Chapter 4

ABO and Rh Blood Group System

BLOOD GROUPING TECHNIQUE, ANTISERA, LECTIN AND OTHER USEFUL REAGENTS AND THEIR USES

Blood Grouping Antisera, Lectin and Other Reagents Used in Blood Centre

- In general blood centering practice three antisera are used for blood grouping.
- Anti 'A'
- Anti 'B'
- Anti 'D' (monoclonal-IgM)
- Extra antisera are used in special conditions (Fig 4.1a).
- **Anti-AB:** For confirmation of presence of subgroups of 'A' or 'B' antigen in case of blood group discrepancy (weak antigen expression).
- **Anti-A_1' lectin *(Dolichos biflorus)*:** For subgroup classification of 'A' and 'AB' subgroup ('A_1', 'A_2' and 'A_1B', 'A_2 B')
- **Anti-'H' lectin *(Ulex europeas)*:** For detection of Bombay phenotype and secretor status studies through saliva.
- **Anti-'D' blend (IgG + IgM):** Used for routine RhD detection and weak D (Du) test of RhD negative donor unit.

Fig. 4.1a: Blood grouping antisera, lectin and other useful reagents

Fig. 4.1b: Minor antisera (anti-e, anti-c, anti-C, anti-E, anti-K) and AHG

- **Anti-'D' monoclonal (IgG):** Used for Du test to confirm D variants, and as a positive control for IAT test and sensitize red cells preparation.
  1. **NB:** If anti-'D' (IgG) commercially is not available, blend (IgG + IgM) anti-'D' can be used for Du test.
  2. **Uses of minor antisera:** The anti-C, anti-E, anti-c, anti-e and anti-K reagents detect the presence of antigens C, E, c, e and K on the surface of red blood cells (Fig. 4.1b).
- **Uses of sensitized red blood cells (Coombs control cells):** To check the validity of DAT and IAT.
- **Uses of papain cysteine reagent:** Enzyme cross-match and to identify the antibodies where multiple alloantibodies are suspected (one is enzyme sensitive and other one is enzyme resistant).
- **Uses of 6% of bovine albumin reagent:** For IgG cross-matching and absorption studies using bovine albumin.
- **Uses of antihuman globulin (AHG) reagent:** For IAT cross-matching, IAT, DAT, Rh antibody detection test, and IAT control.
- **Monospecific AHG (anti-IgG, anti-C_3d) are used in tube test:** Antihuman globulin (anti-IgG and anti-C_3d) is use to detect sensitized RBC (either coated with IgG or C_3d).
- **Use of LISS solution:** LISS is used as a potentiator. It increases the rate of antibody uptake and therefore decreases the incubation time. It is also used for column agglutination technique (Gel card method) and absorption studies.
- Always use two different manufacturer's antisera for blood grouping and Rh (D) typing test which is mandatory according to drug control norm. Always follow the instruction of the blood centre SOP.

ABO Blood Group Systems

The ABO Antigens and Corresponding Antibodies

The red cell membrane carries various types of surface proteins, and proteins that cross the lipid layer of the cell membrane. These surface proteins and glycoproteins carry the blood group antigens and their specificity is mostly determined by the sequence of oligosaccharides (e.g. ABO) or the sequence of amino acids (e.g. Kell, Duffy, Kidd, MNS). These antigens are assigned to blood group systems or collections based on

their relationship to each other as determined by serological or genetic studies. As of November 2023, the red cell Immunogenetics and blood group terminology working party of the International Society of Blood Transfusion (ISBT) recognised 45 blood group system genes containing 362 antigens. In this chapter, the basics of ABO blood group system, and the methods of blood grouping will be discussed.

The ABO antigens and antibodies are the most important for blood transfusion service. It is the only blood group system in which the reciprocal antibodies are consistently and predictably present in the sera of most people, without any previous exposure to human red cells. Transfusion of ABO-incompatible blood may cause severe intravascular hemolysis due to this natural occurring antibody.

Genes at three separate loci (H, Se, and ABO) control the occurrence and the location of the A and B antigens. H gene is responsible for the synthesis of H antigen from which 'A' or 'B' or both or none is produced. As per the synthesis of ABH antigen, the ABO blood group is defined (Fig. 4.2 and Table 4.1).

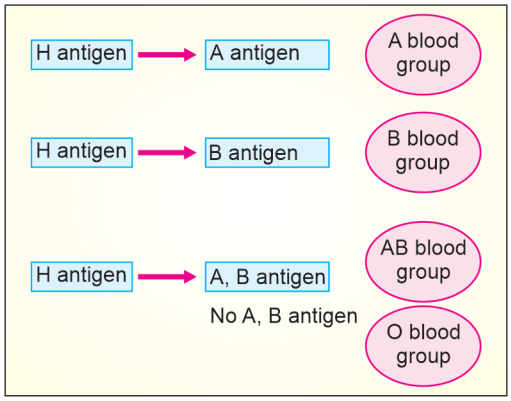

Fig. 4.2: The ABO antigens and corresponding antibodies

Table 4.1: ABO blood group antigens and their corresponding antibodies

Antigen on red cell surface	*Antibody in plasma/serum*	*Blood group*
A	Anti-B	A
B	Anti-A	B
A, B	None	AB
H (No A or B Ag)	Anti-A , Anti-B	O
—No H, A, B Ag	Anti-A, Anti B, Anti-H	Bombay

'O' blood group has anti 'A' and anti 'B' antibodies but no antigen.
'B' blood group has 'B' antigen and anti 'A' antibody.
'A' blood group has 'A' antigen and anti 'B' antibody.
'AB' blood group has 'A' and 'B' antigens but no antibody.

BLOOD GROUPING AND RH TYPING TEST OF UNKNOWN CELLS

- **Blood group:** Genetically determined antigen system.
- Expressed mostly on the RBC surface and it can be detected by potent (efficient) antisera.

