

Division of Blood Transfusion Services

Ministry of Health and Family Welfare



ABO and Rh Blood Grouping & Typing



Teaching Aim

- Understanding inheritance, synthesis, various antigens and antibodies and their clinical significance in ABO & Rh blood group systems
- Understanding practical aspects of ABO & Rh blood grouping

Human Blood Groups

- Red cell membranes have antigens (protein / glycoprotein) on their external surfaces
- These antigens are
 - unique to the individual
 - recognized as foreign if transfused into another individual
 - promote agglutination of red cells if combine with antibody
 - more than 30 such antigen systems discovered
- Presence or absence of these antigens is used to classify blood groups
- Major blood groups – ABO & Rh
- Minor blood groups – Kell, Kidd, Duffy etc



ABO Blood Groups

- Most well known & clinically important blood group system.
- Discovered by Karl Landsteiner in 1900
- It was the first to be identified and is the most significant for transfusion practice
- It is the **ONLY** system that the reciprocal antibodies are consistently and predictably present in the sera of people who have had no exposure to human red cells
- ABO blood group consist of
 - two antigens (A & B) on the surface of the RBCs
 - two antibodies in the plasma (anti-A & anti-B)



Reciprocal relationship between ABO antigens and antibodies

Antigens on RBCs	Antibody in plasma / serum	Blood group
A	Anti-B	A
B	Anti-A	B
AB	None	AB
None	Anti-A, Anti-B	O

Development at birth

- All the ABH antigens develop as early as day 37 of fetal life but do not increase very much in strength during gestational period
- Red cell of newborn carry 25-50 % of number of antigenic sites found on adult RBC
- Although cord red cells can be ABO grouped, the reactions may be a bit weaker than expected
- A or B antigen expression fully developed at 2-4 yrs of age and remain constant throughout life

Expression of ABO Antigens

- Although the ABO blood group antigens are regarded as RBC antigens, they are actually expressed on a wide variety of human tissues and are present on most epithelial and endothelial cells
- ABH antigens are not only found in humans, but also in various organisms such as bacteria, plants, and animals
- Present both on red blood cells and in secretions only in humans and some of the apes (chimpanzee, gorilla)
- In all other mammalian species these substances are found only in secretions



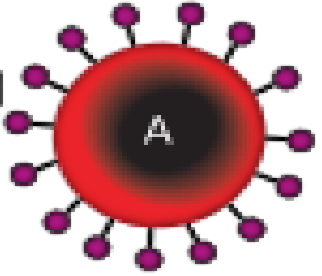
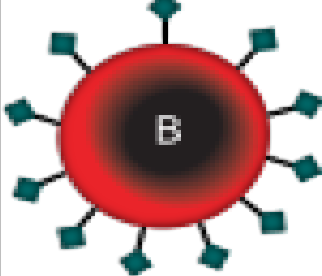
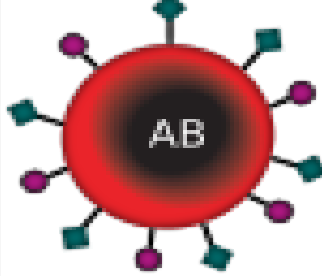
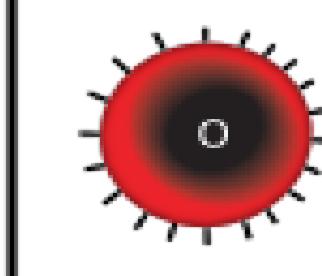






Anti-A and anti-B antibodies

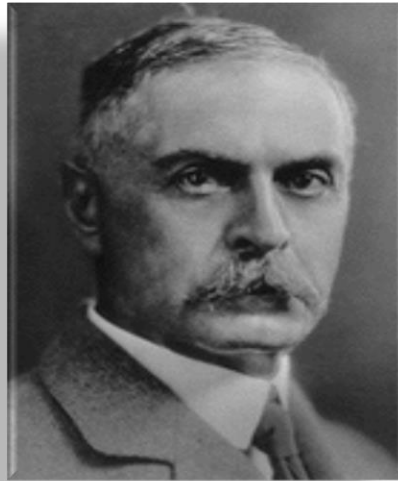
- Not present in the newborn, appear in the first years of life (4-6 months usually), reach adult level at 5-10 years of age, decreases in elderly
- Naturally occurring as they do not need any antigenic stimulus
- However, some food & environmental antigens (bacterial, viral or plant antigens) are similar enough to A and B glycoprotein antigens and may stimulate antibody development

Anti-A and anti-B antibodies (contd...)

- Immunocompetent person react to these antigens by producing antibodies to those absent from their own system
- Usually IgM, which are not able to pass through the placenta to the fetal blood circulation
 - Anti-A titer from group O > Anti-A titer from group B
 - Anti-A titer from group B > Anti-B titer from group A

ABO antigens & corresponding antibodies

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	A antigen 	B antigen 	A and B antigens 	None



'Landsteiner's law : the plasma contains natural antibodies to A or B, if these antigens are absent from the red cells of that person

Inheritance of ABO Blood Groups

- Follows Mendelian principles
- Blood group antigens are “codominant”- if the gene is inherited, it will be expressed.
- There are three allelic genes -A, B & O
- Some aberrant genotypes do occur but they are very rare.
- Understanding of basic inheritance important.

Inheritance of ABO Blood Groups

- Two genes inherited, one from each parent.
- Individual who is A or B may be homozygous or heterozygous for the antigen.
 - Heterozygous: AO or BO
 - Homozygous: AA or BB
- Phenotype is the actual expression of the genotype, ie, group A
- Genotype are the actual inherited genes which can only be determined by family studies, ie, AO.



