# Immunology Lab 5

# ABO Blood Antigens

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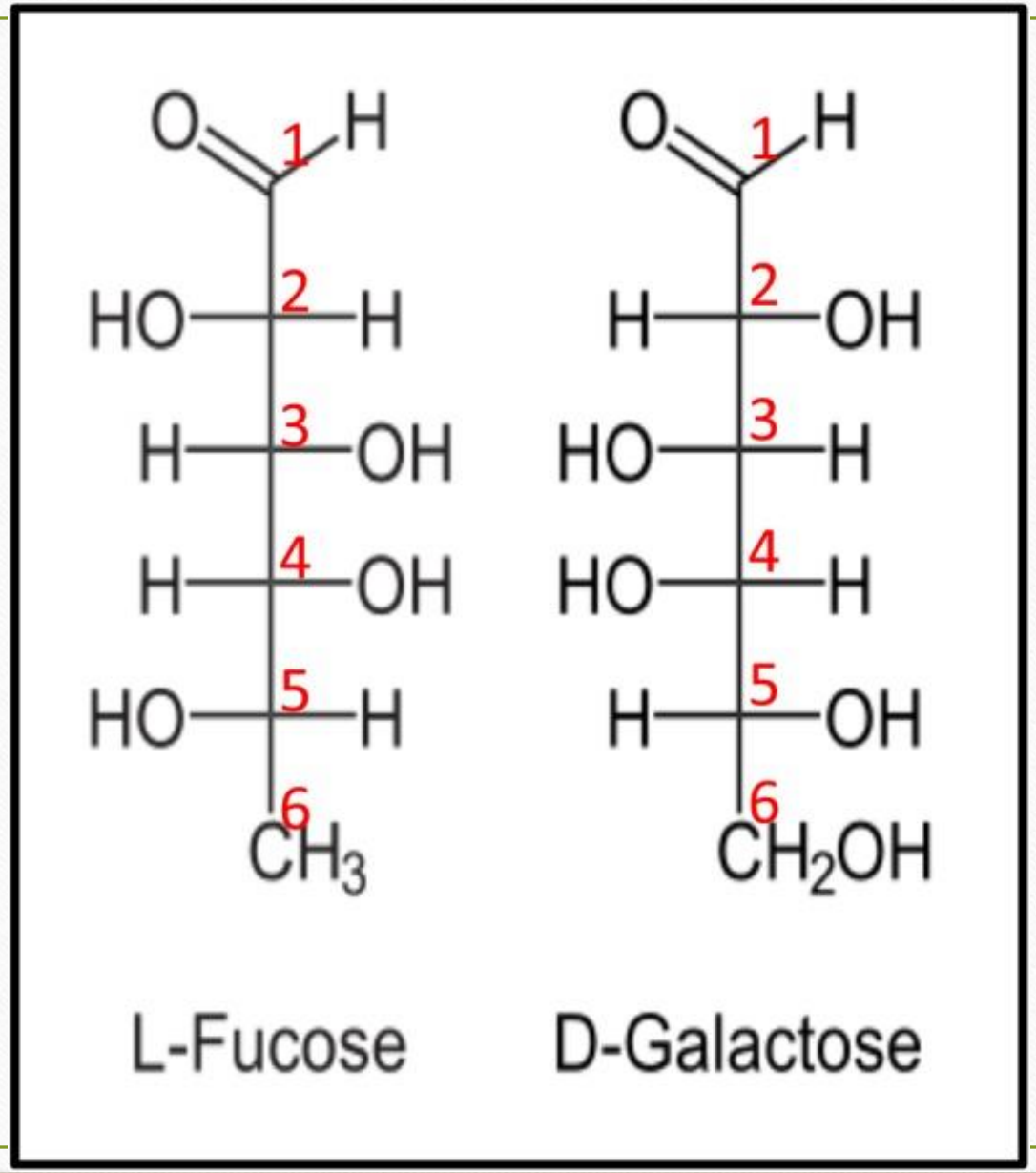
# ABO blood antigens

- The ABO antigens are carbohydrates linked to cell surface proteins and lipids that are synthesized by polymorphic glycosyltransferase enzymes.
- Most individuals possess a fucosyltransferase that adds a fucose moiety to a nonterminal sugar residue of the core glycan, and the resulted fucosylated glycan is called the H antigen (O antigen).
- A single gene on chromosome 9 encodes a glycosyltransferase enzyme that may further modify the H antigen.

**There are three allelic variants of this enzyme**

1. O allele gene product: is devoid of enzymatic activity and can't attach terminal sugars to the H antigen, and express only the H antigen, the precursor of the ABO blood group antigens.
2. A allele– encoded enzyme (N –Acetylgalactosaminyltransferase): transfers a terminal N-acetylgalactosamine moiety onto the H antigen.
3. B allele gene product: transfers a terminal galactose moiety.

The C-6 carbon of l-fucose lacks a hydroxyl group present at the C-6 position of d-galactose. l-Fucose can also be described as 6-deoxy-l-galactose.