Rh Blood Group System

Two highly homologous genes on the short arm of chromosome 1 encode the non-glycosylated polypeptides that express the Rh antigens. These genes are RHD and RHCE which produce 57 antigens. Among these Rh antigens, D, C, c, E, e; are more important. RhD antigen is clinically most significant and its presence is termed as RhD positive and its absence as RhD negative.

Blood Grouping by CTT

Materials/Reagents Required

- Test tube
- Test tube rack
- Table top centrifuge
- Normal saline
- Anti 'A'
- Anti 'B'
- Anti 'D' (monoclonal-IgM)
- Anti D (blend IgG + IgM)
- Anti-AB
- Anti-H
- "A" cell, "B" cell, and "O" cell

Methodology

1. Forward grouping (cell grouping):

a. The principle is agglutination of unknown antigen by known antibodies (commercially available antisera). (Cell/Forward/Direct grouping is based on an agglutination reaction between A- and B-antigen present on RBCs which agglutinates with commercial anti-A and anti-B antisera, respectively.

b. Blood group A is further divided into A_1 and A_2 based on this, the ABO blood group system can be divided into six subtypes group A_1, A_2, B, A_1B, A_2B and O.

c. Only forward grouping (cell grouping) is to be done for newborns baby till 4 months of age.

2. Reverse grouping (serum grouping):

It is the principle of detection of anti-A and anti-B present in the plasma or serum by using pooled red blood cell suspension.

Procedure

- Take standard glass test tubes, labelled it properly and arrange serially from left-to-right direction according to the mark in a test tube rack.
- The wooden rack has been already marked 'A', 'B', 'D1' and 'D2' (Rh) for cells grouping, 'A_1'cells, 'B' cells,'O' cells, for serum grouping (Fig. 4.3).
- Mark sample number on all tubes by marker.

- Prefer fresh samples for testing and do not use the haemolysed serum and haemolysed red blood cells suspension for blood group serology.
- Take two drops (or 100 µl) of respected antisera 'A', 'B', 'D1' and 'D2' (IgM) according to the labelled test tubes.
- Collect unknown test serum (donor/recipient) using pasture pipette or micropipette and place two drops (or 100 µl) in serum grouping in each tube.
- Add one drop (or 50 µl) of prepared 2–5% of red blood cell suspension using clean pasture pipette or micropipette for cells grouping in each tube (prefer 5% of red blood cells suspension for CTT).
- Add 5% 'A_1' cells, 'B' cells, and 'O' cells for reverse grouping in the respective test tubes (Table 4.2).

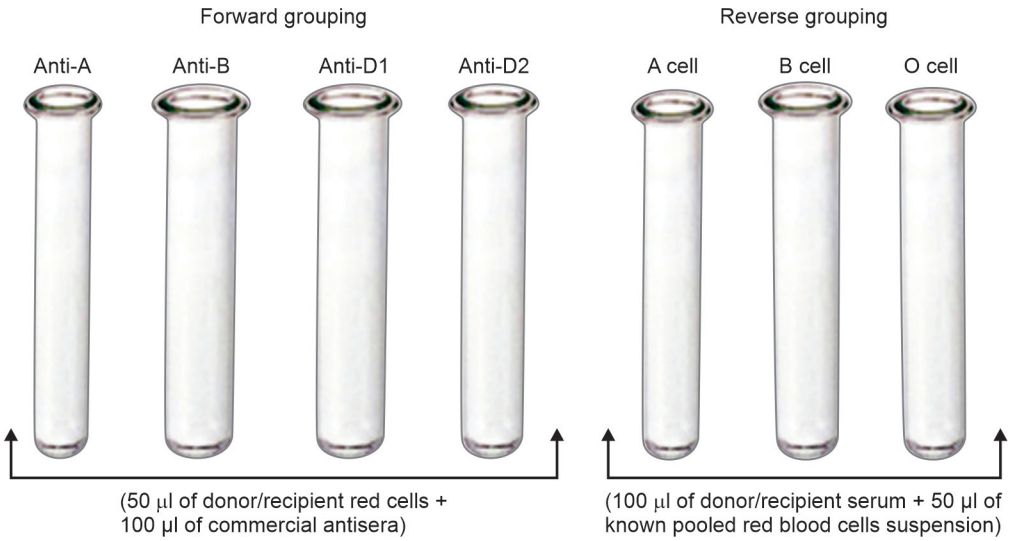

Fig. 4.3: Blood grouping technique through tube method

Table 4.2: Cell grouping and reverse grouping

Cell grouping (Antisera monoclonal)				*Serum/reverse grouping (Antibody screening, donor/recipient)*			
Anti-A	*Anti-B*	*Anti-D1*	*Anti-D2*	*A cell*	*B cell*	*O cell*	*Blood group*
+	0	+	+	0	+	0	A RhD +ve
0	+	+	+	+	0	0	B RhD +ve
+	+	+	+	0	0	0	AB RhD +ve
0	0	+	+	+	+	0	O RhD +ve
0	0	+	+	+	+	+	Bombay RhD +ve*
+	0	0	0	0	+	0	A RhD –ve
0	+	0	0	+	0	0	B RhD –ve
+	+	0	0	0	0	0	AB RhD –ve
0	0	0	0	+	+	0	O RhD –ve

*Agglutination with O cell in reverse grouping warants to test the red cell with anti-H lectin to confirm Bombay (oh) blood group.

INTERPRETATION

Conventional tube technique (CTT) is shown in Fig. 4.4.

Writing for result graded as follows (Fig. 4.4):

++++ Complete single clump.
+++ Several large clumps.
++ Medium size clumps, clear background.
+ Multiple small clumps, turbid background.
+w Weak agglutination.
–Ve (Negative) No clump.

