Blood Group and Crossmatch: Issues and Troubleshoots

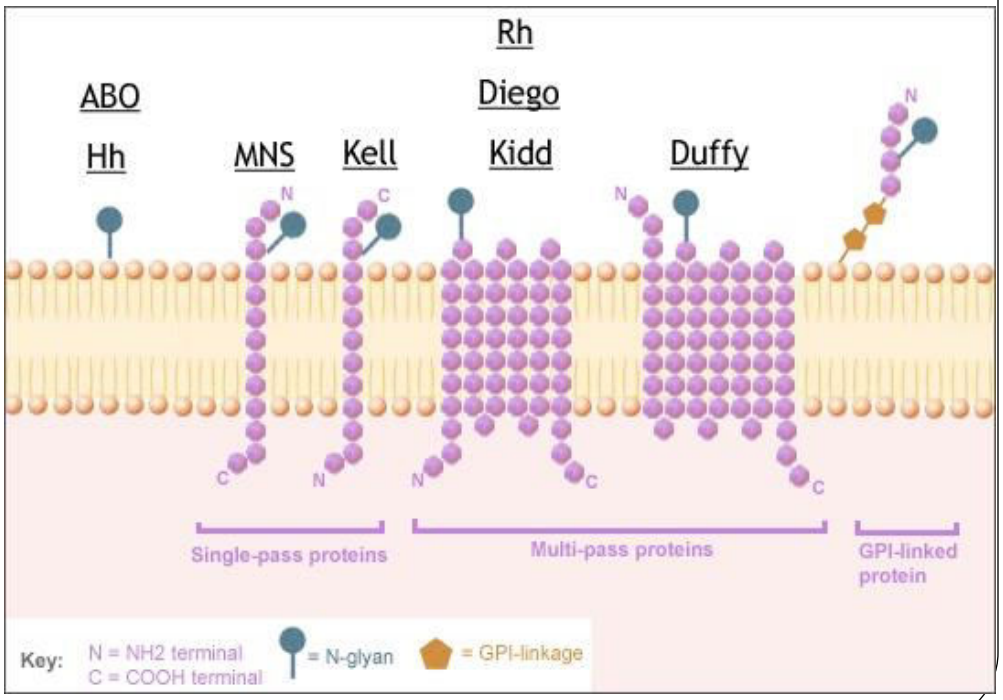
Dr Priti Desai
Associate Professor
Dept of Transfusion Medicine
Tata Memorial Hospital
Mumbai

Introduction:
Blood Group Systems

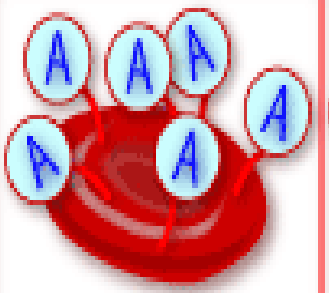
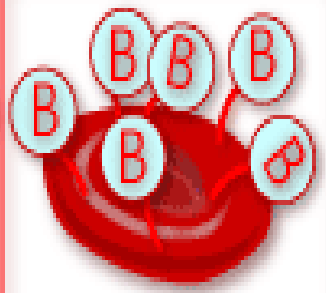
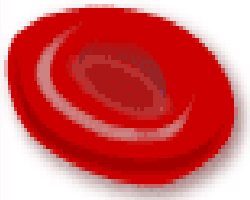
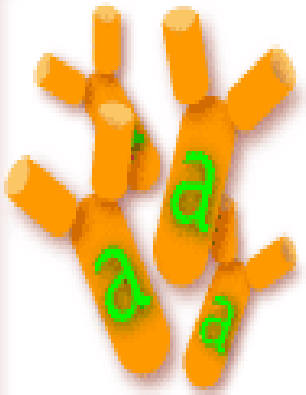
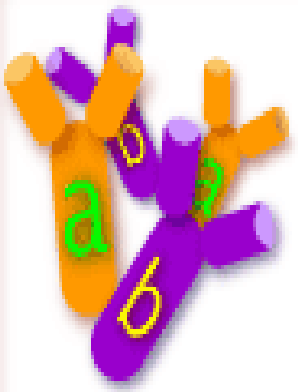
- Karl Landsteiner discovered ABO system - in 1900
- ABO system remains the most significant system till date
- Rh is 2nd most important system after ABO
  - Discovered in 1940