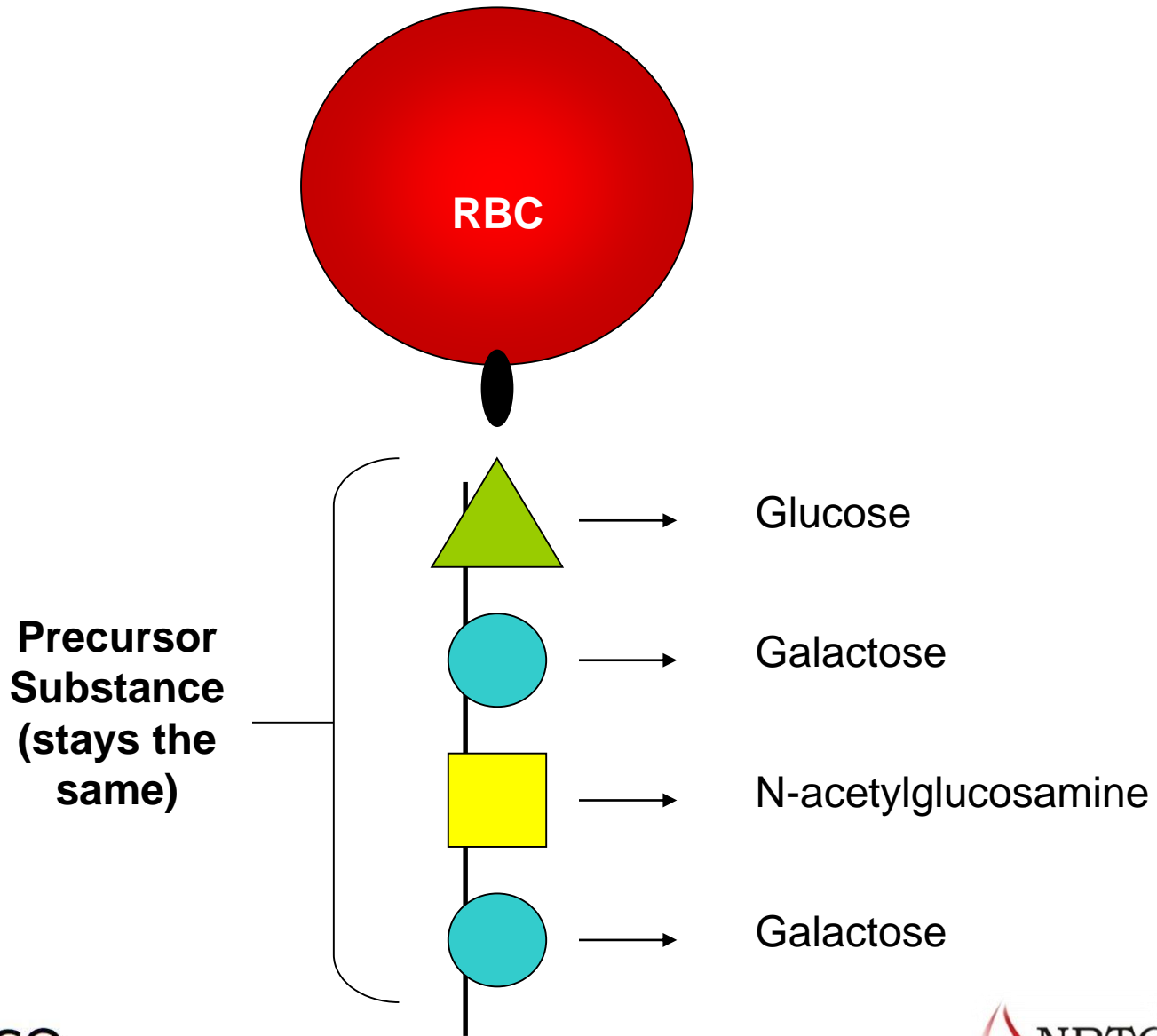
ABO and H Antigen Genetics

- Genes at three separate loci control the occurrence and location of ABO antigens
- Presence or absence of the ABH antigens on the red cell membrane is controlled by the H gene
- Presence or absence of the ABH antigens in secretions is indirectly controlled by the Se gene
 - H gene – H and h alleles (h is an amorph)
 - Se gene – Se and se alleles (se is an amorph)
 - ABO genes – A, B and O alleles

