

# **Division of Blood Transfusion Services**

**Ministry of Health and Family Welfare**



# Antiglobulin Test



# Teaching Aims

- To understand the principle of AGT
- To learn the techniques of AGT
- To know about the AHG reagent



# Antihuman Globulin (AHG)

- Antihuman: antibodies against human antigens
- Globulin: all antibody molecules are globulins
- Antihuman Globulin is antibody directed against the Fc portion of human antibodies and/or complement components.



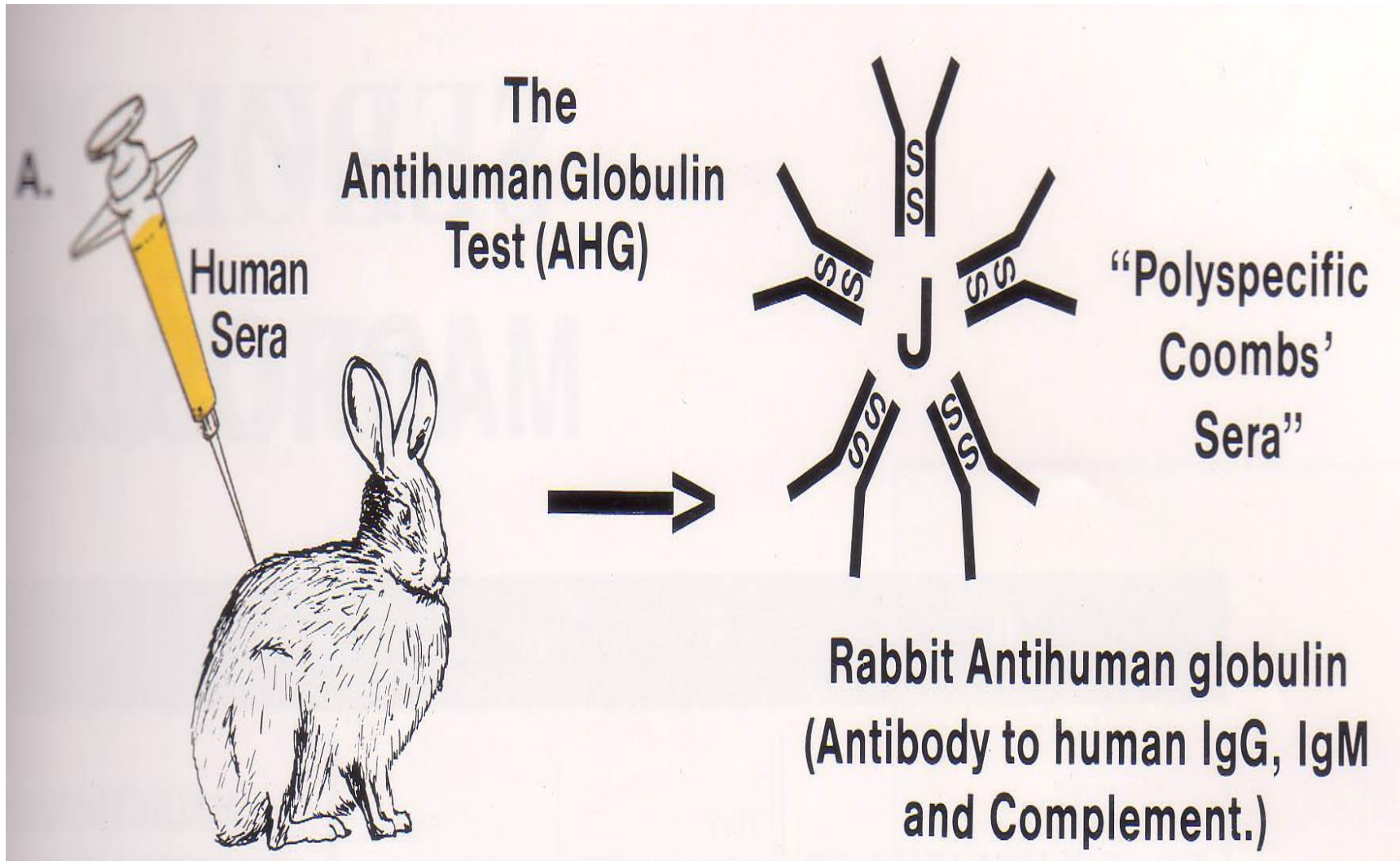
# Anti-globulin Test

- Introduction by Coombs in 1945
- Detects incomplete antibodies
- **Principle of AGT**
  - Antibodies and complement components are globulins.
  - Animals injected with human globulins produce anti-human globulin (AHG).
  - AHG forms bridges between antibody coated red cells.