Introduction : Blood Group System

- Blood group antigens are on RBC
- 35 blood group system known
- ABO & Rh most important
- Others are
  - Kell,
  - Duffy,
  - Kidd,
  - P,
  - MNS etc



The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

Laboratory Determination of the ABO system

Laboratory testing for ABO

- Detection of Antigen on Red cell surface

Cell grouping

- Red cells with unknown antigen tested with known antisera
- Using commercial reagents
  - Anti-A (Blue)
  - Anti-B (Yellow)

- Detection of Antibodies in plasma

Serum grouping

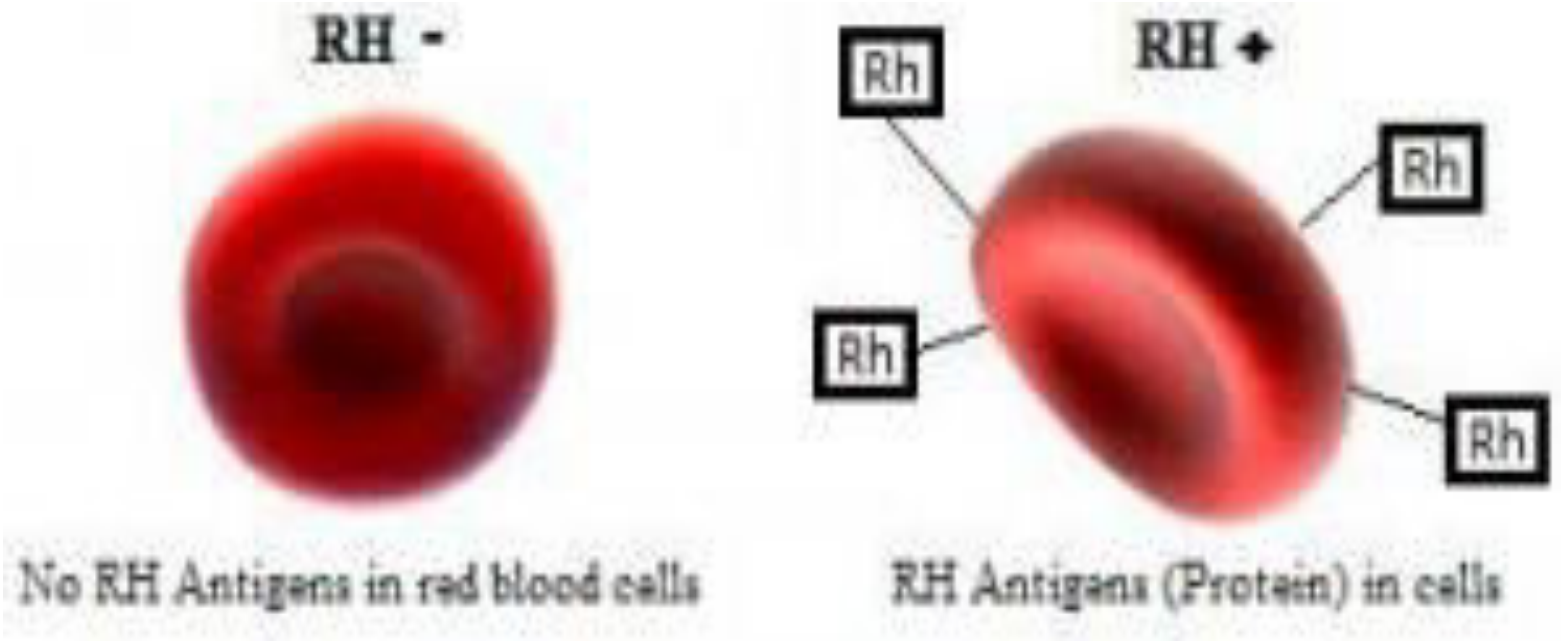
- Serum with unknown antibodies tested with known antigens
- Using reagent red cells
  - A cells
  - B cells

Reaction pattern of ABO group (Cell grouping & Serum grouping)

Red cells tested with			Serum tested with			Interpretation
Anti -A	Anti -B		A cells	B cells	O cells	
4 +	0		0	4 +	0	A
0	4 +		4 +	0	0	B
4 +	4 +		0	0	0	AB
0	0		4 +	4 +	0	O