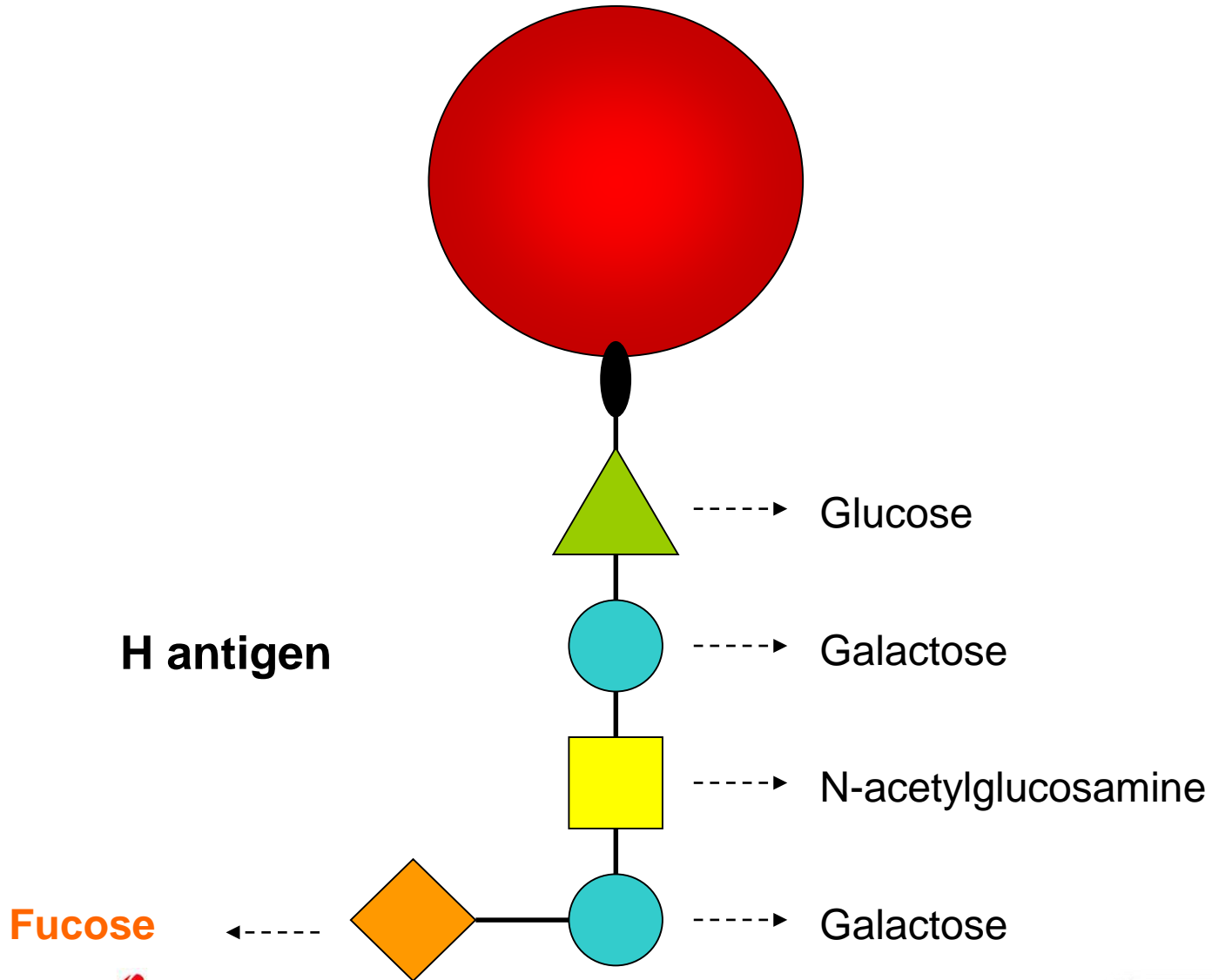
H Antigen

- The H gene codes for an enzyme (fucosyltransferase) that adds the sugar fucose to the terminal sugar of a precursor substance
- The precursor substance (proteins and lipids) is formed on an oligosaccharide chain (the basic structure)
- The H antigen is the foundation upon which A and B antigens are built
- A and B genes code for enzymes that add an immunodominant sugar to the H antigen
 - Immunodominant sugars are present at the terminal ends of the chains and confer the ABO antigen specificity