# Antihuman Globulin Reagents

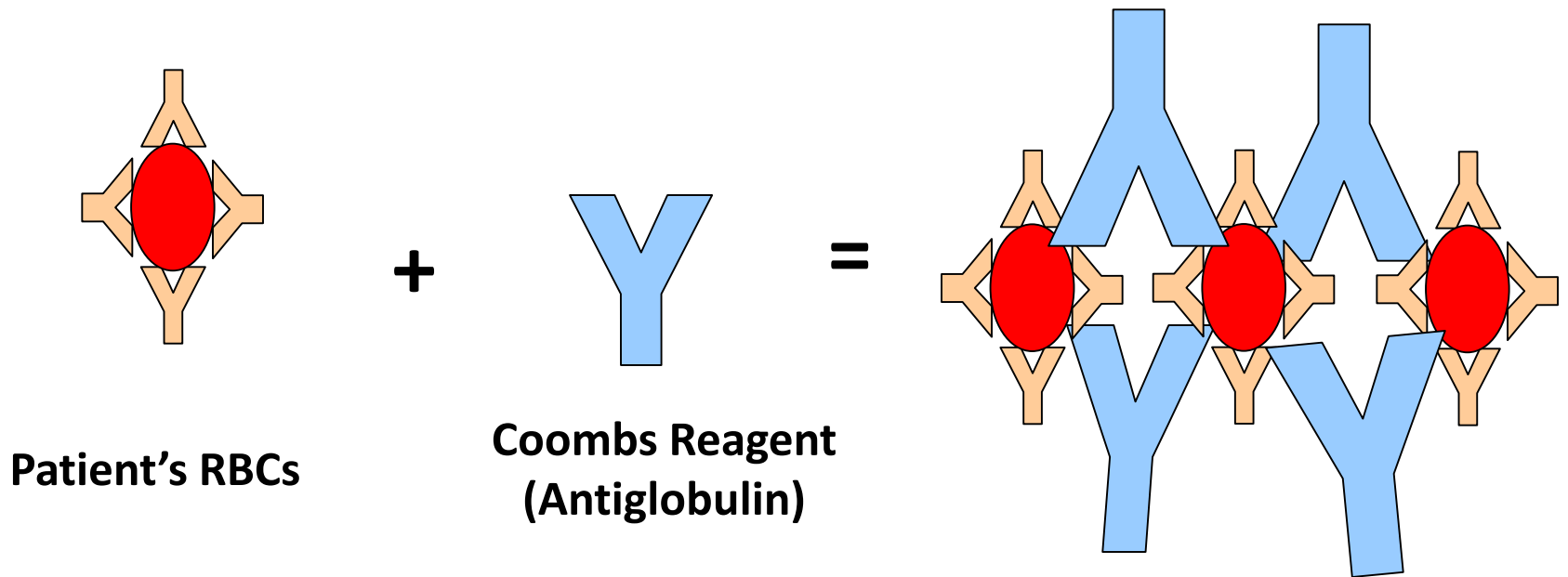
- **Polyspecific**
  - Contains both, anti-IgG and anti-C3d (complement)
- **Monospecific**
  - Contains only one specificity, either anti-IgG or anti-C3d