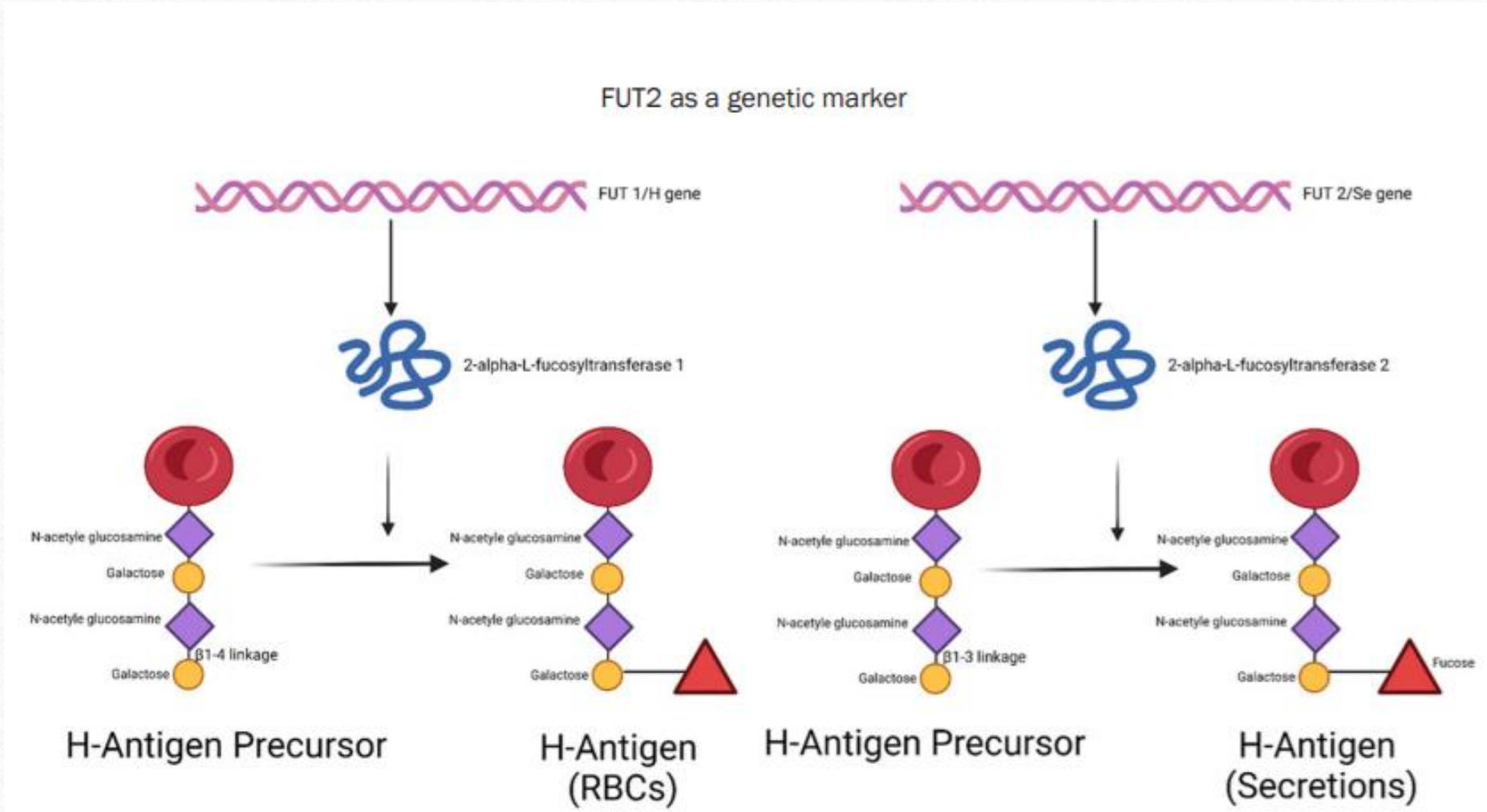
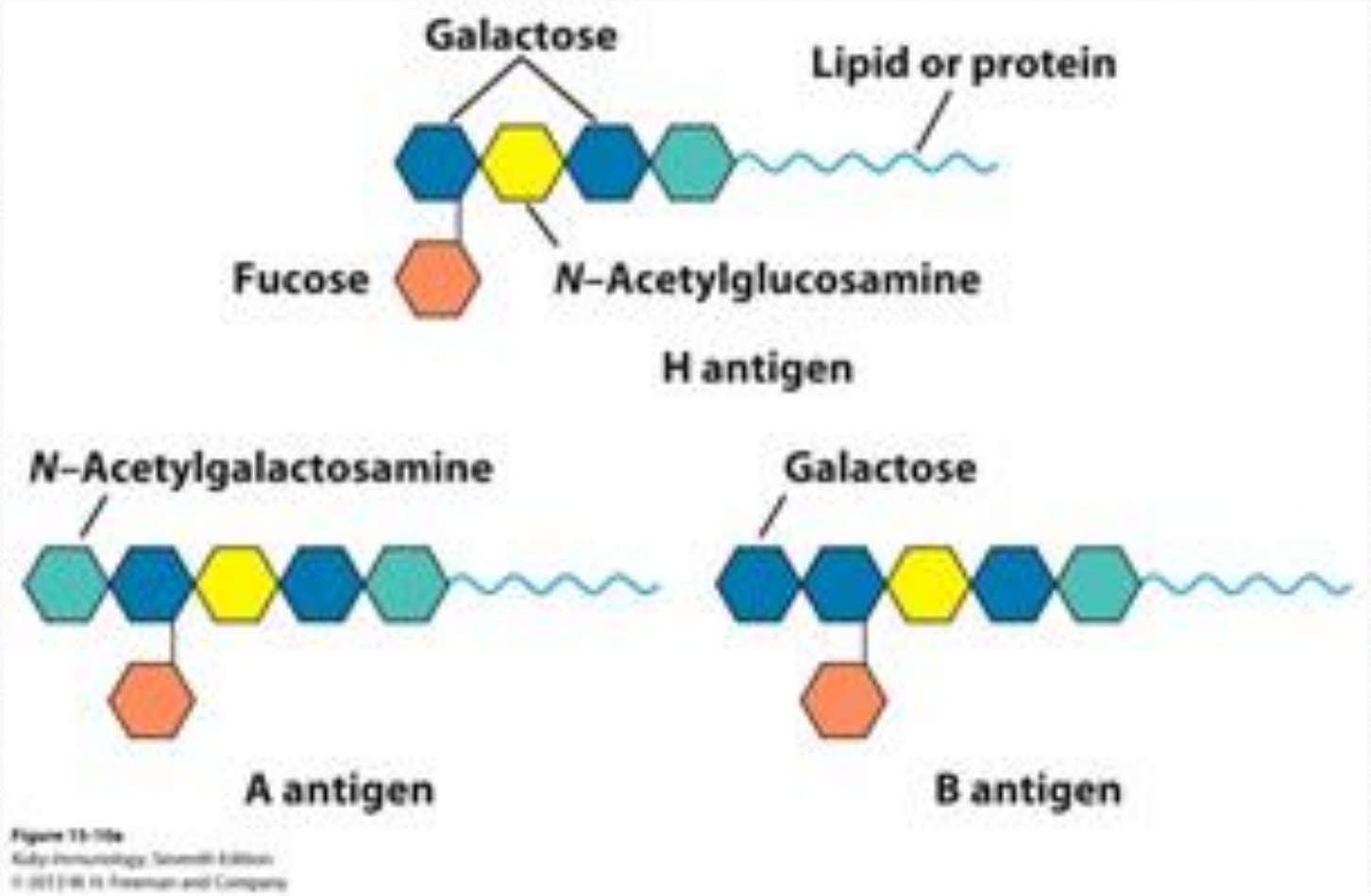
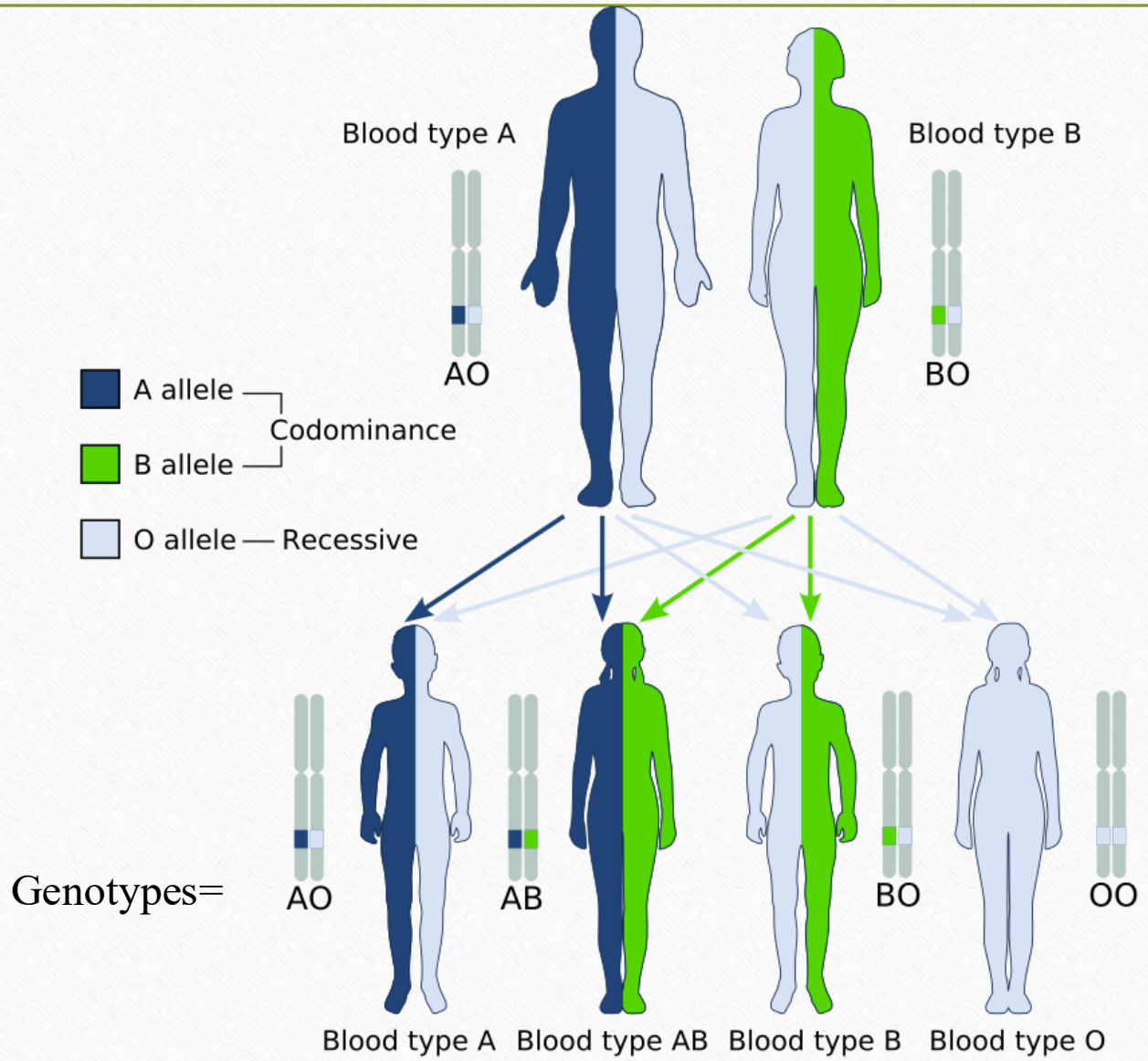