RBC precursor substance



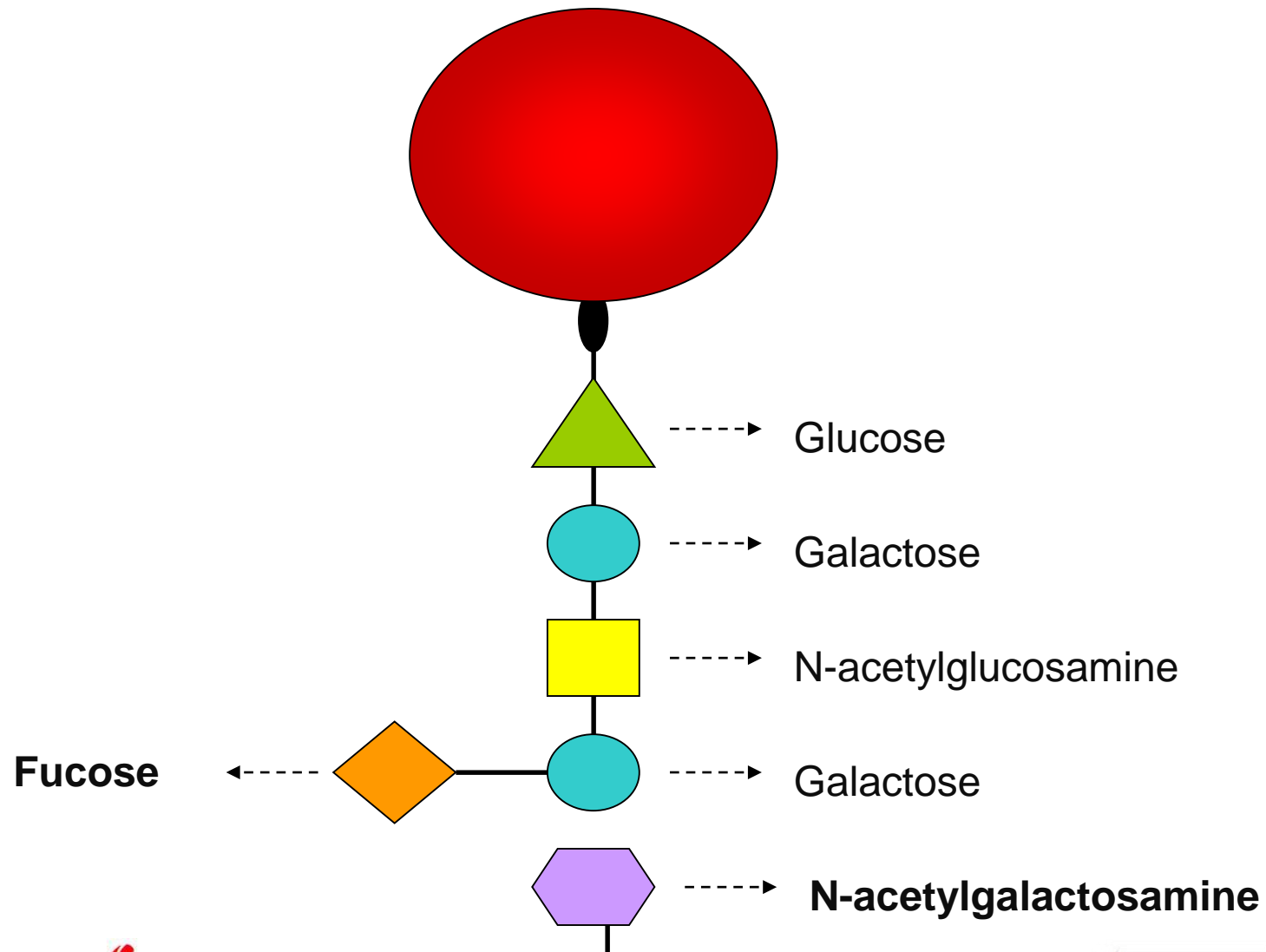
Formation of the H antigen



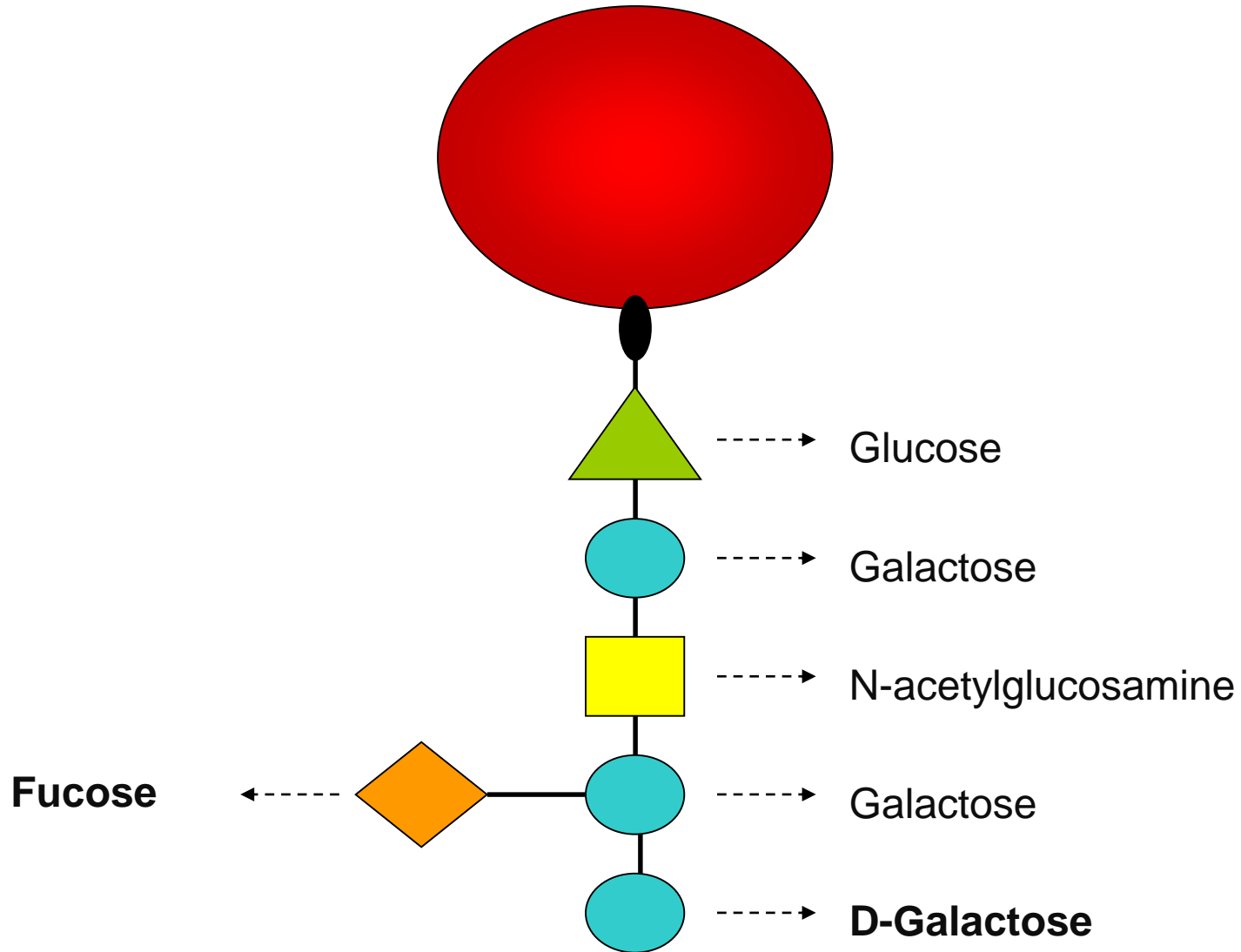
A and B Antigen

- The “A” gene codes for an enzyme (transferase) that adds N-acetylgalactosamine to the terminal sugar of the H antigen
 - N-acetylgalactosaminyltransferase
- The “B” gene codes for an enzyme that adds D-galactose to the terminal sugar of the H antigen
 - D-galactosyltransferase

Formation of the A antigen



Formation of the B antigen



Secretor Status

- A, B, H substances are found in all body secretions (except CSF) in 80% of individuals
- Ability to secrete these substances is determined by the presence of secretor gene (Se) in either homozygous (SeSe) or heterozygous (Sese) state.

Blood Group	Substances Secreted
O	H
A	A & H
B	B & H
AB	A, B, & H
Oh	Nil

Characteristics of Bombay Phenotype

- First reported by Bhende et al in Bombay in 1952.
- Frequency estimated to be about 1 in 7600 in Bombay.
- Absence of H, A & B antigens. No agglutination with anti-A, anti-B or anti-H
- Presence of anti-H, anti-A and anti-B in the serum
- No A, B or H substances present in saliva
- Incompatible with any ABO blood groups, compatible with Bombay phenotype only
- A recessive mode of inheritance (identical phenotypes in children but not in parents)



ABO Subgroups

- ABO subgroups differ in the amount of antigen present on the red blood cell membrane
 - Subgroups have less antigen
- Subgroups are the result of less effective enzymes. They are not as efficient in converting H antigens to A or B antigens (fewer antigens are present on the RBC)
- Subgroups of A are more common than subgroups of B

Subgroups of A

- Two principle subgroups of A are: A_1 and A_2
- Both react strongly with reagent anti-A
- To distinguish A_1 from A_2 red cells, the lectin *Dolichos biflorus* is used (anti- A_1)
- 80% of group A or AB individuals are A_1 and A_1B
- 20% are A_2 and A_2B

A₂ phenotype

- Clinical significance of A₂ phenotype
 - 8% of A₂ and 25% of A₂B individuals may produce anti-A₁ in the serum
 - This may result in discrepancy in blood grouping or incompatibility in cross match
 - However, these anti-A₁ antibodies are cold reacting & therefore may not cause problems routinely.
- Difference between A₁ and A₂
 - It is quantitative
 - The A₂ gene doesn't convert the H to A very well resulting in fewer A₂ antigen sites compared to the many A₁ antigen sites

B Subgroups

- B subgroups occur less than A subgroups
- B subgroups are differentiated by the type of reaction with anti-B, anti-A,B, and anti-H
- B₃, B_x, B_m, and B_{el}

Practical aspects of ABO grouping

- Routine ABO grouping must include both cell & serum testing as each test serves as a check on the other
- Test should be done at room temperature or lower; testing at 37°C weakens the reactions
- Tubes, slides should be dry and labeled properly
- Serum should always be added before adding cells
- Results should be recorded immediately after observation
- Hemolysis is interpreted as positive result

Blood Grouping

- There are 2 components to blood typing:
 - Test unknown cells with known antibodies
 - Test unknown serum/plasma with known red cells
- The patterns are compared and the blood group is determined.

Blood Sample for Blood Grouping

Blood sample

- Clearly labeled blood samples in sterile tubes (plain & EDTA)
- Test should be performed on the fresh sample for best results.

