D antigen may occur during ..... & .....
2. D^u red cells are agglutinated by ..... and not by others
3. Detection of non agglutinated D^u red cells by Anti –D antisera can be done by .....
4. ....., ..., ... and ..... are the four methods of Rh typing.
5. ..... is used for slide grouping.
6. For slide or tube test ..... and for D^u testing a blend of ..... are used.

12.4 SLIDE TECHNIQUE

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

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.

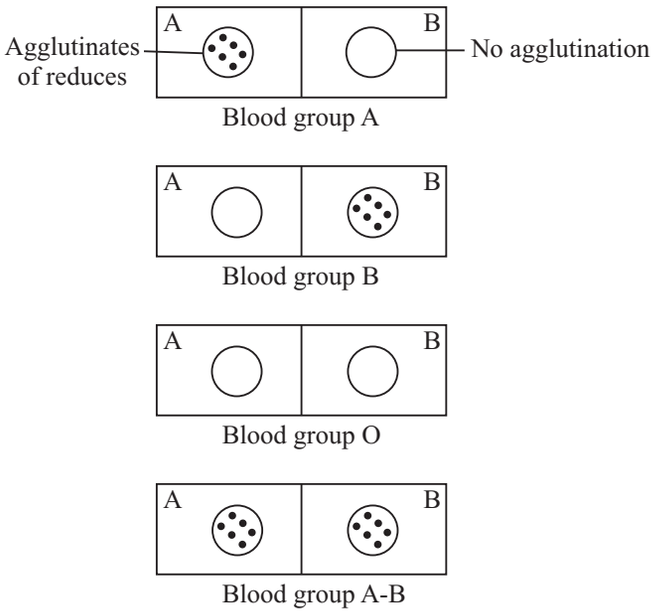
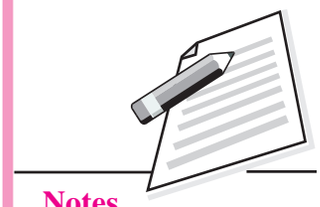


Fig. 12.1: ABO grouping by slide technique



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 × 100mm
3. Glass tubes 75 × 10mm

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 0.2ml/0.5ml for 2 and 5% respectively).
    - Invert gently several times to make an even suspension.

Notes

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 5-10min 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

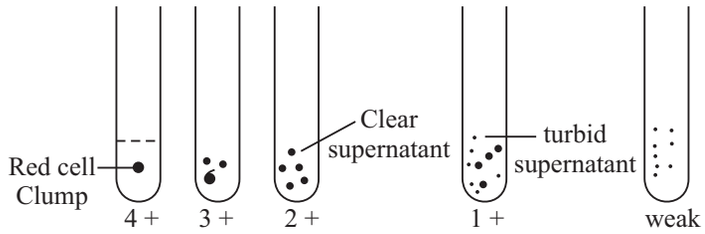


Fig. 12.2: Grading agglutination reactions for blood grouping by tube technique

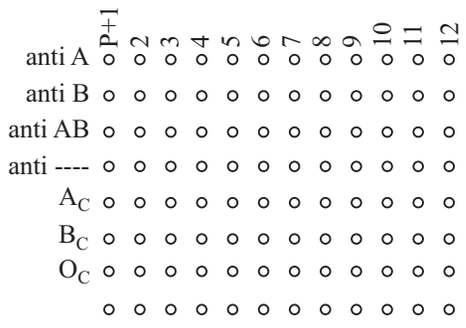


Fig. 12.3: Blood grouping by microplate technique

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 12.1

Group	Cell grouping			Serum grouping		
	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.

WHAT HAVE YOU LEARNT

- The most significant antigen of the Rh system is D because it is highly antigenic. An individual is labeled Rh positive or negative depending on the presence or absence of the D antigen on the surface of the red cells. The D antigen has greater immunogenicity than all other red cell antigens. In India, 95percent of the population is Rh positive and 5percent is Rh negative. Besides antigen D, four other antigens of the Rh system have been

described: ***C,c, E, e.*** Unlike the ABO antigens the Rh antigens are only present on red cells. The Rh antibodies are always immune in nature and form after exposure to red cells containing D antigen. Exposure can occur during pregnancy or after transfusion of an Rh negative individual with Rh positive blood. More than 80 percent of D negative individuals who receive a D positive blood transfusion develop Anti-D. To prevent this, the blood of all recipients and donors is routinely tested for D antigen to ensure that D negative individuals are identified and receive D negative blood only. As Anti-D antibodies can cause hemolytic disease of new born, Rh negative pregnant women are screened during pregnancy for the development of unexpected antibodies. Rh typing can be done by slide, tube, microplate or gel technique.

TERMINAL QUESTIONS

1. Explain the significance of Rh typing
2. Explain the different types of anti-D antisera

ANSWERS TO INTEXT QUESTIONS

12.1

1. ABO grouping
2. D & Antigenicity
3. Red cells
4. RHD & RHCE
5. D activity & C,c,E and e antigens

12.2

1. Pregnancy, transfusion
2. Anti –D antisera
3. AHG technique
4. Slide Tube Microplate & Gel card
5. High protein antisera
6. IgM anti-D antisera, IgM and IgG monocolonal