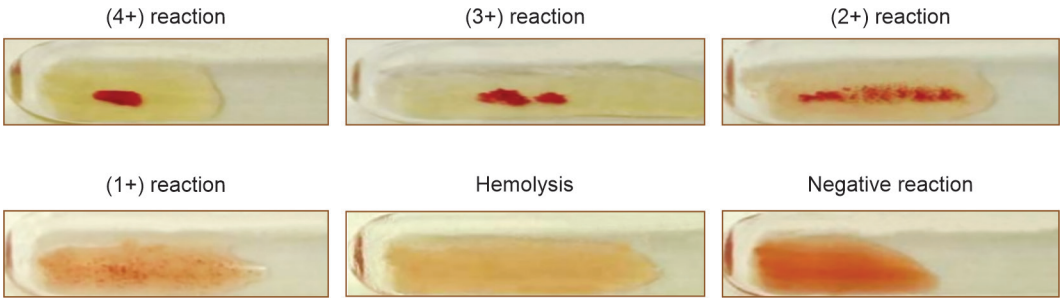

Fig. 4.4: Agglutination grading in conventional tube technique (CTT)

NB: Tubes are centrifuged to enhance the antigen–antibody binding.

Column Agglutination Technique

There are two kinds of methods which are applied for the column agglutination technique. They are:

1. Glass microbeads method
2. Gel card method

1. COLUMN AGGLUTINATION TECHNIQUE BY GLASS MICROBEADS METHOD

Principle

Reis KJ had described the above technique/system for irregular antibodies in the year 1993. The above-mentioned test is performed in a microcolumn prefilled with glass microbeads in the suspension of antihuman globulin serum, any diagnostic reagent, or neutral isotonic solution. During column centrifugation, the sensitized cells are trapped by the microbeads suspension. Detection of sensitized red cells is based upon the sieving effect of glass microbeads. At the upper part of a column over the glass microbeads suspension, red cells, and serum are incubated. During the centrifugation step they retain the agglutinations and the unsensitized cells settles at the bottom.

The follow materials are required for the process:

- BioVue cassettes.
- BioVue centrifuge.
- BioVue incubator.

- BioVue system work rack.
- BioVue liner.
- Micropipettes 10 µl, 40 µl and 50 µl.

Cell suspension preparation for glass micor-beads method:

- **Preparation of 4% red cell suspension:** Take 960 µl of normal saline in a standard test tube and 40 µl of packed RBC of A/B/O cells
- Mix gently to make cell suspension homogenous.

Red blood cell suspensions preparation as per requirement using isotonic saline and packed red blood cells.

Normal saline	*Packed red blood cell volume*	*Red blood cell concentration %*
970 µl	30 µl	3%
960 µl	40 µl	4%
950 µl	50 µl	5%
1.2 ml	10 µl	0.8%
990 µl	10 µl	1.0%

Forward Grouping and Reverse Grouping by Glass Microbeads Method

- Prepare red blood cell suspension
- Allow the cassette to come to room temperature before use.
- Open the wells of the cassette using the liner assembly (Fig. 4.5a).
- The cassette should be used within one hour after insertion of the liner.
- **For forward grouping:** Add 10 µl of 3% to 5% or 40 µl of 1.0% or 50 µl of 0.8% red blood cell suspension to the reaction chambers of the cassette (columns 1, 2, 3, and 4) (Fig. 4.5b).
- **For reverse grouping:** Add 50 µl of 0.8% or 10 µl of 3% pooled cell to the appropriate reaction chambers of the cassette (column 5 and 6).
- Add 40 µl of serum/plasma to the appropriate reaction chambers of the cassette (column 5 and 6).
- Centrifuge the cassette using the BioVue system centrifuge.
- Centrifuge should occur within 30 minutes of addition of the samples to the reaction chamber.
- Read the front and back of the individual columns for agglutination and/or hemolysis upon test completion.

The following are some of the advantages of using glass microbeads technique:

- The incubation period/time is as low 10 minutes for antibody screening or cross-matching.
- Approximately, 5 minutes time for biphasic centrifugation.
- There is no need to wash cells or use sensitized cells for confirmation in AHG test
- There is no tube shaking or re-suspension of cells button leading to variation in reading and grading the agglutination.

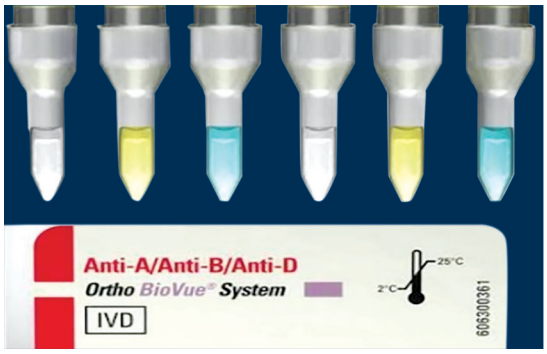

Fig. 4.5a: ABO forward grouping card

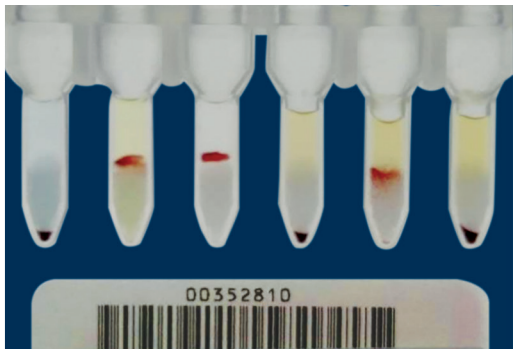

Fig. 4.5b: ABO forward and reverse grouping card

- Due to the provision of a calibrated centrifuge to spin at the optimal speed for a fixed and correct length of time, the margin of error during this phase of the test is less.
- The interpretation of the result is more objective, consistent, and reproducible.

Protocol for Coombs Test (DAT/ DCT) by Glass Microbeads Method in Ortho BioVue System

Preparation of 4% red blood cell suspension:

Add 50 µl of packed RBC cell of test sample (washed once in saline) in 1.0 ml (1000 µl) of normal saline solution and mix gently.

Procedure:

- Open the aluminum foil of the BioVue casstte.
- Add 10 µl of 3–5% RBC suspension.
- Centrifuge the cassette for 5 minutes using BioVue centrifuge.
- Read the result.