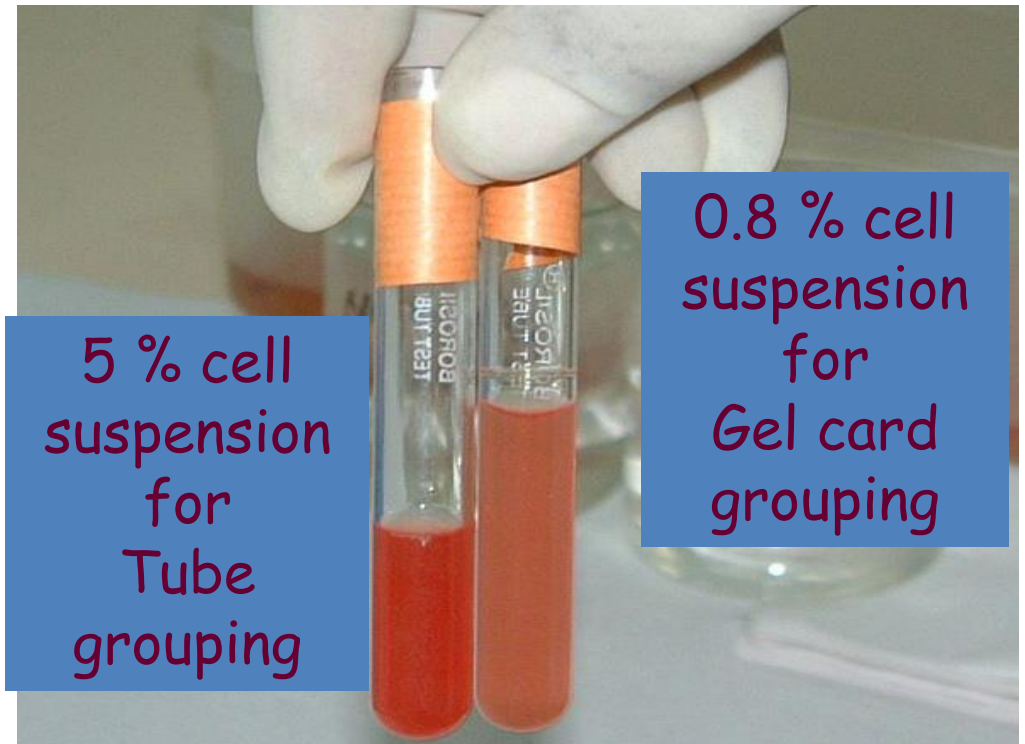
In case the test can not be performed immediately, sample can be stored at 4°C & should be tested within 48 hours

- No signs of hemolysis should be there
- If serum is not completely separated, centrifuge tube at 3000 rpm for 3 min
- Preferably use saline washed red cells and make 2-5% suspension



Red Cell Suspensions for Blood Grouping

- 2-5%: Test Tube Method
- 0.8-1%: Gel technology
- 1%: Microplate



Test Tube Method of ABO Grouping

Recommended method

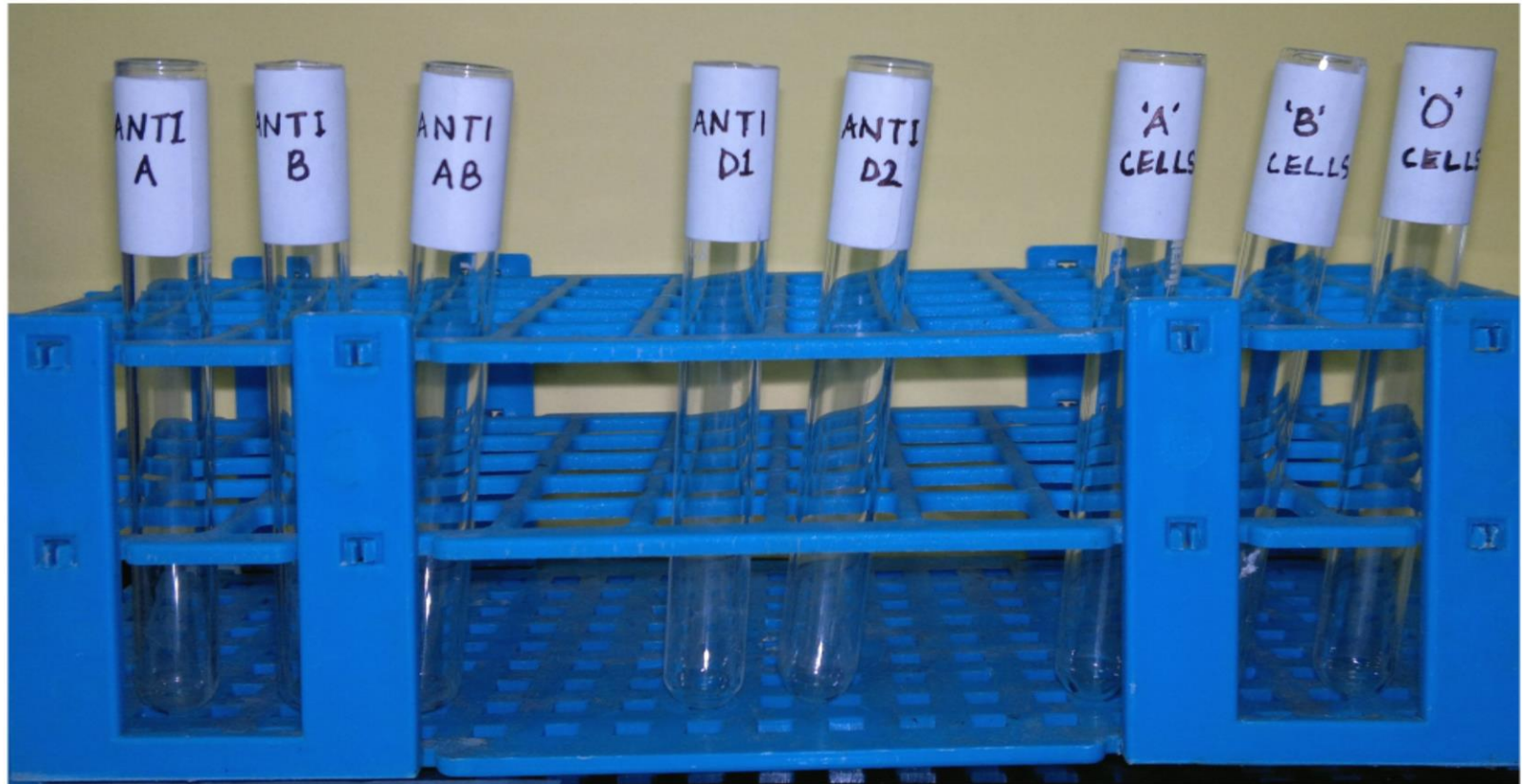
- Allows longer incubation of antigen and antibody mixture without drying
- Tubes can be centrifuged to enhance reaction
- Can detect weaker antigen / antibody