**Figure 1.** Role of FUT1 and FUT2. Fucosyltransferase enzymes H (FUT1) add fucose to the alpha (1, 2) binding of type 2 glycoproteins on RBCs to form H antigen, whereas FUT2 adds fucose to the alpha (1, 2) binding of type 1 glycoprotein chains to make ABH antigens in other body fluids (secretor phenotype).


Galactose
Lipid or protein
Fucose
N–Acetylglucosamine
H antigen
N–Acetylgalactosamine
Galactose
A antigen
B antigen


Blood type A
Blood type B
AO
BO
A allele
B allele
Codominance
O allele — Recessive
Genotypes=
AO
AB
BO
OO
Blood type A
Blood type AB
Blood type B
Blood type O

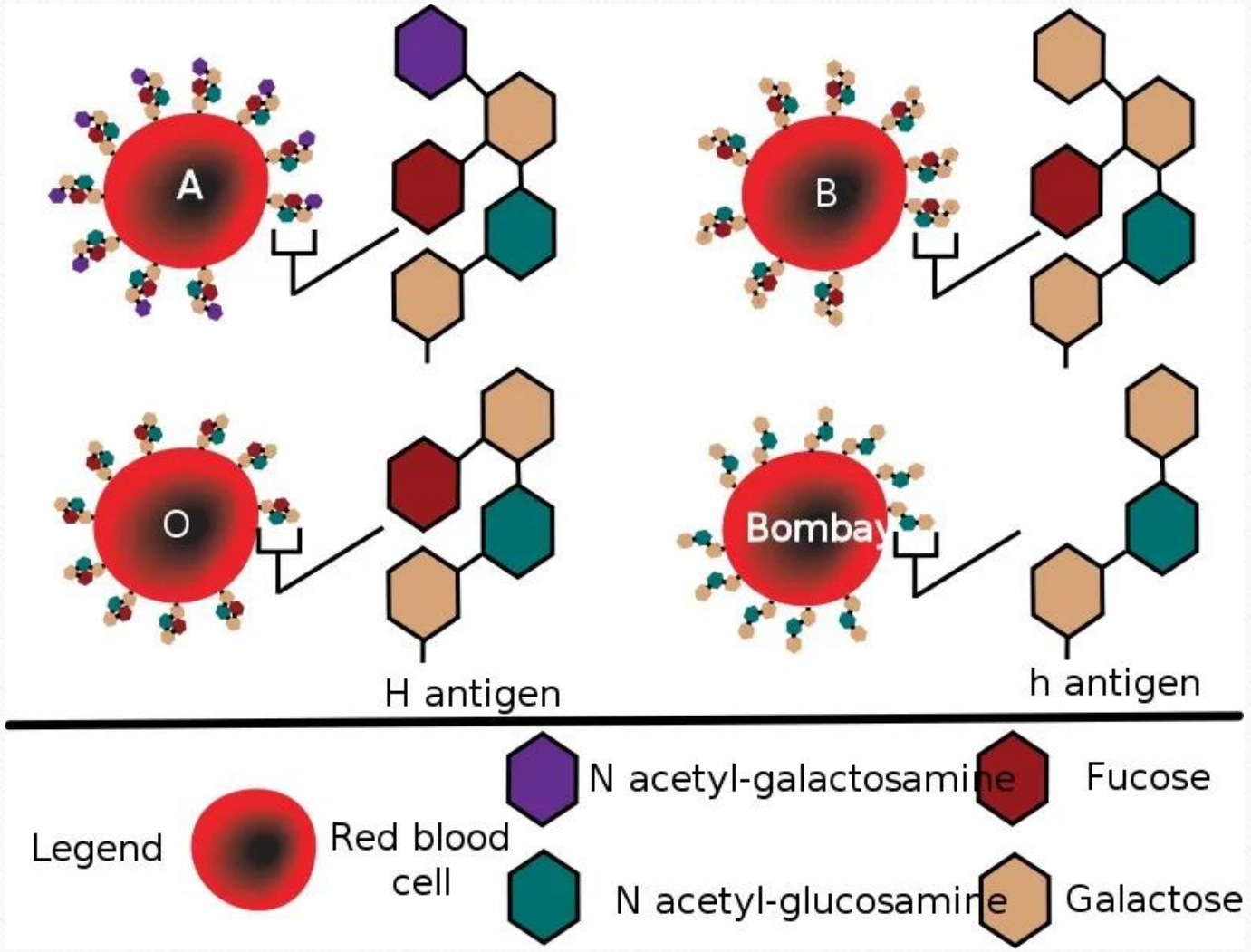



**Diagram showing the carbohydrate chains that determine the ABO blood group**

- Mutations in the gene encoding the fucosyltransferase that produces the H antigen without fucose are rare.
- People who are homozygous for such a mutation are said to have the **Bombay blood group** (h/h, also known as Oh).
- And cannot produce H, A, or B antigens. And can't receive type O, A, B, or AB blood.

| Blood Groups (Antigens and Antibodies) | | |
|---|---|---|
| **Blood Group** | **Antigens** | **Antibodies** |
| **A** | A,H | B |
| **B** | B,H | A |
| **AB** | A,B,H | - |
| **O** | H | A,B |
| **Bombay Blood Group** | - | A,B,H |