Indirect Coombs Test (ICT) by Glass Microbeads Method in Ortho BioVue System

Procedure:

- Centrifuge the sample supplied at 3000 rpm for 3 minutes to get supernatant test serum.
- Open the aluminum foil of the BioVue cassette.
- Add 50 µl LISS reaction chamber.
- Add 10 µl of 3–5% pooled 'O' red cell suspension.
- Add 40 µl of test serum.
- Incubate at 37°C for 10 minutes OWS incubator.
- Centrifuge the cassette for 5 minutes using BioVue centrifuge.
- Read the result.

2. COLUMN AGGLUTINATION TECHNIQUE BY GEL CARD METHOD

- For column agglutination technique (CAT), please follow the manufacturers instruction.
- Red blood cell suspension of 0.8 to 1% is used in CAT testing (whereas 5% cell suspension is used in CTT) and dispensed into the reaction chamber by micropipette at an angle of 45°.
- Serum is dispensed by the micropipette at perpendicular to the gel card.

- Blood grouping, direct antiglobulin test (DAT) and indirect antiglobulin test (cross-matching, antibody screening/identification) can be performed by CAT method which is more sensitive compared to tube technique.
- In CAT, washing step is not required as the unbound antibody cannot interfere in the agglutination process like that of CTT.
- Serum or plasma can be used in (gel card method) column agglutination technique for reverse grouping, cross-matching, antibody screening, and indirect antiglobulin test. Centrifuge the anticoagulated (EDTA) sample and keep that plasma in a separate tube and this plasma is used for the above test. Serum is prefered for antibody screening as complement activating antibodies may be missed while using plasma for the test.
- Strictly follow the time of incubation, check the temperature of the incubator, rpm, and timing of centrifugation according to the manufactures instruction.
- During incubation, check that the incubator is maintaining the temperature at 37°C. Observe the result immediately after completing the test procedure.

BLOOD GROUPING AND REVERSE GROUPING BY COLUMN AGGLUTINATION TECHNIQUE (GEL CARD METHOD)

The gel column acts as a filter that taps agglutinated red blood cells as they pass through the column during the centrifugation of the card. The gel column separates agglutinated red blood cells from non-agglutinated red blood cells based on size.

Reagents/apparatus required:

- Gel card: ABO/D + Reverse grouping card.
- Test tubes (CTT).
- Micropipette.
- Gel card centrifuge machine.
- Gel card warmer (incubator).
- Prepared readymade diluents (LISS solution).
- A_1 cells, B cells, O cell (0.8 to 1% suspension), ready to use for reverse grouping.
- Donor and recipient red blood cells and serum/plasma.
- Tissue paper/cotton/normal saline/distill water.
- Gel card rack and test tube rack.

Instructions of gel card pipetting.

✓ **Right instructions:** Pipetting of red cells suspension at 45° angle and pipetting of serum at 90° angles (Figs 4.6a and b).

× **Wrong instructions:** Pipetting of red blood cells suspension and serum.

Red blood cell washing is not required for cell suspension preparation in the column agglutination technique (gel card method).

Step 1

- Preparation of 1% A,B, O cell suspension for reverse grouping and 5% test red cell suspension for forward grouping.
- Take 1000 µl of readymade diluents (LISS or NS) + 10 µl of donor packed RBCs in a precipitin tube. Take 950 µl LISS + 50 µl red cell (test sample) to prepare 5% cell suspension.

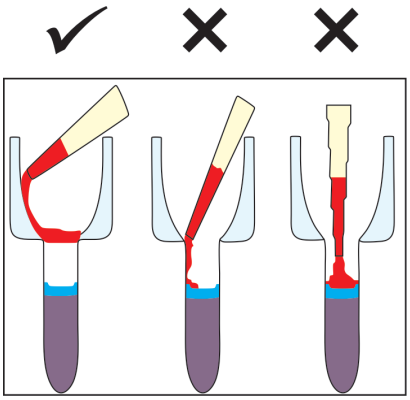

Fig. 4.6a: Pipetting of red ells

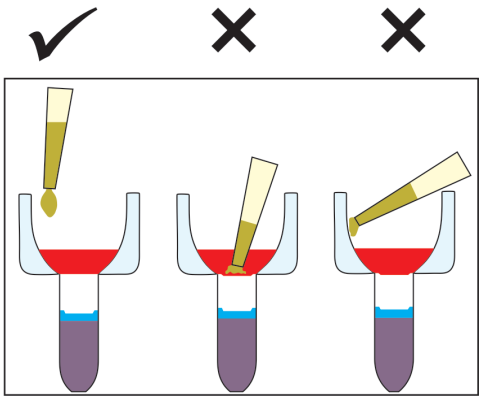

Fig. 4.6b: Pipetting of serum/plasma

Step 2

- Forward grouping.
- Remove aluminum foil of micro-tube in upright position of the card (Fig. 4.7a).
- Add 10–12.5 µl 5% cell suspension (test) red blood cells suspension of sample to be tested in gel micro-tube labelled as A, B, D.

Step 3

- Reverse grouping.
- Add 50 µl of readymade 'A_1' cell, B cells, and O cells to gel micro-tube labelled as A_1, B and O cell (control)
- Add 50 µl of serum/plasma (prefer serum) of sample to be tested in both gel micro-tube labelled as A_1, B and control.

Step 3

Incubate at room temperature for 10 minutes and then centrifugation for 10 minutes in gel centrifuge machine.

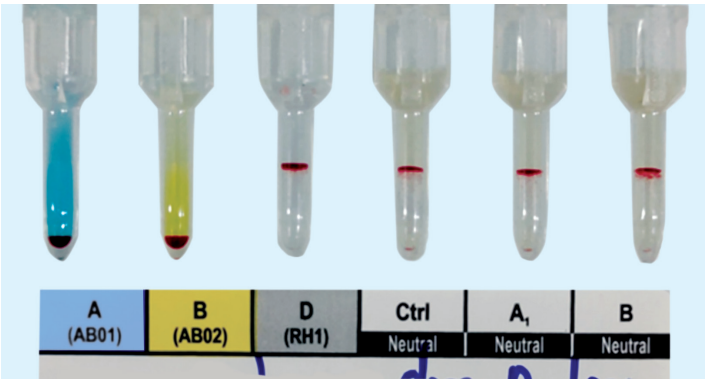

Fig. 4.7a: Blood grouping gel card (Bombay Rh postive)

Note: Details of Bombay blood group is discussed in Chapter 5.