Two steps in ABO grouping

- Cell grouping (Forward grouping)
 - Tests the patients red cells with known Anti-A & Anti-B to determine the antigen expressed
- Serum grouping (Reverse grouping)
 - Test the patients serum with known A & B cells to determine the presence of antibody



Lay Out of Tubes for ABO & Rh grouping



Forward grouping
Cell grouping

Rh grouping

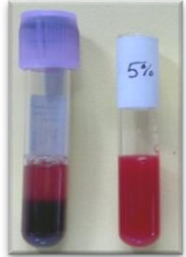
Reverse grouping
Sera grouping



2 vol of anti- A /
anti-B/ Anti-AB



1 vol of 2-5% red
cell suspension



Incubate at room temp
(20-24°C) for 5 min



Forward Grouping

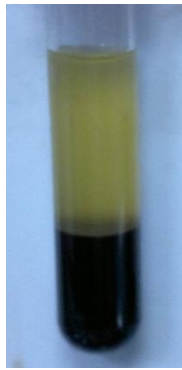


Centrifuge at 1000 rpm
for 1 min



Check for agglutination
against well lighted
background





2 vol of test
serum/plas
ma



1 vol of 5%
suspension of
reagent red cells
in respective
tubes



Shake & leave at room
temp (20-24°C) for 5 min



Reverse Grouping

Centrifuge at 1000 rpm
for 1 min







Centrifuge & record the
results similarly as for
cell grouping



Tube Agglutination Grading

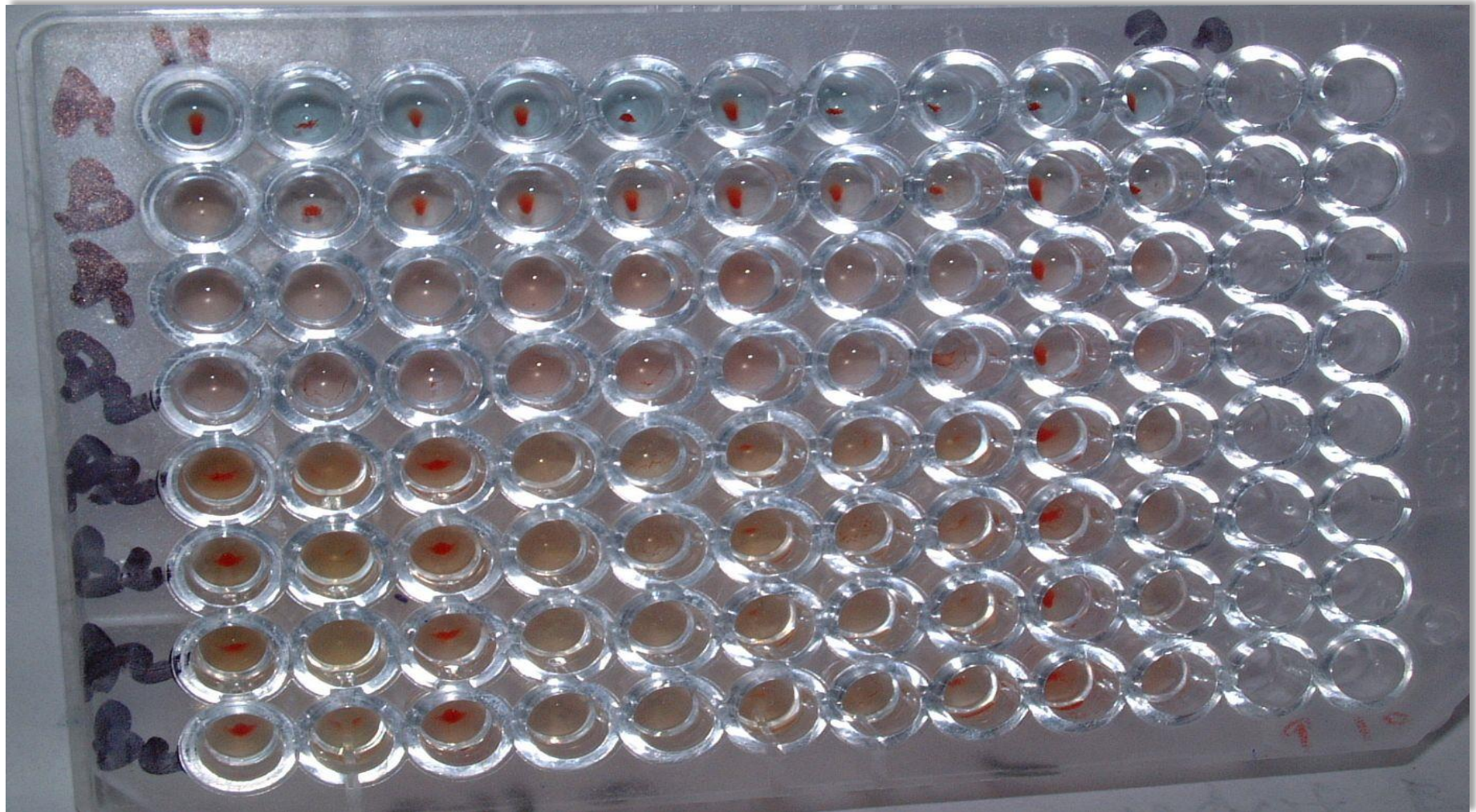
Tube Agglutination Grading Chart

Scale		Macroscopically Observed Findings
0-4	0-12	
4	12	 One solid agglutinate, no free red cells detected.
3	10	 One or two large agglutinates, a strong reaction.
2	8	 Medium size agglutinates, clear background.
1	5	 Small agglutinates, with a lot of free red cells.
+/-	3	 Weak granularity in the red cell suspension.
0	0	 No agglutinates, an even red cell suspension.