**Called (O, hh, Oh)**

# Percentages of the 8 blood groups

AB-negative (0.6 percent)

B-negative (1.5 percent)

AB-positive (3.4 percent)

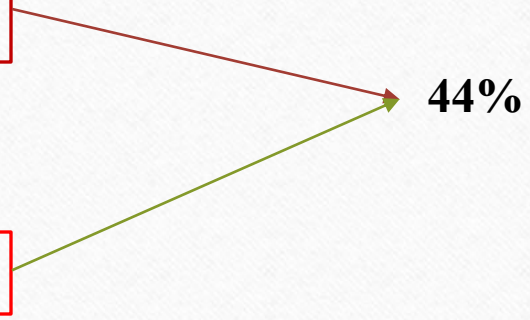
A-negative (6.3 percent)

O-negative (6.6 percent)

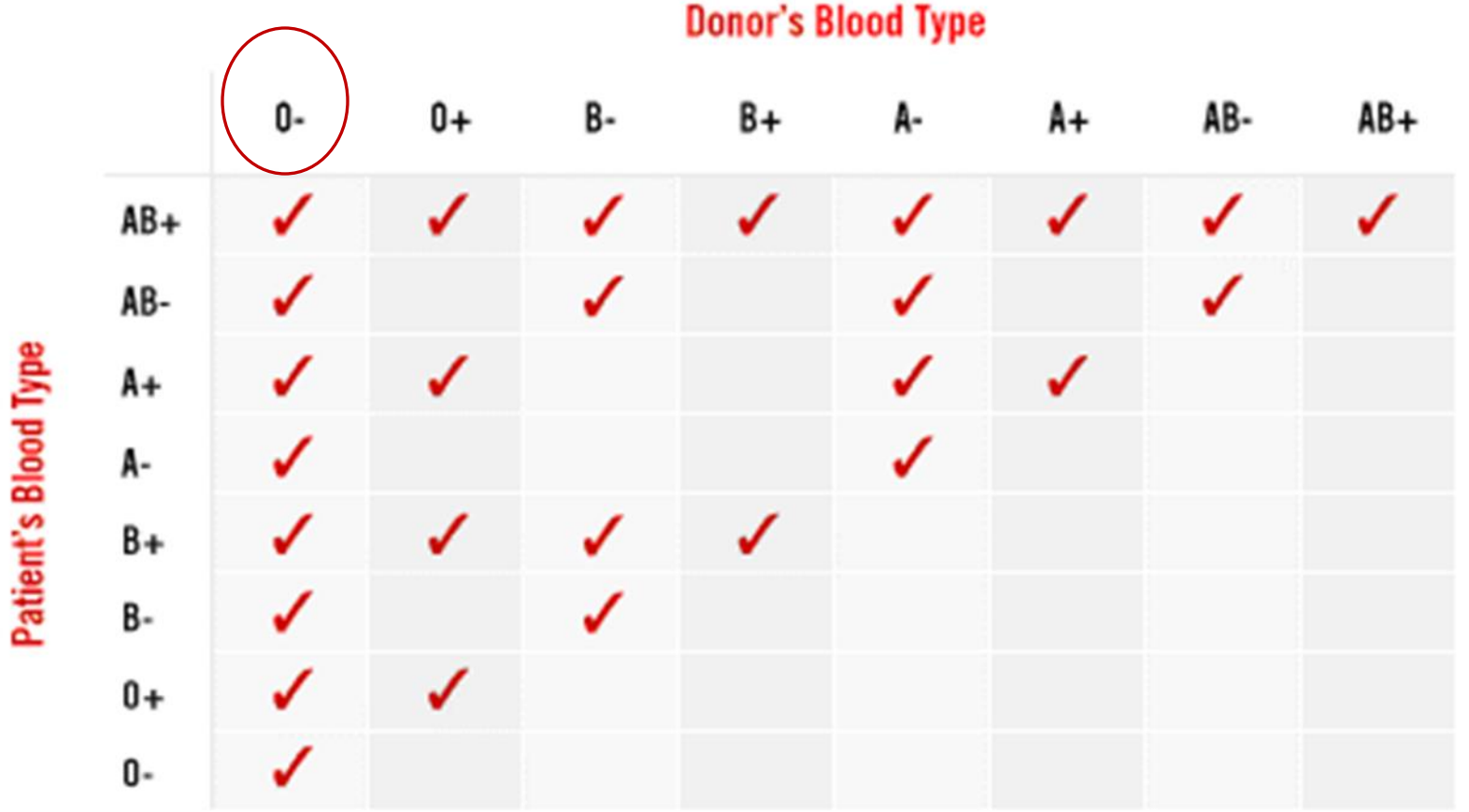
B-positive (8.5 percent)

A-positive (35.7 percent)

O-positive (37.4 percent)

