

Blood Group and Crossmatch: Issues and Troubleshoots

Dr. S.B.Rajadhyaksha

Professor and Head

Dept of Transfusion Medicine

Tata Memorial Hospital, Mumbai

Introduction: Blood Group Systems

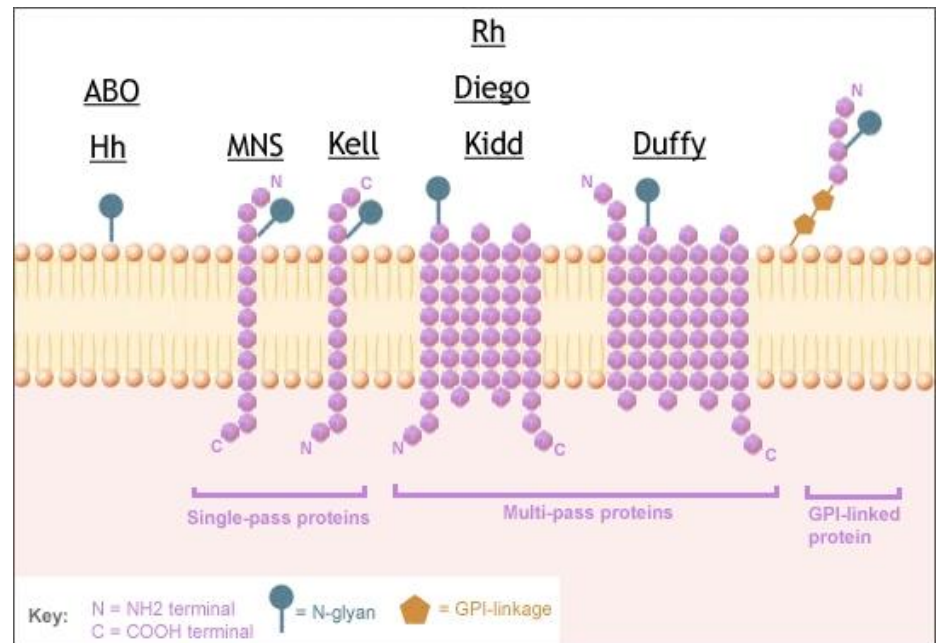
- Karl Landsteiner discovered ABO system - in 1900
- ABO system - most significant system
- Rh is 2nd most important system after ABO
 - Discovered in 1940







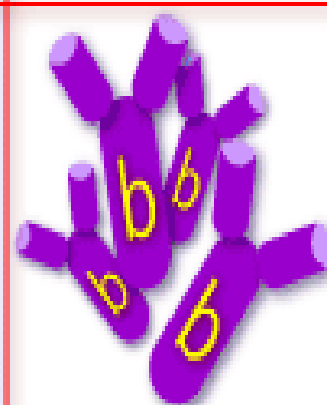
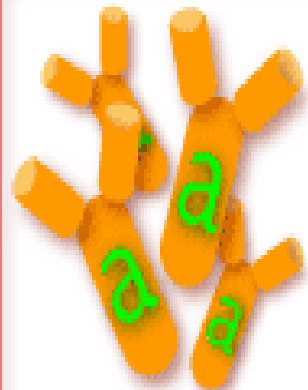

Introduction:

Blood Group Systems

- Blood group antigens are on RBC
- 29 blood group systems known
- **ABO & Rh are most important**
- Others:
 - ❖ 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)	 <p>A agglutinogens only</p>	 <p>B agglutinogens only</p>	 <p>A and B agglutinogens</p>	 <p>No agglutinogens</p>
Plasma Antibodies (phenotype)	 <p>b agglutinin only</p>	 <p>a agglutinin only</p>	<p>NONE.</p> <p>No agglutinin</p>	 <p>a and b agglutinin</p>

Laboratory Determination of the ABO system

Laboratory testing for ABO

- Detection of Ag on Red cell surface

Cell grouping



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

- Detection of Ab in plasma

Serum grouping

- Serum with unknown Ab tested with known Ag
- Using reagent red cells
 - A cells
 - B cells