Microplate Method

- It is ideal for testing large number of blood samples.
- More sensitive to detect weaker antigen-antibody reactions
- Results can be photographed for archival storage
- Microplate can be incubated & centrifuged
- There is significant saving in time and in the cost of disposables and reagents.
- Microplates are intended to be disposable however they can be reused after cleaning them properly making sure that all foreign protein are removed.
- Microplates can be adapted for automation

Microplate Method



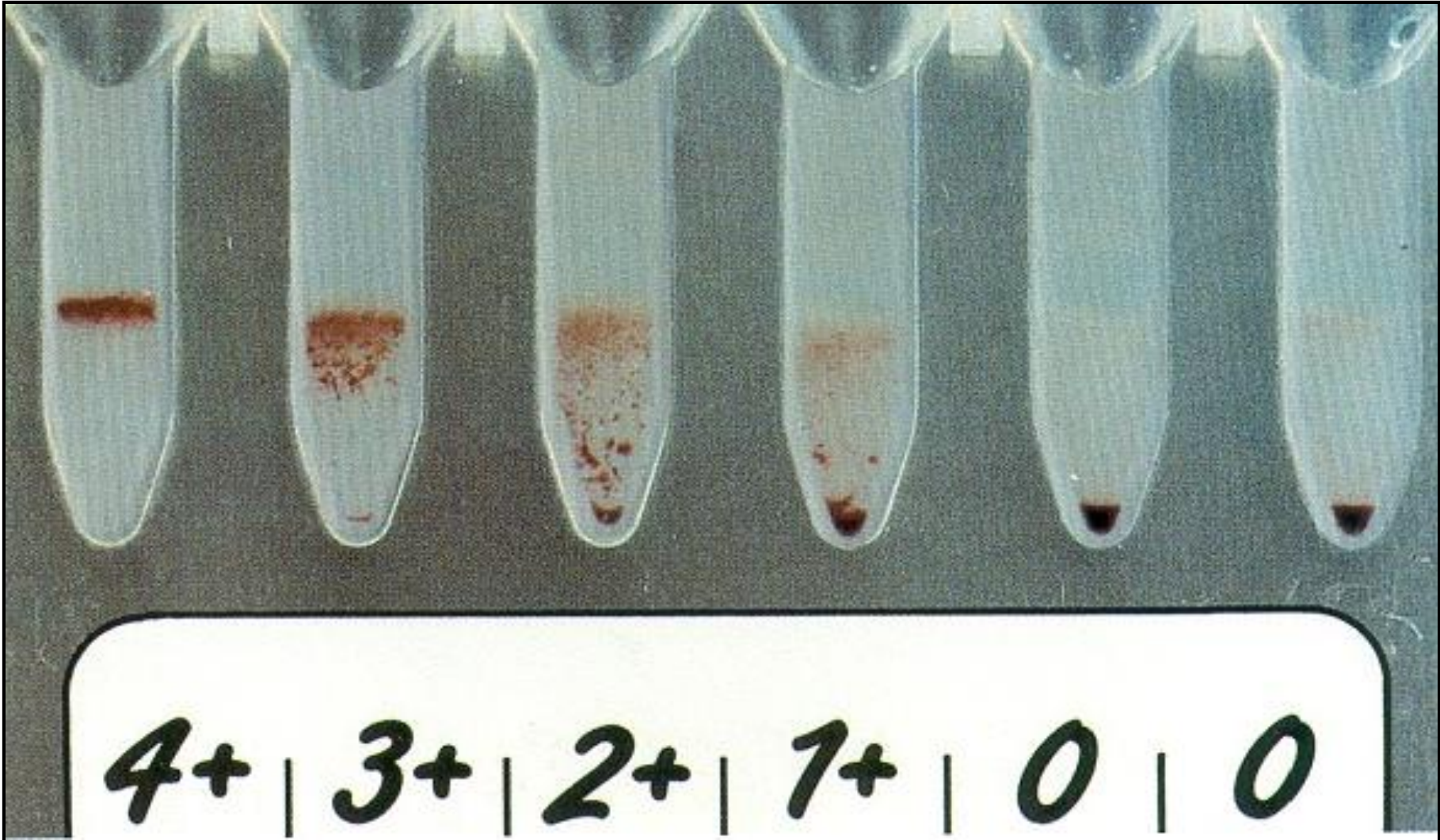
Reaction in the microplate after 1 hour incubation at room temperature

Column Agglutination Technology

- One card is basically a set of 6 microtubes
- Microtubes contain either Sephadex Gel or glass microbeads impregnated with antisera
- Antigen-antibody reaction takes place in the reaction chamber of microtube
- Gel matrix or glass beads act as sieve and allow free cells (un-agglutinated) to pass through and settle at the bottom of microtube while agglutinated cells are trapped in the matrix



Grading Result



Rh Blood Group System



Rh (D) Antigen

- Of next importance is the Rh type.
 - Rh is a blood group system with many antigens, one of which is D.
- Rh refers to the presence or absence of the D antigen on the red blood cell.
- Unlike the ABO system, individuals who lack the D antigen do not naturally produce anti-D.
- Production of antibody to D requires exposure to the antigen.
- The D antigen is very immunogenic, i.e., individuals exposed to it will very likely make an antibody to it.
- For this reason all individuals are typed for D, if negative must receive Rh (D) negative blood.

Rh (D) Antigen (contd...)

- Rh antigens are an integral part of the red cell membrane.
- They are protein in nature with an active phospholipid component
- Rh antigens do not exist in the soluble form and, therefore are not excreted in body fluids.
- Unlike ABO antigens, Rh antigens are present only on red blood cells. These antigens are not found on other blood cells including platelets and leukocytes

Rh (D) Antigen (contd...)

- A very potent antigen (50% may form antibody to exposure)
- Frequency in Indian population
 - 92-95% Rh positive
- The most important patient population to consider is females of child-bearing age.
- If immunized to Rh (D) antigen the antibody can cross the placenta and destroy Rh (D) positive fetal cells resulting in death.
- This is why Rh negative women are given injection anti-D after birth of Rh positive baby.