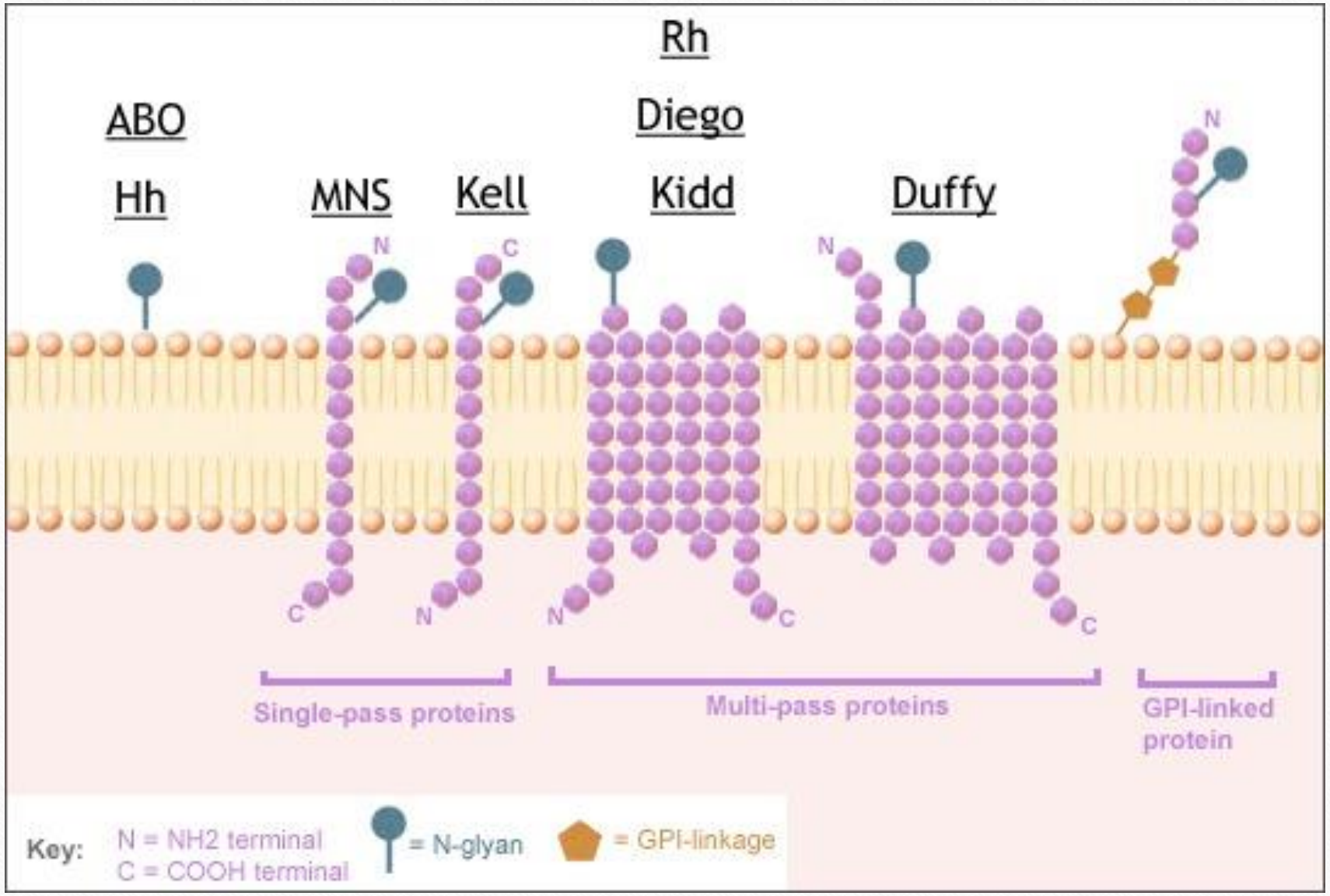


# Most Common Transplantation
# -Blood Transfusion-



- ✓ O-negative is the **universal blood type**, meaning any other blood type may receive it (see our blood type compatibility chart here).
- ✓ This can quickly deplete the stores of O-negative that blood centers have on the shelves.
- ✓ While 44% of the population is type O, less than 7% is O-negative. So as you can see, the most needed type of blood is also the hardest to collect.
- ✓ AB negative is the rarest of the eight main blood types - just 1% of our donors have it. Despite being rare, demand for AB negative blood is low

| Blood grouping System | System symbol | Epitope or carrier, notes | Chromosome |
|---|---|---|---|
| ABO | ABO | Carbohydrate) N-Acetylgalactosamine ,galactose .(A, B and H antigens | 9 |
| MNS | MNS | Main antigens M, N, S, s. carried on sugar-bearing proteins called glycophorins. | 4 |
| Rh | RH | Protein. C, c, D, E, e antigens (there is no "d" antigen; lowercase "d" indicates the absence of D | 1 |
| Kell | KEL | Glycoprotein. $K_1$ can cause hemolytic disease of the newborn (anti-Kell ,( which can be severe. | 7 |
| LI | Li | Polysaccharide | 6 |
| Duffy | FY | Protein) chemokine receptor .(Main antigens $Fy^a$ and $Fy^b$ .Individuals lacking Duffy antigens altogether are immune to malaria caused by *Plasmodium vivax* and *Plasmodium knowlesi*. | 1 |


Rh
ABO
Diego
Hh
MNS
Kell
Kidd
Duffy
N
C
N
C
N
C
N
C
N
C
Single-pass proteins
Multi-pass proteins
GPI-linked protein
Key:
N = NH2 terminal
C = COOH terminal
= N-glyan
= GPI-linkage

# RH blood antigen

- Rh antigens are non-glycosylated, hydrophobic cell surface proteins found in red blood cell membranes.
- 15% of the population has a deletion or other alteration of the RhD allele.
- Rh status is inherited from our parents, separately from our blood type.
- If you inherit the dominant Rhesus D antigen from one or both of your parents, then you are Rh-positive (85% of us). If you do not inherit the Rhesus D antigen from either parent, then you are Rh-negative (15% of us).

# Rh System

***Rh Antigens and Encoding Genes***

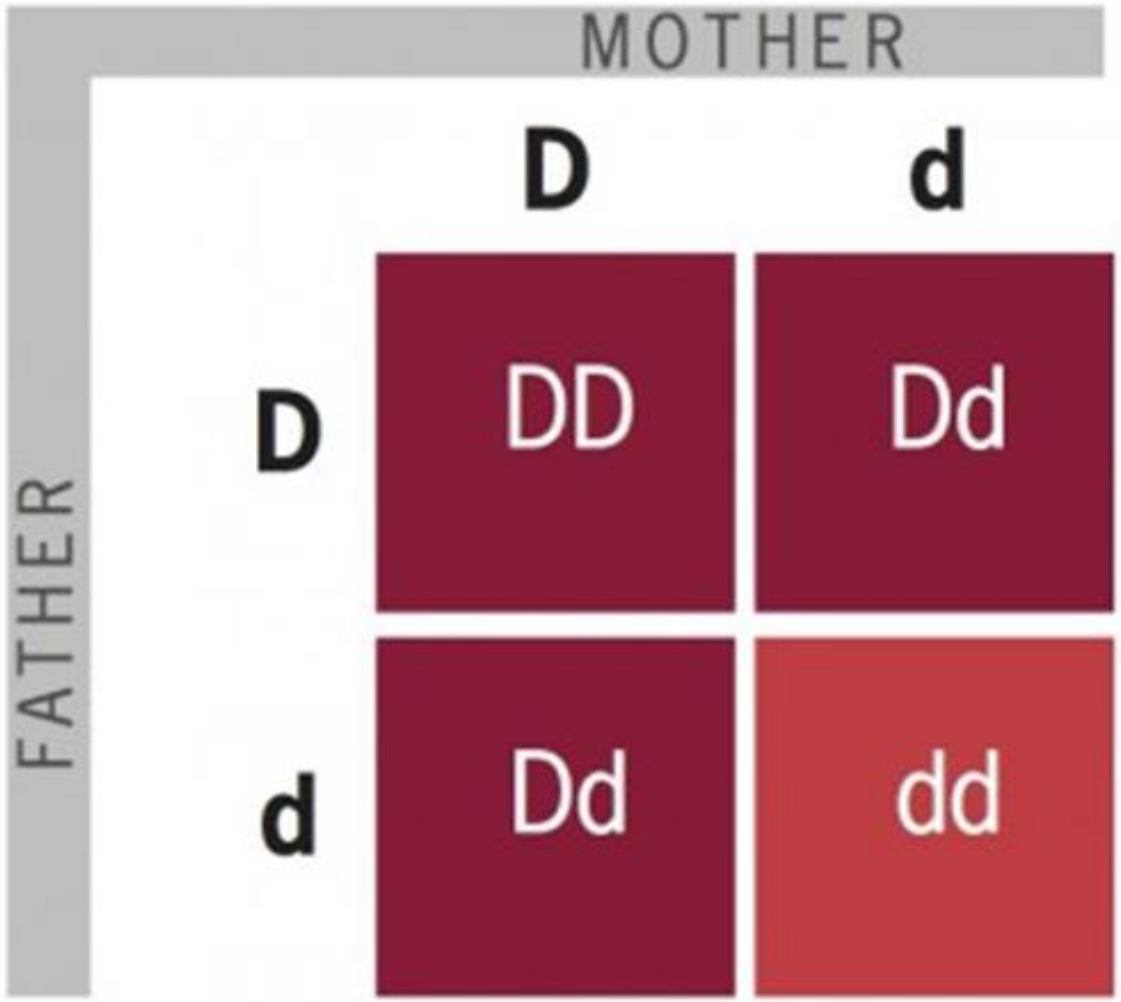
- Subsequently it was confirmed that the RH locus is on chromosome 1 and comprises two highly homologous, very closely linked genes, RHD and RHCE.
- The Rh blood group system consists of 49 defined blood group antigens, among which the five antigens (D, C, c, E, and e) are the most important.
- There is no d antigen. D antigen is the main that its presence or absence mean Rh+ or Rh- respectively.
- The main antigens are D, C, E, c and e, which are encoded by two adjacent gene loci, the RHD gene which encodes the RhD protein with the D antigen and the RHCE gene which encodes the RhCE protein with the C, E, c and e antigens
- The RHCE gene has four main alleles; CE, Ce, ce and cE.
- This concept of D and CcEe genes linked closely and transmitted together is consistent with the Fisher nomenclature.