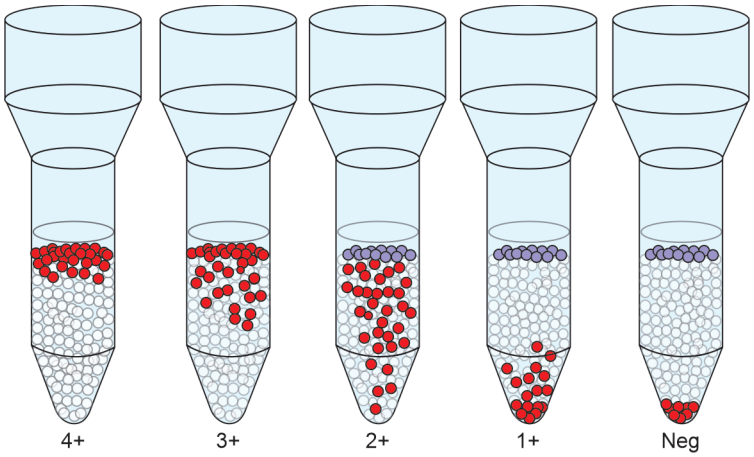

Fig. 4.7b: Grading of agglutination

Step 4

Read results and grades the result as mentioned in Fig. 4.7b.

The control test is always negative. If the control shows agglutination that indicates clot, contamination, or auto-agglutination of the sample.

Newborn Blood Grouping

Reagents/apparatus required

- ABD/Rh for newborn gel card (Fig. 4.8).
- Readymade diluent (LISS solution).

Follow the above mentioned blood grouping and an extra ABD/Rh newborn gel card is needed for newborn grouping. (Reason—newborn blood sample is cord blood sample containing Whartons jelly which may produce a false positive reaction.)

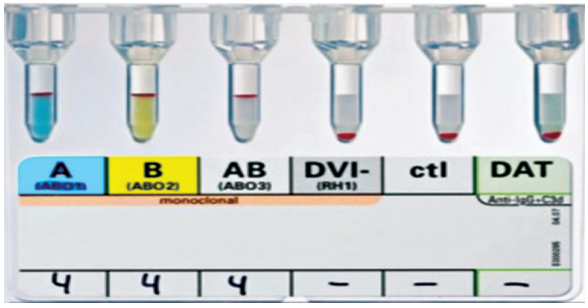

Fig. 4.8: Newborn grouping and DAT

Step 1

0.8–1% red blood cell suspension preparation.

Take 1000 µl of readymade diluents (LISS solution) + 10 µl of the newborn patient's packed RBCs in a precipitin tube. Mix gently.

Step 2

Forward grouping and DAT:

Remove the aluminum foil of the micro-tube in the upright position of the card.

Add 50 µl of the above-suspended cell to all 6 micro-tubes of gel card.

Step 3

Centrifugation for 10 minutes in a gel centrifuge machine.

Result interpritetion: Blood group is reported as per the agglutination observed in the gel cards.

Rh and Kell Subtyping by Column Agglutination Technique (Gel Card)

Reagents/apparatus required

Rh subgroup + Kell gel card (Fig. 4.9).

And follow the same above-mentioned blood grouping and an extra Rh subgroup + Kell gel card is needed for Rh and kell subtyping.

Step 1

5% red blood cell suspension preparation.

Take 500 µl of readymade diluents (LISS solution) + 25 µl of patients-packed RBCs in a precipitin tube. Mix gently.

Or

1% cell suspension:

Take 950 µl of readymade diluents (LISS solution) + 50 µl of patients-packed RBCs in a precipitin tube. Mix gently.

Step 2

Add 50 µl of above 1% red blood cell suspension of samples (10 µl of 5%) to be tested in a micro-tube labelled as C, c, E, e, Kell, and control.

Step 3

Centrifugation for 10 minutes in a gel-centrifuge machine.

Result interpritaion: Agglutination in the gel column is considered as positive and the Rh, Kell phenotype is reported accordingly.

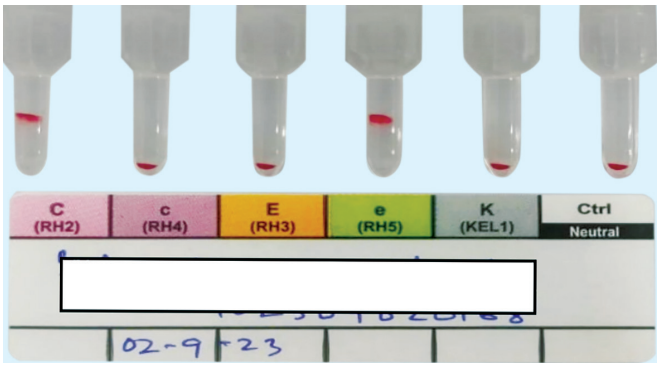

Fig. 4.9: Gel card for Rh subgroup and Kell

Chapter 5

Resolution of Blood Group Discrepancy

- ABO blood grouping can be performed by simple technique and it involves two basic steps—cell grouping (forward grouping) that establishes the presence or absence of A- and/or B-antigens on the red blood cells (RBCs) and serum grouping (reverse grouping) that demonstrates the presence or absence of ABO antibodies in the serum.
- Determination of the correct ABO blood group needs both cell grouping and serum grouping because of the existence of a mutual association between cell grouping and serum grouping.
- Blood group discrepancies are usually encountered when there is a deviation from the expected pattern of antigen in cell grouping and antibody in serum grouping. In other words, blood group discrepancy is the mismatch between the expected forward and reverse grouping.
- These blood group discrepancies occur due to weaker expression of A- or B-antigens or missing or low titer of ABO antibodies. Other reasons may be related to technical error or clerical error, miscellaneous, or rouleaux formation, which gives rise to the blood group discrepancy.
- As per the standard classification of ABO discrepancy, blood group discrepancy is divided into four major categories—type I, type II, type III, and type IV ABO blood group discrepancy