# Preparation of AHG reagent



# Direct Antiglobulin Test

**Detects in vivo sensitization of red cells**





# Method of DAT

Wash test red cells 3-6 times with saline



Decant supernatant saline from the last wash



Make 5% suspension of washed red cells



Add one drop of 5% washed cells to a labeled tube



Add one drop of AHG reagent



Mix well and centrifuge at 1000 RPM x 1 min



Note the results

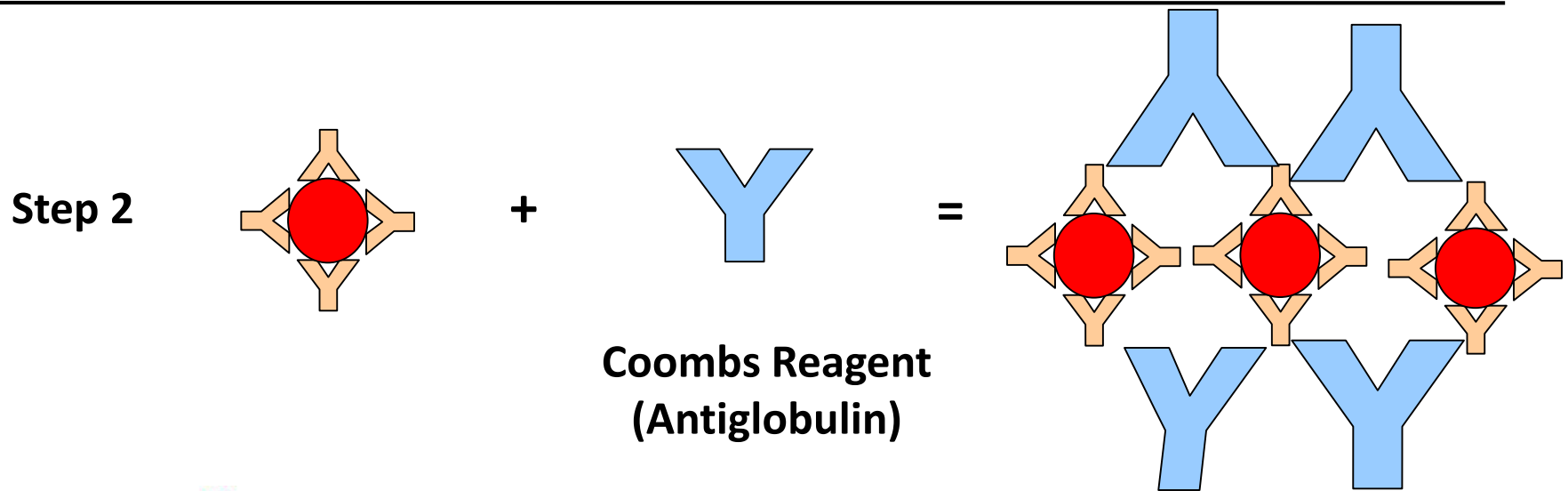
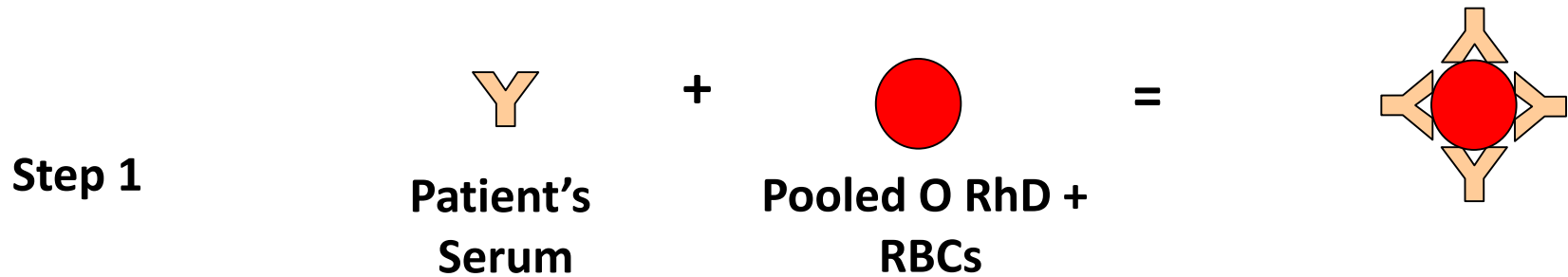
# Applications of DAT

- Diagnosis of HDN in newborn
- Diagnosis of autoimmune hemolytic anemia
- Diagnosis of drug induced immune hemolytic anemia
- Investigation of hemolytic transfusion reaction



# Indirect Antiglobulin Test

Detects free antibodies in the serum



# Method of IAT

Add 2 drop of test serum to a test tube



Add 1 drop of 5% suspension of pooled O + red cells



Incubate at 37°C for 60 minutes



Wash the red cells 3-6 times with normal saline



Add 1 drop of AHG reagent to red cell button



Look for agglutination



Negative results should be confirmed  
by adding 1 drop of check cells

# Applications of IAT

- Detection and identification of unexpected antibodies in the serum
- Cross matching
- Typing of minor red cell antigens such as Duffy, Kell, Kidd
- Detection of weak D (earlier Du test)
- Titration of antibodies
  - Anti-D in maternal serum in HDN

# Factors affecting AGT

- Temperature - 37°C
- Ratio of serum to cells – 2:1
- Incubation time- 60 min
- Reaction medium –Saline/ LISS
- Washing of cells – 3 to 6 times
- Addition of AHG reagent – remember to add
- Centrifugation for results – speed & time
- Quality of AHG reagent – QC of reagent

# Sources of errors: false negative results in AGT

- Inadequate washing of red cells.
- Test is interrupted or delayed.
- Problems with AHG reagent
  - Bacterial contamination of reagent
  - Improper storage.
  - Failure to add AHG reagent.
  - Decreased reactivity of AHG reagent.