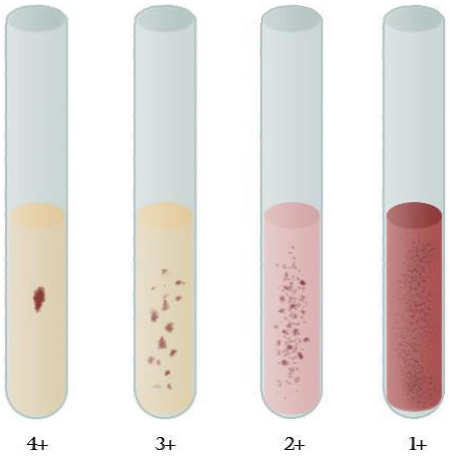
Laboratory testing for Rh

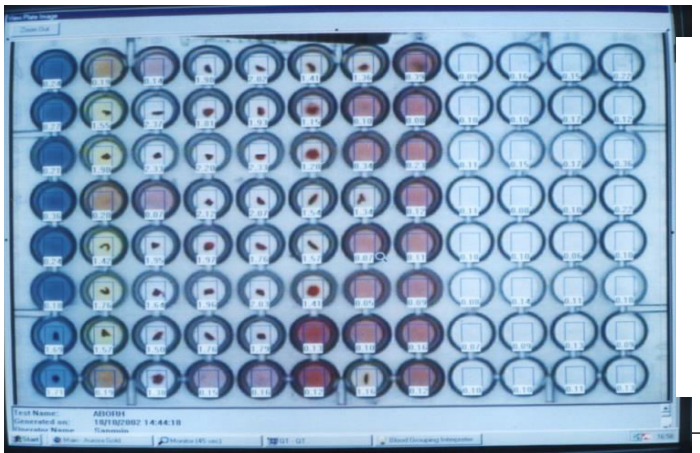
- D antigen is most immunogenic
- Routine testing for D antigen
- Using commercial Antisera (Anti-D)
  - Rh Positive
  - Rh Negative

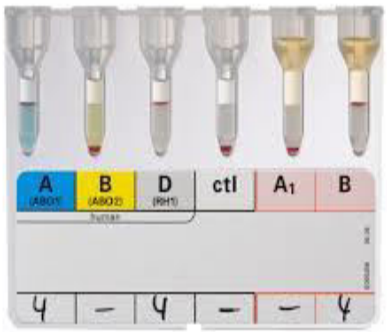


Techniques

- Tube technique
- Microplate technique
- Column agglutination technique







Issues and Troubleshoots in Routine Blood Grouping

Issues in Blood Grouping

- Blood Grouping
  - ABO grouping problems
    - Discrepancy in Cell and Serum Grouping
  - Rh grouping problems
    - Weak D/Partial D

ABO Grouping problems

- When cell and serum grouping do not match
- Which one is right ??
- Interpretation ??
- Such situations are called discrepancy

Important to note :

- Discrepant results must be recorded
- Interpretation must be delayed till discrepancy resolved
  - If donor sample : do not release the unit
  - If patient sample : If transfusion is urgent group O unit may be issued

Discrepancy in ABO grouping

- Cell grouping
  - Weak or missing red cell reactivity
  - Extra red cell antigen reactivity

- Serum grouping
  - Weak or missing reactivity
  - Extra reactivity

Interesting problem cases:

- 21 yr /F , Clinical diagnosis AML, M1
- Blood group results

Anti-A	Anti-B	Ac	Bc	Interpretation
0 to 1+	0	0	4+	? A ? Subgroup of A

- Cell grp- weak reaction for A ag
- Serum grouping- A group
- Possibilities :
  - Subgroup of A
  - Weakening of A ag due to disease

- To resolve:
  - ✓ Previous bld grp report if kn
  - ✓ Detail clinical history
  - ✓ Special techniques

Interesting problem cases:

- 2 months /M, posted for Surgery on next day
- Blood group results

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	4+	0	0	0	? B ?AB

- Cell grp- B
- Serum grouping- AB
- Possibilities:
  - Weak antibodies
    - ✓ Newborn:
    - ✓ Old age:
    - ✓ Hypogammaglobulemia

To resolve:

- ✓ Check age of the pt
- ✓ Clinical diagnosis
- ✓ Modification of techniques –extended incubation, alter cell serum ratio etc

Interesting problem cases:

- F/43, T cell lymphoma, Hb 5.6
- Blood group results

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	3+	3+	3+	3 +	? Irregular Antibodies

- Alloantibodies
- Autoantibodies
- Others : abnormal proteins, fibrin clot, recent infusion of immunoglobulins etc

To resolve:

- ✓ Alloantibodies: identification by using reagent red cell panel
- ✓ Auto antibodies: test at different temperature, prewarm technique,
- ✓ Abnormal high proteins: alter cell to serum ratio

Interesting problem cases:

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
4+	1+	0	4+	0	? A ? AB

Cell Typing : Extra Reactivity

Possibilities:

- Antibody coated red cells: AIHA
- Acquired B phenotype : associated with gram negative bacterial infection, Ca colon etc
- False positive due to contamination of reagents

Resolve by

- ✓ Check clinical diagnosis
- ✓ AIHA: clinical history, lab Ix, DAT, IAT,
- ✓ Use different batch of reagent if contamination is suspected

Interesting problem cases:

- 10 yr M, case of NHL, Hb 7.0, on chemotherapy
- Blood group results

Anti-A	Anti-B	Ac	Bc	Oc	Interpretation
0	0	3+	3+	3 +	? O ? Bombay

To confirm:

1. Test with Anti-H
2. Test with various batches of anti-A, anti-B, anti-AB, anti-H
3. Family study
4. Secretor status

Bombay Phenotype (Oh)

- Discovered in Bombay by Bhende et al in 1952
- Absence of A, B and H antigen
- Presence of anti-A, anti-B & anti-H
- Should be transfused only with Bombay blood group

Rh typing problems

- All Rh negative samples are tested for weak D
- Weak D :
  - extended incubation and test with AHG
- Partial D
- Significance in donor and patient

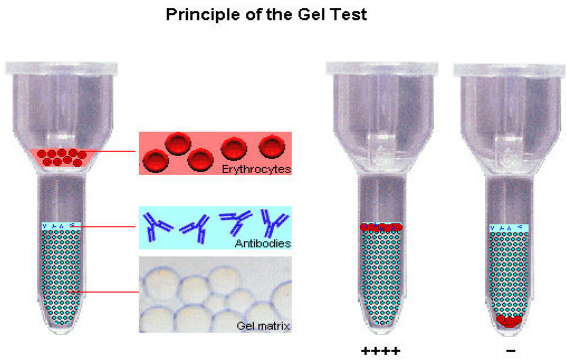
Compatibility testing

Compatibility testing

- Set of procedures required before blood can be issued
- To make sure that there are no antibodies present in patient serum which react with donor red cells
- This is the final check on compatibility between donor & recipient
- It includes:
  - ABO & Rh grouping of Patient & Donor
  - Screening for irregular antibodies
  - Cross-matching

Techniques for compatibility

- Routine procedure
  - Saline RT & 37^0 C
  - Antiglobulin test 37^0 C
- Method
  - Test tube
  - Column agglutination