Reaction pattern of ABO group (Cell grouping & Serum grouping) - "Type and Screen"

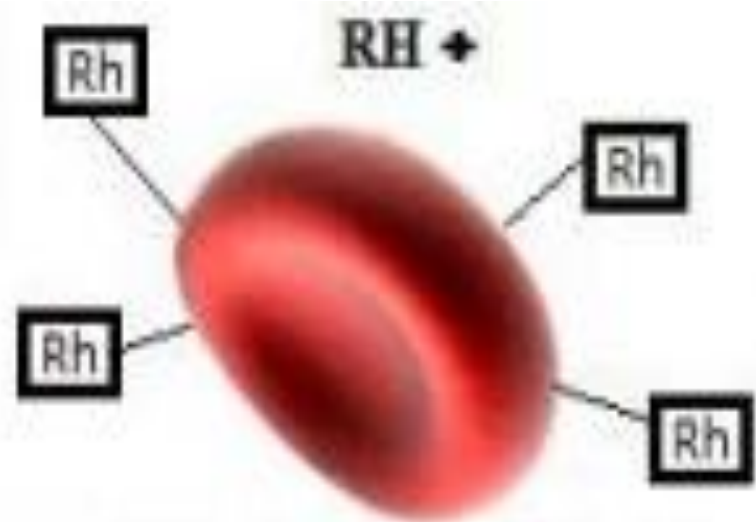
Red cells tested with		Serum tested with			Interpretation (Blood Group)
Anti -A	Anti -B	A cells	B cells	O cells	
4 +	0	0	4 +	0	A
0	4 +	4 +	0	0	B
4 +	4 +	0	0	0	AB
0	0	4 +	4 +	0	O

Laboratory testing for Rh

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

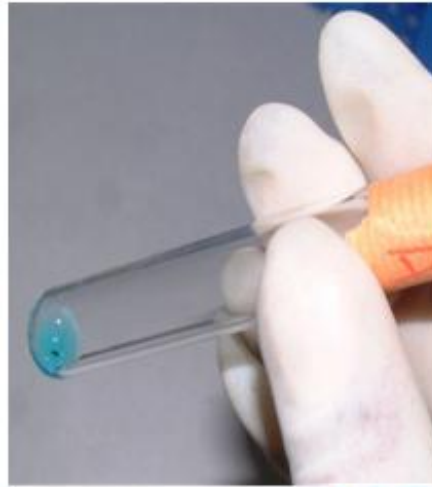


No RH Antigens in red blood cells



RH Antigens (Protein) in cells

Machine or Manually



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 it is called DISCREPANCY
- Which one is right ?? Interpretation ??
- French hospitals- Discrepancy 1:3400 blood groupings
(*Transfusion 2004*)

Important to note :

- Discrepant results must be recorded
- Interpretation held back until discrepancy resolved
 - If donor sample : do not release the unit
 - If patient sample : Transfuse group O unit, if urgent

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:

- F/21, 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
 - Chimerism
(O group transfusions to A)
- To resolve:
 - ✓ Previous blood group report if known
 - ✓ Detailed clinical history
 - ✓ Using appropriate lectins
 - anti -A1 lectin (*Dolichos biflorus*)
 - anti-B lectin (*Bandeiraea simplicifolia*)
 - ✓ Special techniques: salivary grouping

Interesting problem cases:

Anti -A	Anti -B	Anti -AB	Anti -A ₁	Ac	Bc	Oc	Interpretatio n
2+	4+	4+	0	1+	0	0	? AB ? B

Interesting problem cases:

Anti -A	Anti -B	Anti -AB	Anti -A ₁	Ac	Bc	Oc	Interpretation
2+	4+	4+	0	1+	0	0	? AB ? B

A₂B with anti-A₁

Anti-A₁ occurs in:

- 8% of A₂
- 22 to 35% of A₂B individuals

Interesting problem cases:

- M Ch/ 2 months, posted for surgery next day
- Blood grouping 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:

- Acquired B phenotype : associated with gram negative bacterial infection (P.mirabilis), Ca colon
- Antibody coated red cells: AIHA
- 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
- ✓ Acidifying B reagent (pH 6.0)

Interesting problem cases:

- M/10, case of NHL, Hb 7.0 g/dl, 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 lectin *(*Ulex europeus*)
2. Test with various batches of anti-A, anti-B, anti-AB, anti-H
3. Family studies
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

RESOLVING ABO DISCREPANCIES

-A	-B	Ac	Bc	Oc
3+	0	0	w	0

Unexpected Negative

-A	-B	Ac	Bc	Oc
w	w	0	0	0

Serum

Red Cells

Repeat Testing with following modifications

- Include Auto- control
- ↑ Serum:cell ratio
- ↑ Incubation time,
- ↓ Incubation Temp

- ↓ Incubation Temp
- ↑ Incubation time,
- Use different Anti-sera
- Use of Lectins

possibilities

confirmation

possibilities

Age
Diagnosis
Medication

Resolved

Subgroups
↓ antigen expression
Advanced age
Diagnosis

RESOLVING ABO DISCREPANCIES

-A	-B	Ac	Bc	Oc
3+	0	0	4+	3+

Unexpected Positive

-A	-B	Ac	Bc	Oc
4+	1+	0	4+	0

Serum

Red Cells

Repeat Testing with following modifications

- Include Auto- control
- antibody screen (AbS)