Rh Antibodies

- All Rh antibodies are immune in nature, developed after immunizing event
- React at 37°C and require anti globulin test to demonstrate the reaction
- Generally do not react at room temperature in saline
- Most are IgG in nature and therefore can cross the placenta
- Generally, do not fix complement and cause extravascular hemolysis
- All are important in HDN and delayed HTR

Rh typing

- Normal typing for Rh antigens only includes typing for Rh (D).
- The result of this typing determines the Rh status of the cells (Rh - positive or Rh - negative).
- Some Rh typing sera is diluted in high protein solutions and may require a negative control.
- It is recommended to use two monoclonal anti-D sera from two different manufacturers labeled as D1 and D2, especially to confirm all Rh negatives

Monoclonal Anti-D

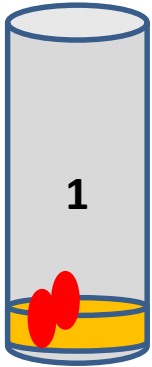
Three types

1. IgM anti-D monoclonal reagent
 2. Blend of IgM and IgG monoclonal antibodies reagent
 3. Monoclonal IgG anti-D
- IgM antibodies are highly specific and saline reacting equally at RT and 37⁰ C but unreliable for detection of weak D
 - Blended antibodies are now routinely used and can be used for detecting weak D

Tube Technique for Rh Typing

- Prepare 5% washed red cell suspension of test sample.
- Take three clean test tubes and label tubes 1 & 2 as “test” and tube 3 as “control”.
- Place 1 drop of anti-D (D1) in tube 1 and 1 drop of anti-D (D2) in tube 2.
- Place 1 drop of 22% bovine albumin / control in tube 3.
- Add 1 drop of 5% test cell suspension to each tube.
- Mix well, centrifuge at 1000 rpm for 1 min.
- Resuspend cell button & look for agglutination
- Control tube should show no agglutination
- For all RhD negative test on blood donor, D^u test recommended

Method for Rh Typing



1 drop anti-D1



1 drop anti-D2

+
1 drop 5% red
cells

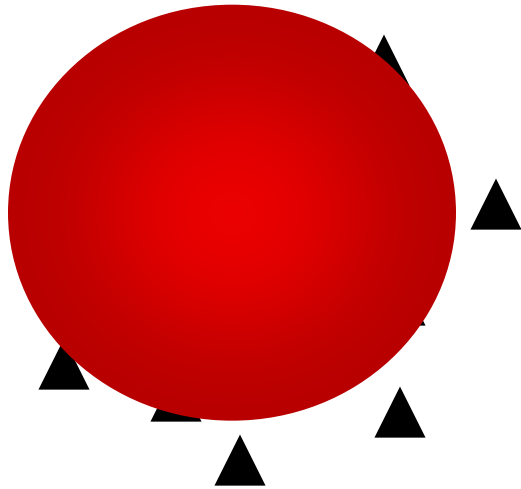
+
1 drop 5% red
cells

Mix well and centrifuge at 1000 RPM x 1 min

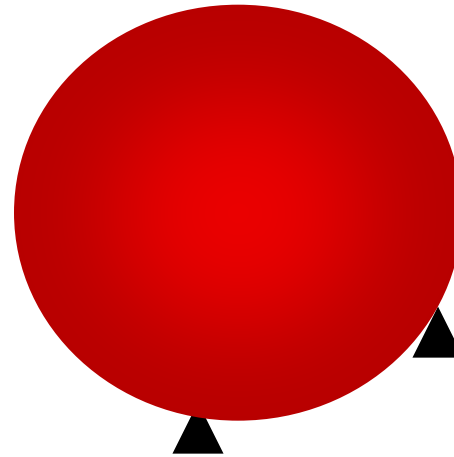
↓
Look for agglutination

Weak D

Inheritance of D genes which result in lowered densities of D Antigens on RBC membranes, gene codes for less D.



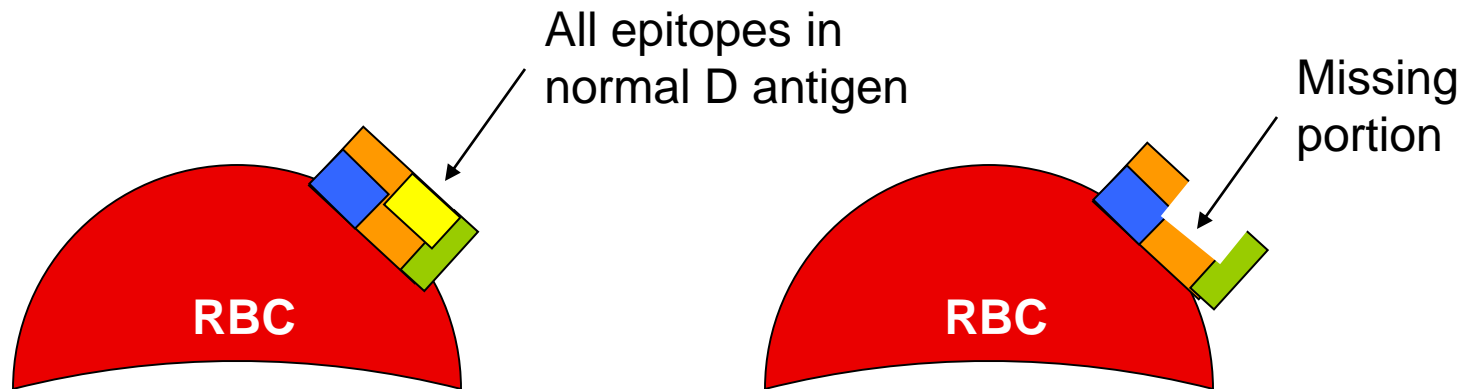
RBC with normal expression of D antigen



Decreased number of D antigens in Weak

Partial D

- Absence of a portion of the total material that comprises the D antigen (qualitative defect)
- If the partial D patient is transfused with D positive red cells, they may develop an anti-D alloantibody to the part of the antigen (epitope) that is missing



Method for Weak D Testing

- Add 1 drop of 2-5 % suspension of D negative red cells to a test tube and add 2 drops of Anti D (blend of IgG + IgM)
- Incubate at 37°C for 30 minutes.
- Wash three times with normal saline.
- Make dry red cell button and add polyspecific AHG reagent.
- Look for agglutination.

Results:

- If there is agglutination Du Positive.
- If there is no agglutination Du Negative.



Significance of Weak D

Donors

- Weak D testing on donors required.
- Labeled as D positive
- But as recipient D negative

Patients

- Weak D testing on patients not required.
- Standard practice to transfuse with D negative

Significance of Weak D

- Weak D is much less antigenic in comparison to D, however, such red cells may be destroyed if transfused to a patient already having anti-D. Hence, weak D donor units are labeled as Rh positive.
- The weak D positive recipients are classified as Rh negative and safely transfused with Rh negative blood
- D^u positive infant can suffer from HDN if the mother possess anti-D antibodies
- Rh immunoprophylaxis is recommended for the Rh negative mother if the newborn is D^u positive.



Learning Outcome

- You should now be able to perform ABO & Rh grouping on the donor and recipients sample
- You should be able to resolve discrepancies in the blood grouping
- You should be able to perform weak D testing if required