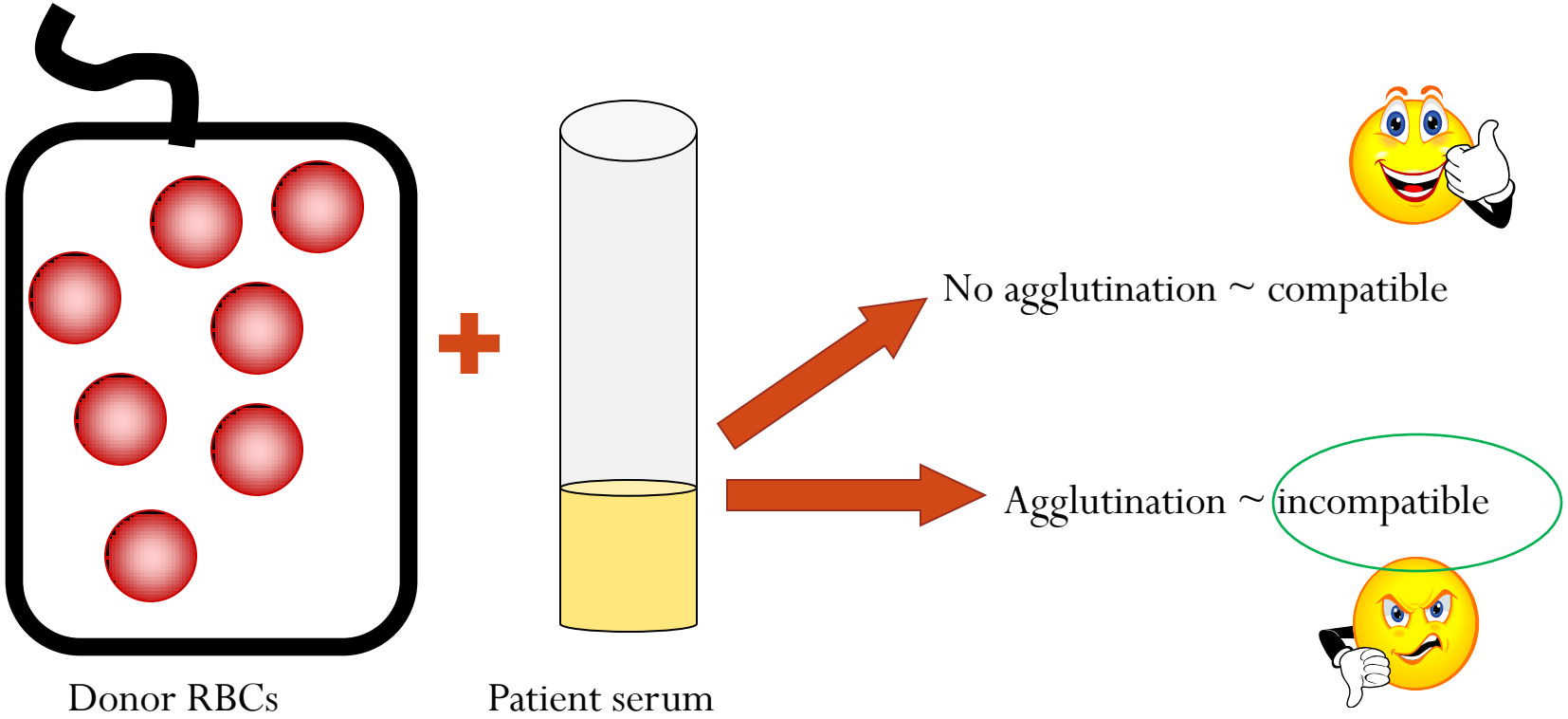
Testing of patient sample

- Verification of previous result
- If discrepancy – obtain new sample
- ABO grouping – most critical step
- Rh typing – most critical step

Issues related to Compatibility testing

Crossmatch

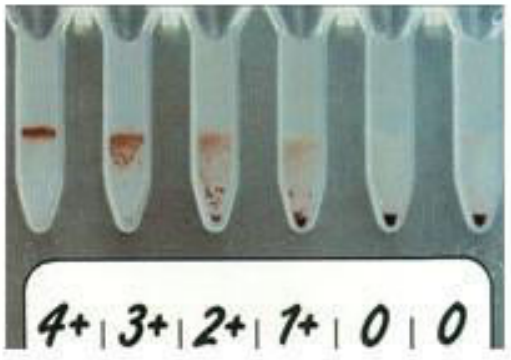
- Primary objective of crossmatch is to detect presence of antibodies in recipient's serum, which could destroy the donor red cells



Resolving incompatibilities

Causes of positive crossmatch results are

- ✓ Incorrect ABO grouping of patient or donor
- ✓ Presence of alloantibodies in patient's serum
- ✓ Presence of autoantibodies
- ✓ Abnormalities in patient serum
- ✓ Prior coating of donor red cells
- ✓ Contaminants in the test system



Incorrect grouping of patient or donor

- Due to procedural error
- Sampling error
- Repeat the blood grouping on patient and donor sample
- If require ask for new sample and also check blood group in previous record

Presence of Alloantibodies

- Antibody screening positive
- Incompatible with many donor unit
- Detail clinical history
- DAT, IAT and autocontrol
- Antibody identification
- Find out antigen negative unit

Presence of autoantibody

- Autocontrol positive
- Test at different temperature
  - (RT, 37^0c, 4^0c)
- DAT, IAT
- Titre of antibody
- Auto adsorption- to remove the autoantibodies- perform compatibility

Abnormalities in patient's serum

- Altered A/G ratio in certain disease condition-may cause RBCs to stick together giving appearance of stacks of coins- Rouleaux formation
- Mimic agglutination
- Resolved by saline replacement procedure
- High molecular weight dextrans, plasma expanders may give false positive results

Key points

- ✓ Follow standard procedures & manufacturer's instruction
- ✓ Use appropriate equipment and reagents
- ✓ If there is discrepancy
- ✓ Repeat test on same sample
- ✓ Still it persists
- ✓ Obtain clinical diagnosis, previous bld grp report, transfusion history, medication
- ✓ Obtain fresh sample
- ✓ Review results of allo or auto antibodies

Thank You !!!