Auto - pos

Auto - neg

Auto - neg

AbS - pos

AbS -neg

AbS -pos

Saline

Replacement

- Subgroup with anti A1
- Low incidence Ab

Allo-antibody

Positive

Negative

Auto-antibody

Rouleaux

- Different antisera
- Transfusion history
- Diagnosis

Mix Field

Chimerism
Recent Tx
BMT

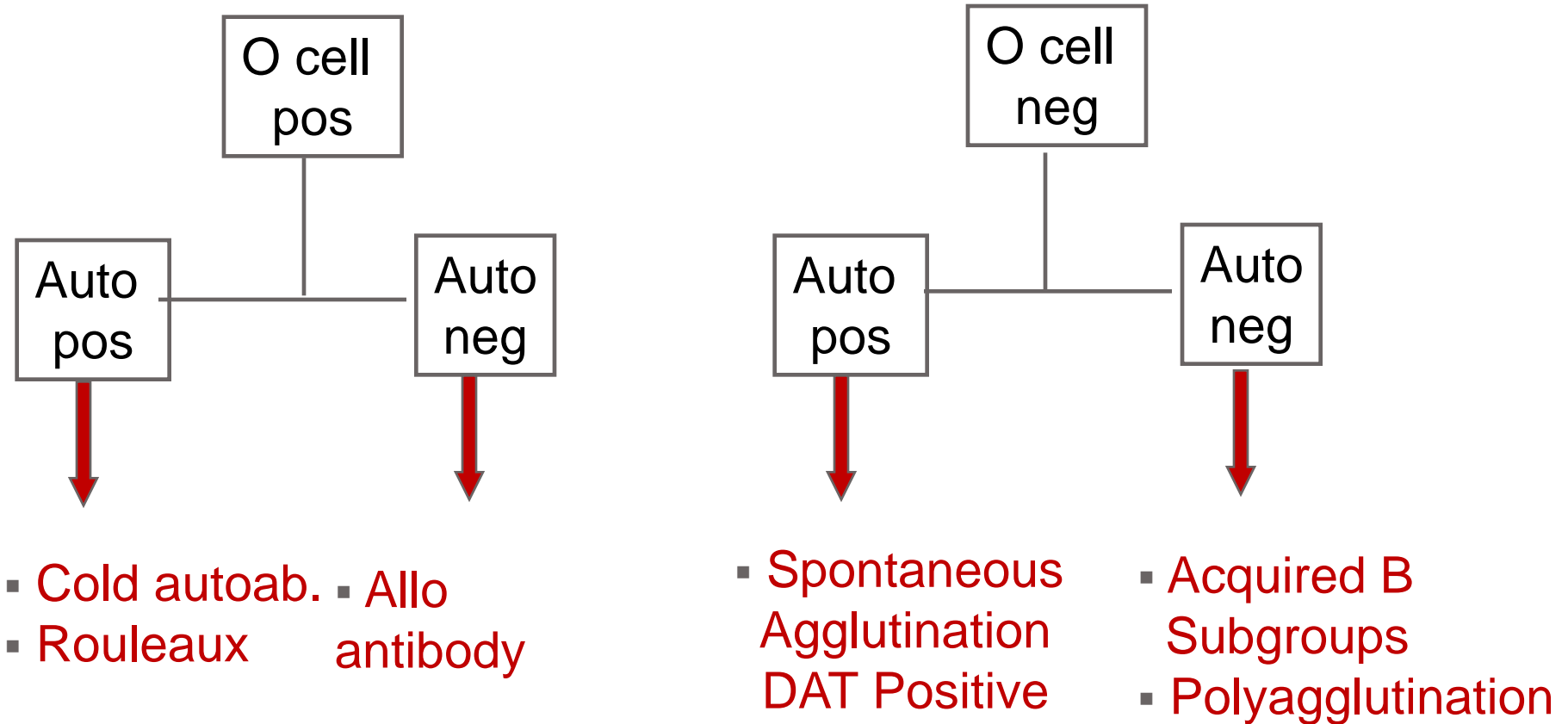
DAT

Positive
AIHA

Polyagg

use lectins

RESOLVING ABO DISCREPANCIES



Compatibility Testing
or
Pretransfusion Testing

Compatibility testing

- Set of procedures required before blood can be issued
- To make sure that there are no Ab 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° C
 - Antiglobulin test 37° C

Phase	Purpose	Detects
Saline	IgM; cold agglutinins	ABO incompatibilities
37°C, Enzyme	IgG Antibodies	Potent Rh Antibodies
AHG	IgG Antibodies	Rh, Duffy, Kidd, others

Use of Proteolytic Enzymes (Papain)

activity enhanced
activity suppressed

Rh, Kidd, Kell
Duffy, MNS

Testing of patient sample

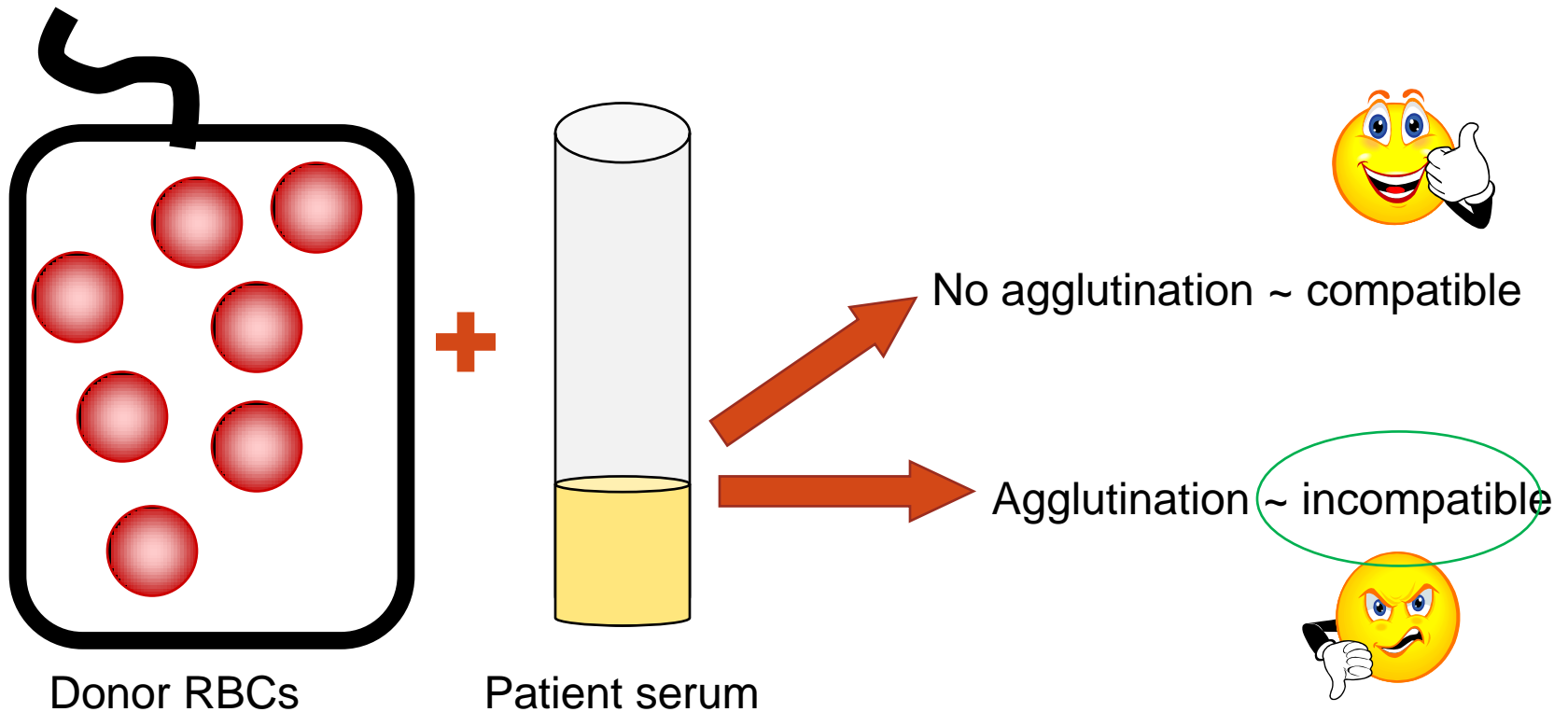
- Verification of previous result
- If discrepancy - obtain a new sample
- ABO grouping - most critical step
- Rh typing - most critical step