**Examples on antigens in Rh+ and Rh -**

D− C+ E+ c− e+ (RhD−)

D+ C+ E− c− e+ (RhD+)

- ✓ Each locus has its own set of alleles which are Dd , Cc , and Ee . The D gene is dominant to the d gene, but Cc and Ee are co-dominant (meaning that all of the inherited alleles lead to expression of the coded antigens).
- ✓ Antibodies to Rh antigens can be involved in hemolytic transfusion reactions and antibodies to the Rh(D) antigens confer significant risk of hemolytic disease of the fetus and newborn.


MOTHER
D
d
FATHER
D
d
DD
Dd
Dd
dd

# Rh System

- **Antibodies**

✓ Antibodies directed against all Rh antigens, except d, have been described: anti-D, anti-C, anti-c, anti-E and anti-e.

✓ Rh antigens are restricted to red cells and Rh antibodies result from previous alloimmunization by previous pregnancy or transfusion.

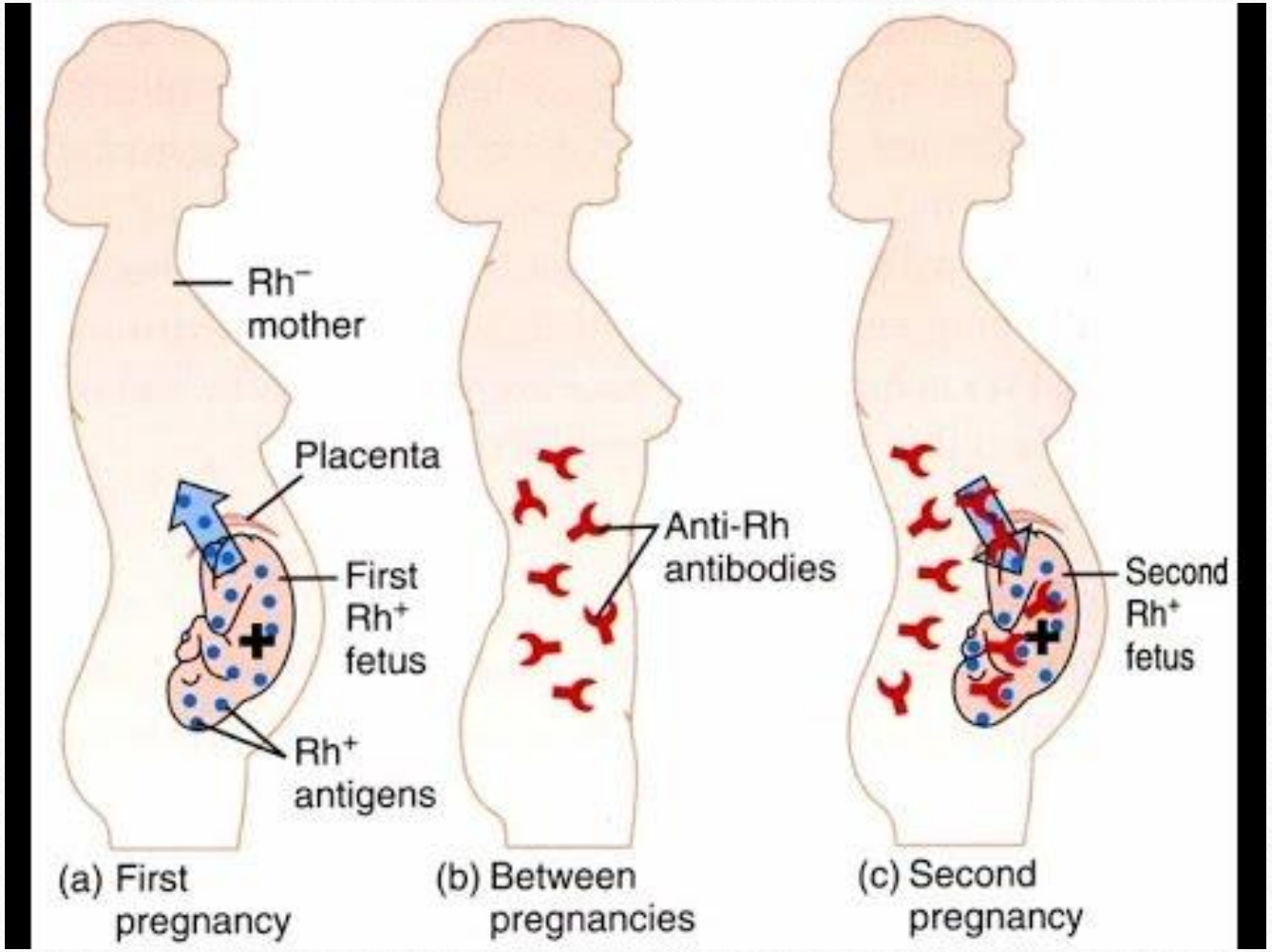
✓ Immune Rh antibodies are predominantly IgG.

# …Rh Antibodies

- ✓ Anti-D is clinically the most important antibody.
- ✓ It may cause hemolytic transfusion reactions and was a common cause of fetal death resulting from hemolytic disease of the newborn before the introduction of anti-D prophylaxis.