RESOLUTION STEPS FOR BLOOD GROUP DISCREPANCY

- Always ask for a fresh sample to repeat the blood group.
- Check for clerical error.
- Rule out wrong blood in the tube (WBIT means the blood inside the sample tube or vacutainer is of different patient and the name and registration number mentioned on the tube is different.)
- Usually, higher strength of agglutination is considered as true agglutination and the missing agglutination is considered for further test.
- If the blood group discrepancy is due to missing antibody in the plasma, then increase the serum to cell ratio that is four-part serum instead of two-part and one part cell. If still there is no agglutination, increase the duration of incubation or incubate the cell-serum mixture at 4°C, then check for agglutination.
- If the blood group discrepancy is due to missing antigen, then increase the cell to serum ratio that is one part serum and cell each. If still there is no agglutination,

Table 5.1: Types of ABO discrepancy and their causes

Type I	*Type II*	*Type III*	*Type IV*
Weak or missing antibodies	*Weak or missing antigen*	*Rouleaux formation*	*Miscellaneous or irregular antibody*
• Newborn • Elderly patients • Patient with leukemia or lymphoma • Patients on immunosuppressive drugs • Patients with immunodeficiency diseases • Patients with bone marrow transplant	• Subgroups of A or B • Leukaemia and lymphoma • Excess antigen of blood group soluble substances • Acquired A- or B-antigens and Hodgkin's lymphoma	• Elevated levels of plasma globulins: Multiple Myeloma, Waldenstrom's macroglobulinemia, and Hodgkin's lymphoma • Elevated levels of fibrinogen • Use of plasma expanders such as dextran • Wharton's jelly in cord blood samples	• Poly agglutination • Patient with cold autoantibodies • A_2 or A_2 B individual with anti-A_1 antibodies • Naturally occurring or irregular antibodies reacting at room temperature • Cis-AB (Cis-AB, a rare ABO variant, is caused by a gene mutation that results in a single glycosyltransferase enzyme with dual A and B glycosyltransferase activities)
RESOLUTION			
• Increase serum: Cell • Increase incubation time • Incubate at 4°C	• Increase cell: Serum • Increase incubation time • Incubate at 4°C	• Saline replacement technique is the established method to resolve rouleaux. (After centrifugation, remove the plasma and add equal volume of saline) Rouleaux will no longer be seen when the plasma proteins are removed.	• Monoclonal antisera and use of lectin • Poly-agglutination • Antibody screening/identification • Pre-warming technique • DTT treatment (Dithiothreitol) which breaks IgM antibody

increase the duration of incubation or incubate the cell-serum mixture at 4°C, then check for agglutination.

- Even then there is no agglutination is detected in both the types of discrepancy, elution-adsorption technique is performed.
- **Saline replacement technique:** This method is required when false agglutination is suspected in reverse grouping in a patient of multiple myeloma or paraproteinemia. Do the reverse grouping by CTT as described previously (Chapter 4). Do not check for agglutination. Rather, remove the supernatant plasma from all three test tube and add equal volume of normal saline. If there is true agglutination, it will remain as it is. But the false agglutination will disperse.
- **Pre-warming technique:** If the blood group discrepancy is due to a cold antibody, then keep the disposable syringe, needle, vacutainer, normal saline and pipette at 37°C. Immediately after collecting the sample, separate the plasma from cell. Keep the serum at 37°C for one hour and then repeat reverse grouping. Wash the cell with the warm saline for at least six times and repeat the forward grouping. Even then if the blood group discrepancy is not resolved, cell can be treated with DTT to remove the cold antibody (dithiothreitol, DTT breaks the IgM antibody).

PRACTICAL PROTOCOL FOR RESOLUTION OF BLOOD GROUP DISCREPANCY

Blood grouping and unknown irregular antibodies screening test of unknown serum (recipient/donor) through tube technique (CTT).

Procedure

- Prepare 2–5% of red blood cells suspension for forward grouping, Rh typing and autocontrol (prefer 5% of red blood cells suspension for serological tube testing).
- Prepare 5% of A_1', 'A_2' and 'B' blood group pooled red blood cells for reverse grouping and 'O' pooled red blood cells for unknown antibody and Bombay group screening (for donor and recipient).
- Take precipitin test tubes or standard glass test tubes and arrange serially from left-to-right direction according to the mark in a wooden rack.
- The wooden rack has been already marked 'A', 'B', AB', 'A_1', 'H', 'D_1' and 'D_2' (Rh) for cells grouping, 'A_1' cells, 'A_2' cells, 'B' cells, for serum grouping, 'O' cells, 'O' cells + Papain cysteine enzyme for antibody screening and Auto control (Donor/Recipient, self serum and self cell). Label the test tubes similarly.
- Mark sample number on all tubes by marker.
- Add prepared 2–5% of red blood cell suspension using clean pasture pipette or micropipette and place one drop (or 50 µl) of red cells for grouping, Rh typing and auto-control according to the labelled test tubes (prefer 5% of red blood cells suspension for serological tube testing).
- Collect unknown test serum (donor/recipient) using pasture pipette or micropipette and place two drops (or 100 µl) in serum grouping, antibody screening and auto-control in each tube.
- Prefer fresh samples for testing and do not use the hemolysed serum and hemolysed red blood cells suspension for blood centre serological tests.
- Take two drops (or 100 µl) of respected antisera 'A', 'B', 'AB', 'A_1', 'H', 'D_1' (IgM) and 'D_2' (Blend IgG + IgM) according to the labelled test tubes and in 5% of 'A_1' cells,

'A_2'cells, 'B' cells for reverse grouping and 'O' blood group pooled red blood cells for irregular unknown antibody (IgM) screening.