# Sources of errors: false positive results in AGT

- Autoagglutination or polyagglutination of red blood cells.
- Improper washing of glassware.
- Over centrifugation.
- Presence of other antibodies in the AHG reagent.
- Contaminated reagents
- Saline contaminated by heavy metals or colloidal silica.





# Preparation of Check Cells

- Perform doubling dilution of commercially available monoclonal anti-D (IgG)
- Select the highest dilution which gives +2 reaction with ORhD positive red cells
- For eg. For dilution of 1:64, add 630  $\mu$ L of NS to 10  $\mu$ L of undiluted anti-D
- Take one volume of diluted anti-D to which add equal vol of pooled O RhD positive red cells

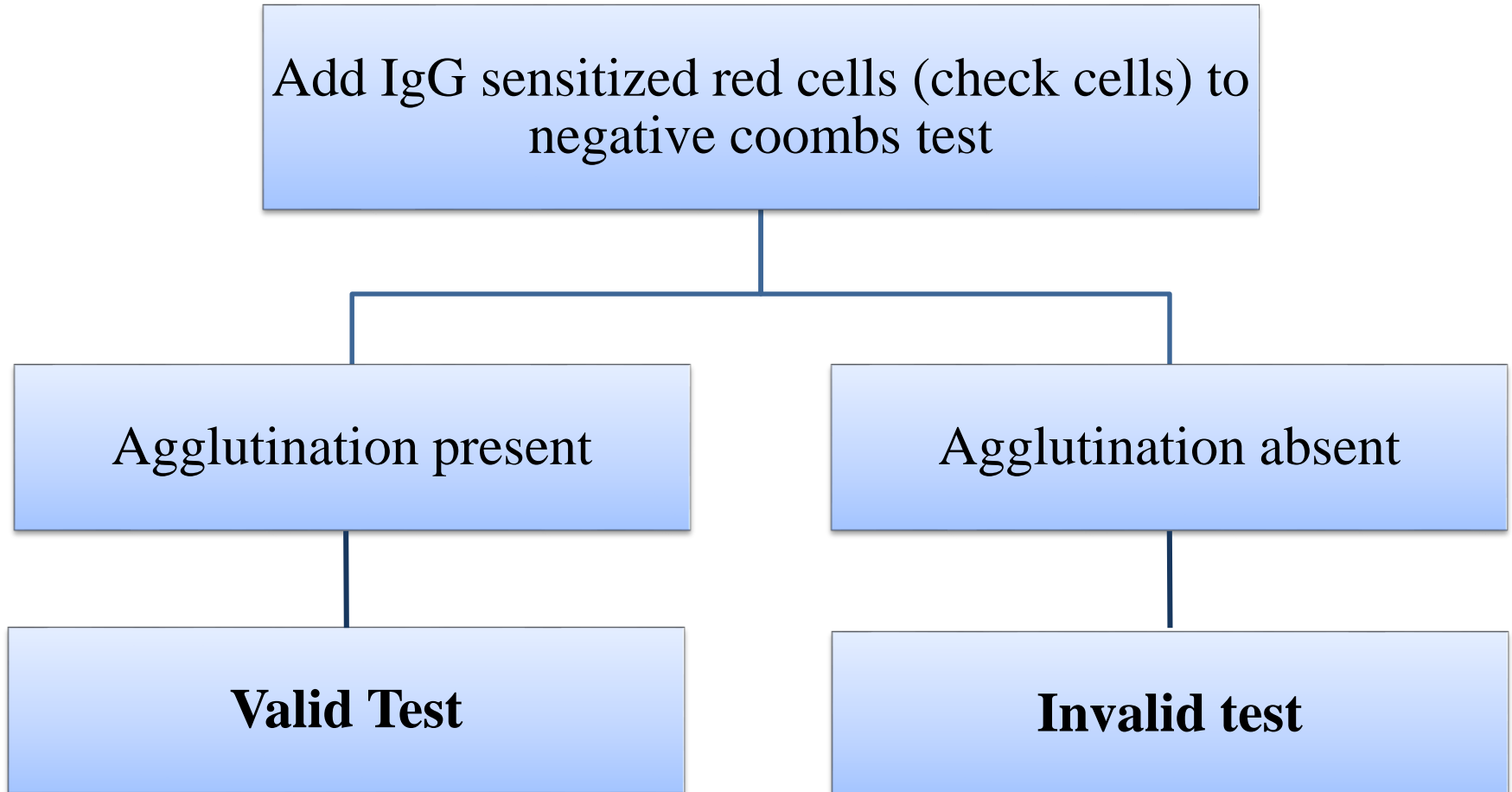


## Preparation of Check Cells (contd...)

- Incubate at 37<sup>0</sup>C for 45 min
- Wash 3 times with NS
- Add 1 drop of AHG to 1 drop of 5% suspension of washed red cells
- Check cells can be stored in Alsever's solution for 1 week at 4<sup>0</sup>C



# Validation of negative AGT



## Points to remember...

- a) Even 10 mL of AHG can be neutralized by a tiny amount of free serum protein.
- b) A technologist can forget to add AHG to a test tube.
- c) A couple of drops of residual saline can dilute the AHG reagent below detectable levels.
- d) Normally people do **NOT** produce unexpected antibodies. Therefore the test should normally be negative.



# What are reagent red cell panels?

- Red cell suspensions used in tests employing the principles of hemagglutination and hemolysis for the detection and identification of blood group antibodies.

Commercial

- **Sources**
  - In-house
    - Regular donors
    - Staff members



# Applications of Reagent Red Cells

- Reverse ABO grouping
- Antibody screening
- Antibody identification
- Antibody titration
- Allogenic adsorption
- Control of AHG technique



# Reagent cells for antibody screen of donors

- Pooled reagent screening cells used only for testing samples from blood donors
- Group O red cells
  - Naturally occurring anti-A or anti-B do not interfere with detection of unexpected antibodies
- As a minimum the following antigens should be expressed:  
D; C; c; E; e; K.



# Reagent cells for antibody screen of patients

- Un-pooled cells from a minimum of two donors must be used
- One reagent red cell should be  $R_2R_2$ ; other  $R_1R_1$ .
- Must express  $K;k;Fy^a;Fy^b;Jk^a;Jk^b;S;s;M;N;P_1;Le^a$  and  $Le^b$ .