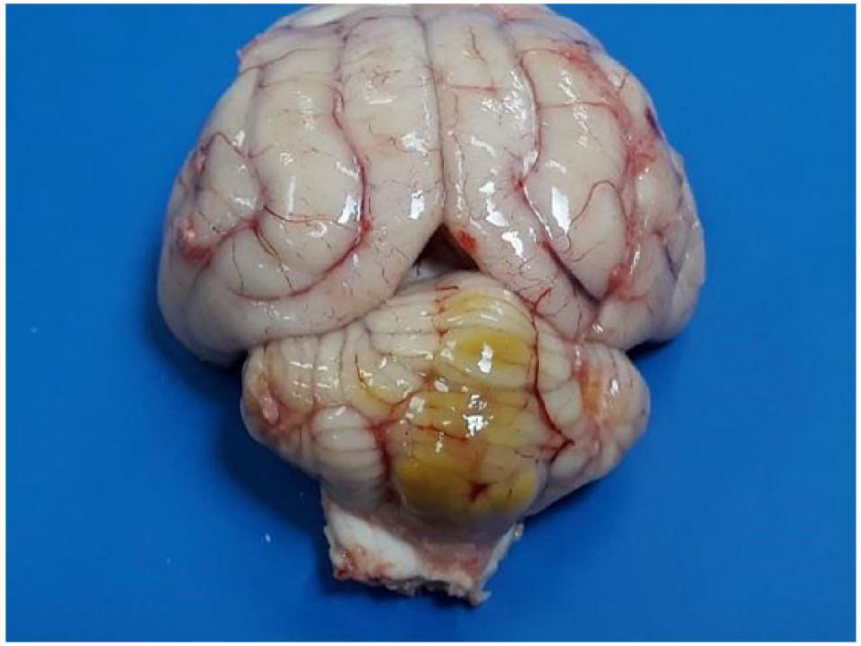
# *hemolytic disease of the newborn*

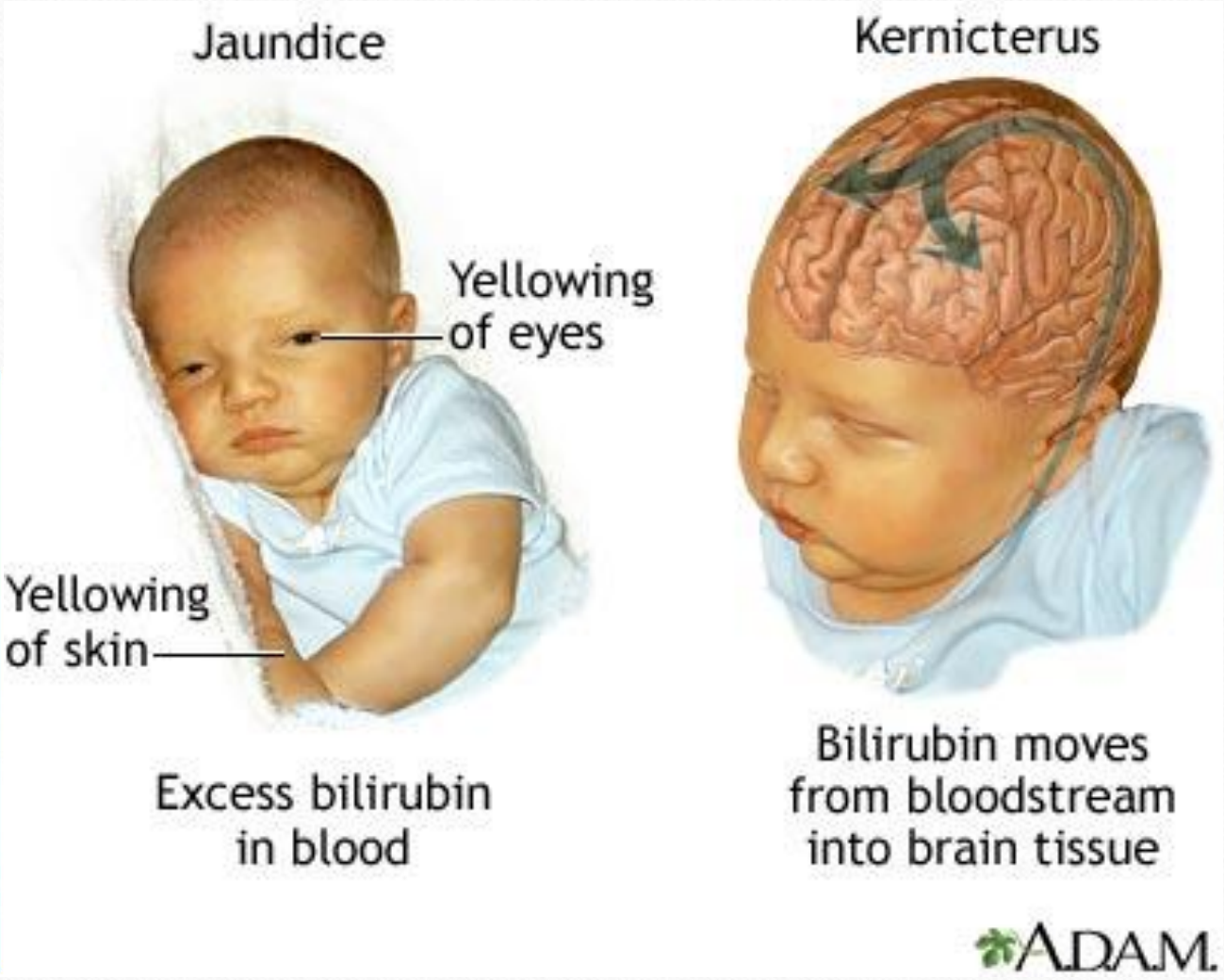
- When the condition is caused by the RhD antigen-antibody incompatibility, it is called Rh D Hemolytic disease of the newborn.
- The major clinical significance of anti-Rh antibodies is related to hemolytic reactions associated with pregnancy that are similar to transfusion reactions.
- (**Rh-negative mothers**) carrying an Rh-positive fetus can be sensitized by fetal red blood cells that enter the maternal circulation, usually during childbirth. IgG antibodies are generated in Rh-negative mothers.
- Subsequent pregnancies in which the fetus is Rh positive are at risk because the maternal (anti-Rh D) IgG antibodies can cross the placenta and mediate the destruction of the fetal red blood cells. This causes anemia, dyspnea, jaundice and erythroblastosis fetalis.




**Erythroblastosis Fetalis**

**Kernicterus:** is a type of brain damage that can result from high levels of bilirubin in a baby's blood.





# 3- Coombs test

- The key component of agglutination in coombs test is antibody to human globulin that is made in animals or by means of hybridoma techniques (Coombs reagent).
- The ***direct antiglobulin test ( coombs test)*** is used to demonstrate in vivo attachment of antibody to an individual's red blood cells.
- Patient RBC sample is mixed with coombs reagent, if agglutination occurs this mean that the RBCs have antibody on their surfaces (sensitized)
- This test serves as an indicator of :

✓ autoimmune hemolytic anemia

✓ hemolytic disease of the newborn

✓ sensitization of red blood cells caused by the presence of drugs, or a transfusion reaction.

# 5-Indirect coombs test

- The ***indirect antiglobulin test (indirect coombs test)*** is used to determine the presence of a particular antibody in a patient.