- Take one drop (or 50 µl) of papain cysteine enzyme before adding 'O' +ve pooled red blood cells suspension in serum tube for irregular unknown antibody (IgG) screening test.
- Take one drop (or 50 µl) 5% of red blood cell suspension (donor/recipient) in last serum tube for auto-control test and mix all the tubes gently.
- For Rh typing shake 'D' tube gently and centrifuge at 1000 rpm for one minute and read agglutination immediately macroscopically and microscopically and follow the manufacturer's instruction documented in the literature.
- Always use two different lots of same manufacturer or two different manufacturer's anti-'D' (monoclonal-IgM) and anti-D (blend IgG + IgM) for Rh (D) typing test which is mandatory according to drug control norm.
- 5% of pooled 'O' group red blood cells should be always used for the reverse grouping to rule out unknown antibodies and Bombay group (H).

NB: Spin method for cells and serum grouping—for cells grouping and serum grouping all the tubes are shaken gently and kept for minimum 15 minutes at room temperature as per model standard operating procedure for blood transfusion service guidelines and also follow the manufacturer's instruction. The test should be done at room temperature (20 to 24°C). And centrifuge at 1000 rpm for one minute and observe the agglutination.

Practical instruction

Forward grouping, reverse grouping + antibody screening + autocontrol (Figs 5.1, 5.2 and Table 5.2).

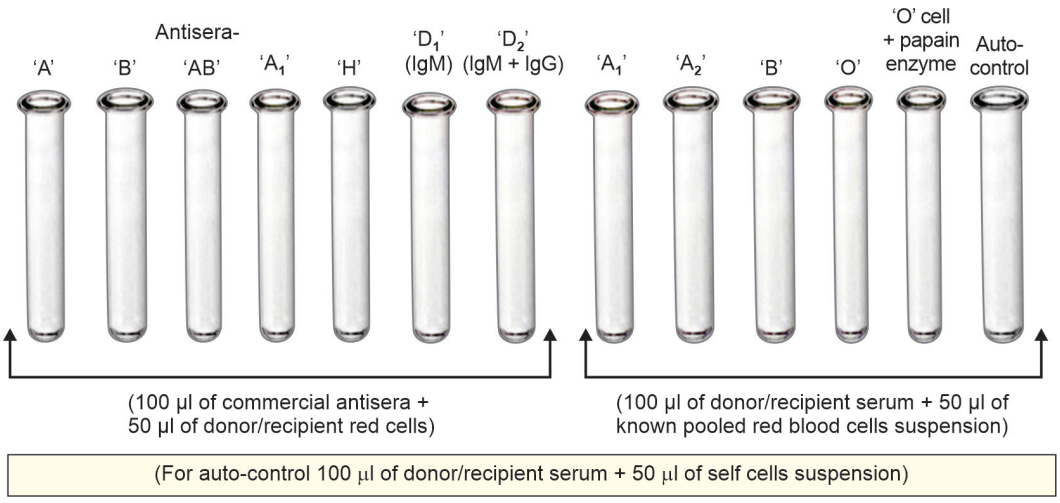

Fig. 5.1: Forward grouping, reverse grouping + antibody screening + autocontrol

BOMBAY GROUP

Discovered in 1952 in Mumbai by scientists Dr Bhende and Dr Bhatia.

There are always non-secretors of A, B, and H substances.

Table 5.2: Forward grouping, reverse grouping + antibody screening + autocontrol

Cell grouping (Antisera monoclonal)							*Serum/reverse grouping (Antibody screening, donor/recipient)*							
Anti 'A'	*Anti 'B'*	*Anti 'AB'*	*'A_1' lectin*	*'H' lectin*	*Anti 'D_1'(Rh) (IgM)*	*Anti 'D_2' (Rh) (IgM + IgG)*	*Group*	*'A_1' cell*	*'A_2' cell*	*'B' cell*	*'O' cell*	*'O' cell + Papain enzyme*	*Auto-control (self serum + self cell)*	*Group sustain*
+	–	+	+	+w	+	+	**A_1**	–	–	+	–	–	–	**'A_1' Rh +ve**
+	–	+	–	+	–	–	**A_2**	–	–	+	–	–	–	**'A_2' Rh –ve**
–	–	–	–	+	+	+	**O**	+	+	+	–	–	–	**'O' Rh +ve**
–	+	+	–	+w	+	+	**B**	+	+	–	–	–	–	**'B' Rh+ve**
+	+	+	+	+w	+	+	**A_1B**	–	–	–	–	–	–	**'A_1 B' Rh +ve**
+	+	+	–	+	+	+	**A_2B**	–	–	–	–	–	–	**'A_2 B' Rh +ve**
–	–	–	–	–	+	+	**Oh**	+	+	+	+	–	–	**'Oh' Rh +ve**

Note:

1. O cell with enzyme is not used routinely.
2. IAT of donor plasma/serum is tested with pooled O cell to detect presence of unexpected antibody and the result should be mentioned in the master record

It resembles 'O' blood group in the forward grouping but red blood cells additionally show a negative reaction when tested with anti-H lectin. But in reverse grouping agglutinates with 'O' red cells.

Anti 'H' antibody is always present in the plasma of the Bombay group person. 'H' antigen is missing. 'H' antigen is more visible agglutination in the case of 'O', 'A_2' and 'A_2 B' blood group, but 'A_1', 'A_1 B', and 'B' visible weaker agglutination.

'H' Ag strength O > A_2 > A_1 > A_2B > A_1B.

Other antisera like anti-AB, lectines anti-A_1 is used to resolve blood group discrepancy.

UNKNOWN IRREGULAR ANTIBODIES SCREENING TEST OF UNKNOWN SERUM (RECIPIENT/DONOR) BY STANDARD TUBE TECHNIQUE

Principle

The antibody screening test is used in the detection of unexpected antibodies. In this test, the donor's/patient's serum is tested using pooled 'O' red blood cells or the antibody-screening reagent red blood cells. The addition of a potentiating medium (enzyme/albumin) helps to promote the interaction of red blood cells and antibodies allowing antibody/antigen reactions to occur early. Positive reactions (hemolysis or agglutinations) in any tests indicate the presence of an all antibody or autoantibody in the serum.