Issues related to Compatibility testing

Crossmatch

- Primary objective:

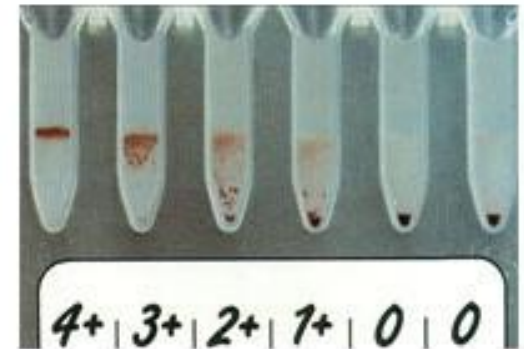
Detect presence of antibodies in recipient's serum, which could destroy the donor RBCs



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 grouping on patient and donor samples
- If required ask for a new sample and also check previous record of Blood Group

Presence of Alloantibodies

- Antibody screening positive
- Incompatible with many donor units

- Detailed clinical history
- DAT, IAT and autocontrol
- Antibody identification
- Find out antigen negative unit

Presence of autoantibody

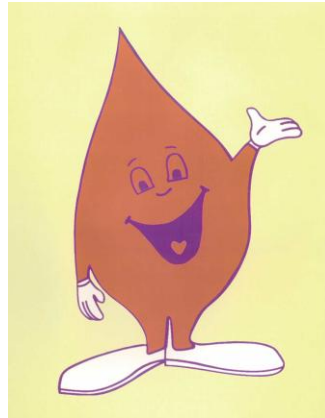
- Autocontrol positive
- Test at different temperatures (RT, 37°C, 4°C)
- DAT, IAT
- Titre of antibody
- Auto adsorption- to remove autoantibodies to perform crossmatching

Abnormalities in patient's serum

- Altered A/G ratio in certain disease conditions 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 instructions
- ✓ Use appropriate equipment and reagents
- ✓ If discrepancy
 - Repeat tests on same sample
 - If still persists
 - Obtain fresh sample
 - Previous blood group report
 - Obtain clinical diagnosis
 - History of transfusions, medication
 - Review results of allo or autoantibodies



THANK YOU

and Best wishes