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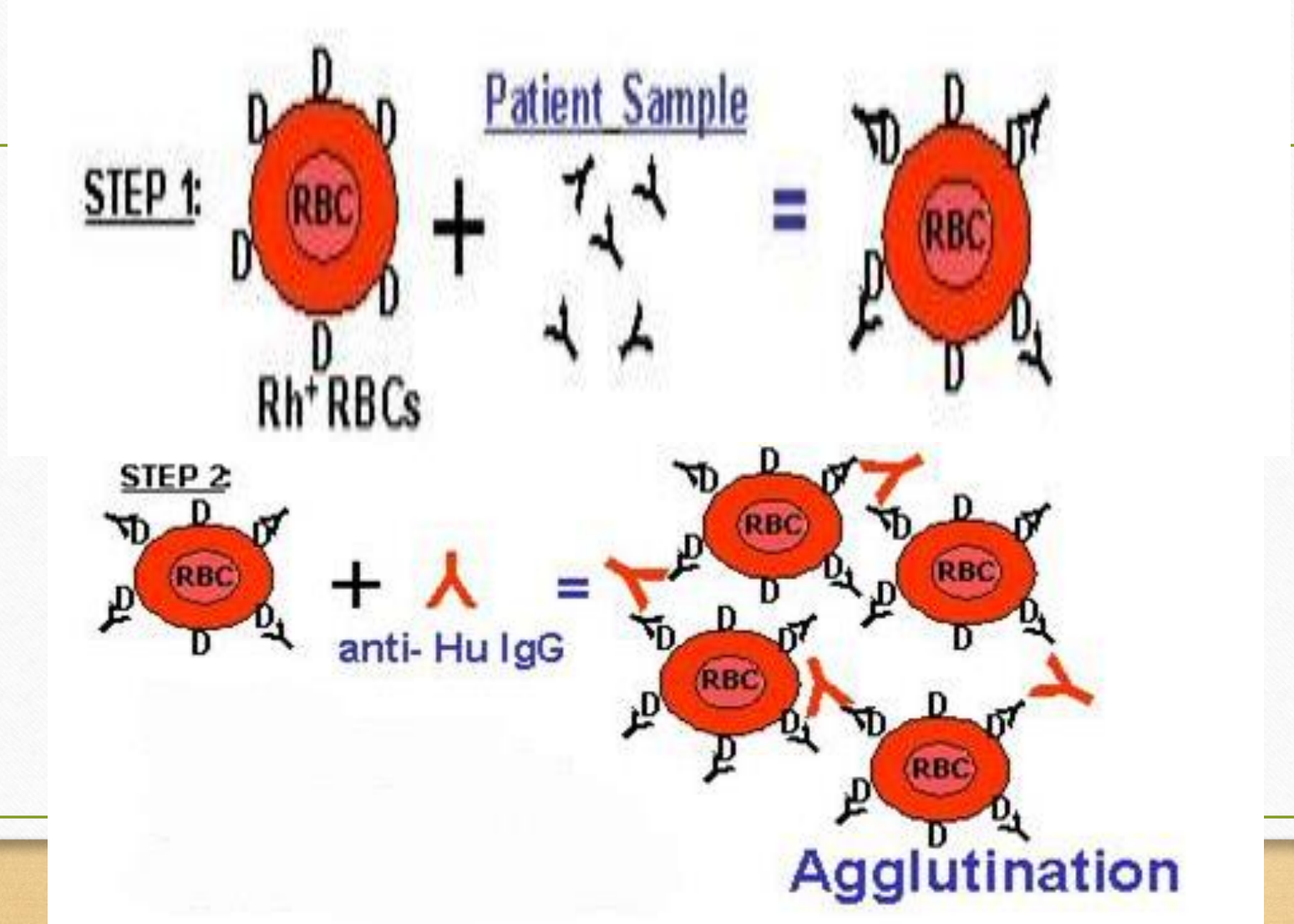
- This is a two-step process.
- Patient serum is mixed with RBC have the RH antigen, and the cells are then carefully washed again to remove any unbound antibody.
- coombs reagent is added, a visible reaction occurs if the patient is positive for the antibody
- is used In antenatal care, to screen pregnant women for antibodies that may cause hemolytic disease of the newborn , Pregnant serum + RH+ RBC
- and to screen for antibodies in the preparation of blood for blood transfusion (Cross match). Recipient serum + donor RBC

# cross match

- To perform a cross match, a small amount of the recipient's serum is mixed with a small amount of the donor RBCs. The mixture is then examined under a microscope. If the proposed transfusion is incompatible, the donor RBCs are agglutinated by antibodies in the recipient's serum.

In Preparation for blood transfusion: recipient serum mixed with



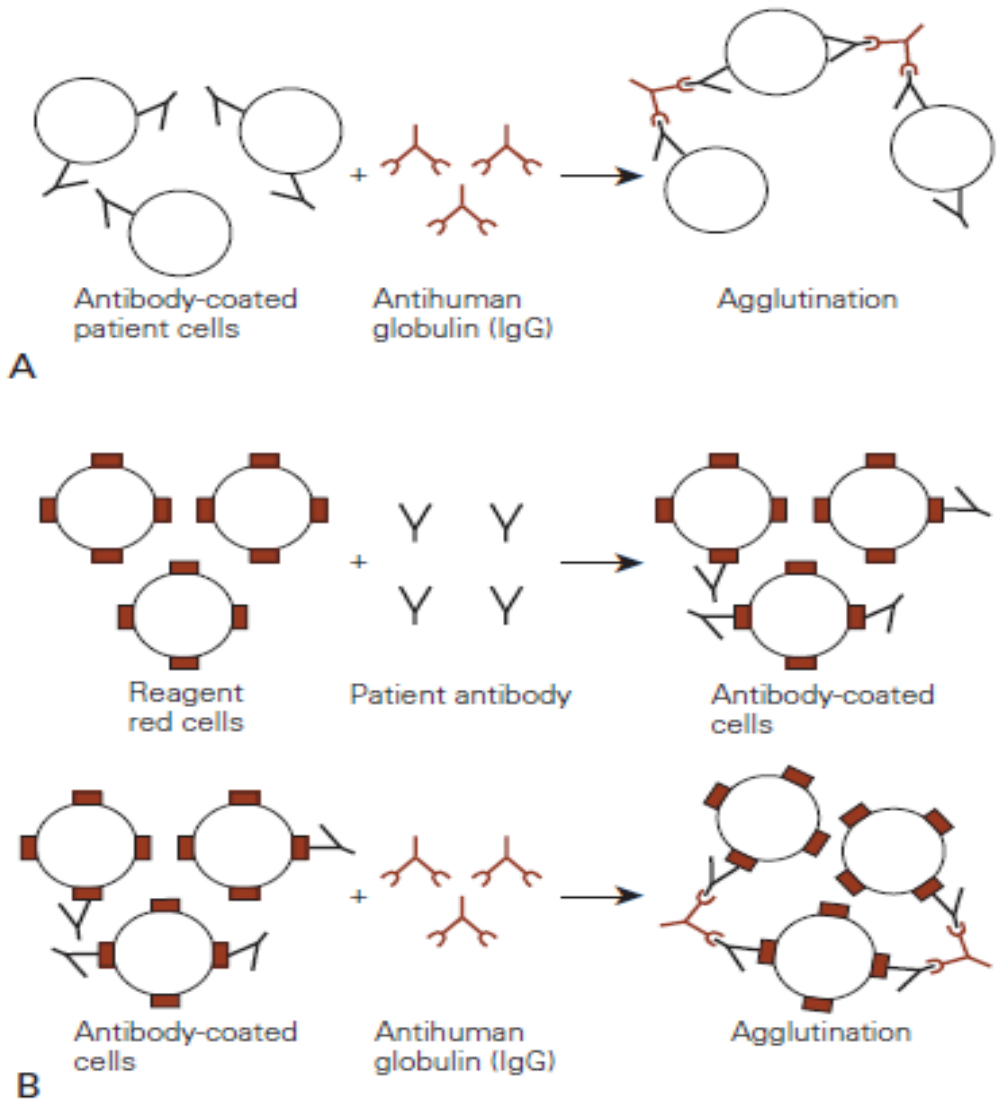



FIGURE 9–6. Direct and indirect antiglobulin tests. *(A)* Direct antiglobulin test (DAT). Antihuman globulin is combined with patient cells that have become coated with antibody in vivo. *(B)* Indirect antiglobulin test (IAT). Reagent cells are reacted with patient antibody. These are washed, and then antihuman globulin is added to enhance agglutination.