1. Usually, two volumes of serum and one volume of 2–5% of red blood cells suspension in normal saline are used for optimum reaction (prefer 5% of red blood cells suspension for serological tube testing, whereas an equal volume of serum and 2–3% red cell suspension in LISS is used.
2. If antibodies screening cells shows positive in saline, enzyme, and IAT then further proceed for identification of atypical antibody detection through antibody identification panel cells.
3. Detection and identification of atypical antibodies. Possibility of antibodies present in serum.
   a. Incompatible cross-match
   b. Transfusion reaction
   c. Positive DAT result of the patient following transfusion
   d. Blood group discrepancy due to extra reaction with 'O' cell in reverse grouping
   e. History of multiple transfusion
   f. Pregnancy
   g. Identified alloantibody in the previous transfusion
   h. Rh incompatibility
4. System reacts through saline (IgM): M, N, P1, Le^a and Le^b,
   Enzyme (IgG): Rh system reacts like C, c, D, E, e.
   IAT (IgG): Ss, Kell, Kidd, Jk^a and Jk^b, Fy^a and Fy^b, Le^a and Le^b, and also reacts Rh system like C, c, D, E, e.

- Antibody screening (IgM) by 5% of 'O' blood group pooled red blood cells suspension + serum tubes are kept for 45 minutes at room temperature (22°C), centrifuge 1000 rpm post-incubation for one minute and observed for agglutination. It is used for detecting irregular unknown antibodies in the saline phase (IgM). Also, follow the instruction of the blood centre SOP.

- If serum + 'O' blood group pooled red blood cells suspension tubes show agglutination, then the Bombay group should be ruled out by testing for 'H' antigen with anti-H lectin.
- Used one special tube of 5% of 'O' pooled red blood cells suspension (+ papain cysteine enzyme if available) for detection of irregular unknown antibody (IgG).
- Take one drop (or 50 µl) of papain cysteine enzyme before adding 5% of 'O' +ve pooled red blood cells suspension in the serum tube and mix the tube gently.
- **Antibody screening (IgG):** Incubate the tube having serum + 5% of 'O' pooled red blood cells suspension + papain cysteine enzyme at 37°C for 45 minutes, centrifuge at 1000 rpm for one minute and observe the agglutination.
  - If agglutination is present, allo-antibody is suspected.
  - If the antibody specificity is in the Rh, Kidd, lewis blood group system, there is an enhanced reaction using the papain cysteine enzyme.
  - Certain red blood cell antigens including M, N, S, Xg^a and Fy^a and Fy^b are denatured (destroyed) when exposed to proteolytic enzymes.
- Antibody screening (IgG) serum +5% of 'O' pooled red blood cells suspension incubate at 37°C for one hour, centrifuge at 1000 rpm for one minute, and observe the agglutination.
  - If there is no agglutination, then it has to be taken ahead for IAT.
  - Wash the content thrice with normal saline and decant the supernatant completely and then add AHG.
  - Observe for agglutination. If agglutination is present, alloantibody is suspected.
  - **Temperature:** IgM antibodies react optimally at or below 22°C where as IgG reacts best at 37°C.
- **Use of autocontrol:** Autocontrol means incubation of the patients own cells and serum. Self-serum and self 2–5% wash red blood cells suspension is taken (prefer 5% of red blood cells suspension for serological tube testing).

Autocontrol is tested to rule out the presence of autoantibody detection and group discrepancies. Autocontrol is tested at three different temperatures, i.e. 4°C, 22°C, and 37°C to know the thermal activity of the autoantibody. If agglutination is detected at 37°C, it is a warm antibody. If agglutination is detected at 4°C, it is a cold antibody. The mixed antibody is reactive at both 4°C and 37°C (two drops of patient self-serum and one drop of self 5% red blood cells' suspension) in the precipitin tube, mixed well, incubate as per blood centre SOP and centrifuged at 1000 rpm for one minute and observe the agglutination.

Result

- If there is no agglutination, then autoantibody is absent.
- If agglutination is present then possibility of autoantibody is there and further DAT and IAT tests are done for the detection of incomplete antibody. If DAT strength (gradation of agglutination/clumps) is more than IAT, it is most suggestive of autoantibody.
- If IAT strength (gradation of agglutination/clumps) is more than DAT, alloantibody is suspected, for which antibody screening and identification is required.
- In the case of autoantibody, underlying alloantibody should be ruled out by autologous or allogenic adsorption test.

- Autocontrol is positive with serum consisting of antibodies against preservative (anti-LISS).

Figure 5.2 shows wooden rack with precipitin tubes or standard tubes.

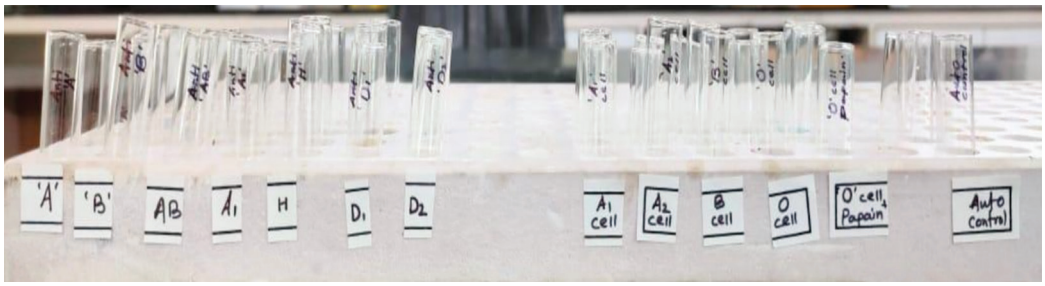

Fig. 5.2: Wooden rack with precipitin tubes or standard tubes