- Homozygous expression of some of these antigens is essential for reliable detection of weak antibodies.





# Antibody Screening

- An anti-gram listing the antigen makeup of each cell provided with each lot of screening cells issued from a manufacture.
- Lot number on the screening cells must match with the lot number printed on the anti-gram because antigen make up will vary with each lot.
- The “ideal” screening cells have red cells with homozygous expression of as many antigens as possible.



## Three cell screening panel

	Rh					Kell		Duffy		Kidd		Lewis		P	MNS			
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	p	M	N	S	s
I	+	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+
II	+	0	+	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+
III	0	0	0	+	+	0	+	+	0	+	0	0	+	+	+	+	+	0

All circled antigens are in homozygous state

# Antibody Screening in Test Tubes

Take 3 test tubes and mark them as 1,2 and 3



Add 2 drop test serum in all tubes



Add 1 drop of screening cells in respective tubes



Add 2 drops of LISS to test tubes



Incubate at 37°C for 15 min- 30 min



Centrifuge at 1000 rpm for 1 min.



Wash cells 3 times with saline



Add 2 drops of AHG reagent



Centrifuge at 1000 rpm for 1 min.



Examine for Agglutination

# Antibody identification

What is the importance of identification of antibody?

- To determine specificity of antibody
- To provide antigen negative blood for transfusion
- To determine clinical significance of antibody
- To investigate hemolytic disease of newborn
- To investigate transfusion reactions



# Potentiators

- Used in antibody screening and identification to enhance antigen-antibody reaction
- Low-ionic strength solution (LISS)
- Bovine serum albumin (BSA)
- Polyethylene glycol (PEG)
- Proteolytic enzymes
  - Papain
  - Ficin
  - Bromelin



# Potentiators in Red Cell Serology

Potentiator	Action	Detects
22% albumin	Reduces zeta potential between red cells	IgG antibodies
LISS	Low ionic strength environment increases antibody uptake by red cells	IgG antibodies
Enzymes	Destroys some red cell antigens and enhances other red cell antigens	Destroys Fya, Fyb, MNS. Enhances Rh, Kidd, P, Lewis antibodies
PEG	Macromolecules reduce distance between two red cells	IgG antibodies

# Learning Outcomes

- You will now understand the principal of AGT
- You will be able to carry out AGT
- You must have known about the reagent in use

