

Blood Biochemistry BCH 471[Practical]

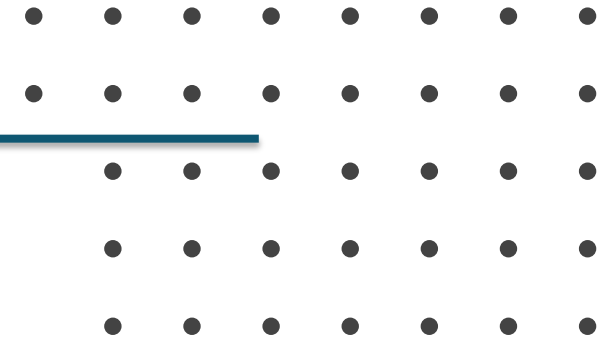
**Lab (3) ABO Blood Grouping & Rh Groups**



# Objectives

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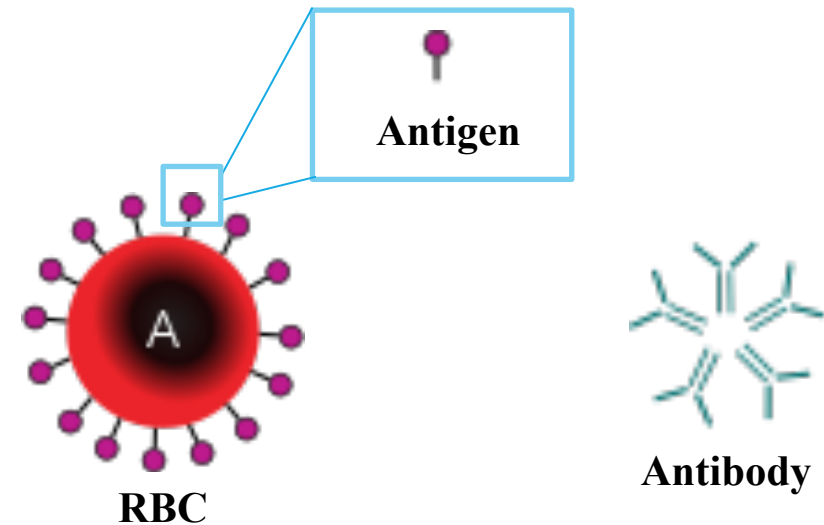
1. To determine the blood group according to the **ABO system**.
2. To test for the availability of the **Rh factor (D antigen)**.



# Blood Group Substances

Remember !!  
Antigen X Antibody

- The differences in human blood are due to the presence or absence of certain protein molecules called **antigens** and **antibodies**.
- The **antigens** are glycoproteins located on the surface of the red blood cells.
- The **antibodies** are proteins present in the plasma to attack foreign antigens, resulting in clumping (**agglutination**).
- **ABO blood grouping consists of:**
  1. Two antigens (**A & B**) on the surface of the RBCs
  2. Two antibodies in the plasma (**anti-A & anti-B**)


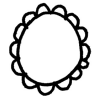
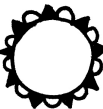



Remember !!  
Antigen X Antibody

# ABO Blood Type System

- The **ABO** blood type system is the **major** blood type classification system.
- The **four blood types** in the ABO system (**A**, **B**, **AB**, and **O**) refer to different versions of **glycoproteins** which are present on the surface of RBCs.

## Blood Types:

Blood Type	Surface antigens	Plasma antibodies	
Type A	A-surface antigens	Anti-B	
Type B	B-surface antigens	Anti-A	
Type AB	A and B antigens	No antibodies	 → Universal Recipient
Type O	No surface antigens	Anti-A and anti-B	

↓  
Universal Donor

## Importance of The ABO System

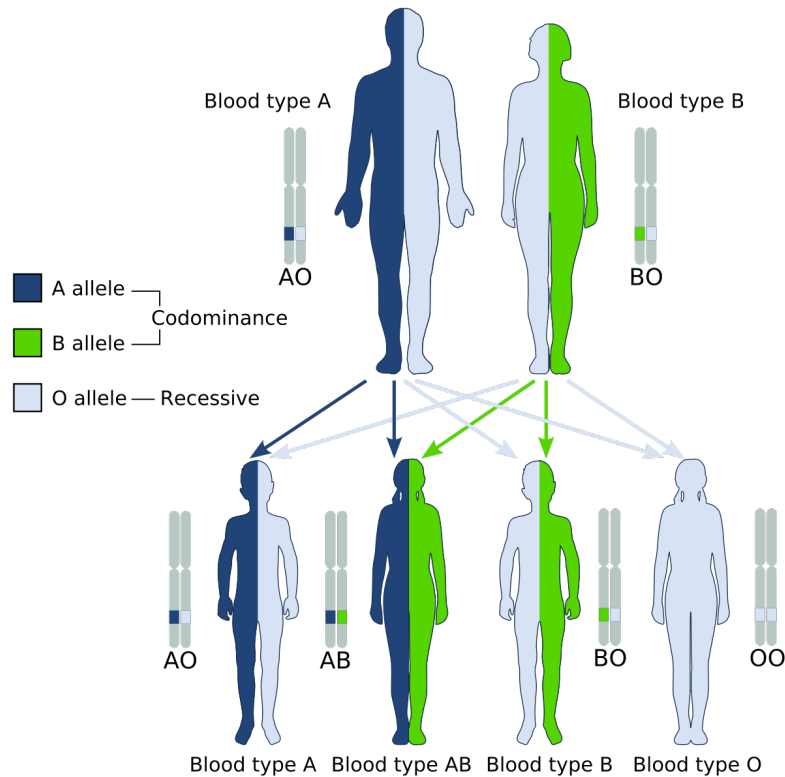
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- Blood group antigens must be determined to secure a safe practice of **blood transfusion**.
- They are also useful in determining familial relationships in **forensic medicine**.



# Genetics of Blood Types

- Individuals inherit a **gene** which codes for specific antigen(s) to be added to the red cell.
- You have two copies of this gene, one inherited from your **mother** and the other inherited from your **father**.
- There are **3** versions 'alleles' for blood type: **A**, **B** and **O**.
- Since we have **2 genes**, there are **6 possible combinations (genotypes)**.
- The **A** and **B** genes are **dominant** and the **O** gene is **recessive**.



Father	Mother			Genotypes	Blood type
	A	B	O		
A	AA	AB	AO	A+A A+O	A A
B	BA	BB	BO	A+B B+B B+O	AB B B
O	OA	OB	OO	O+O	O

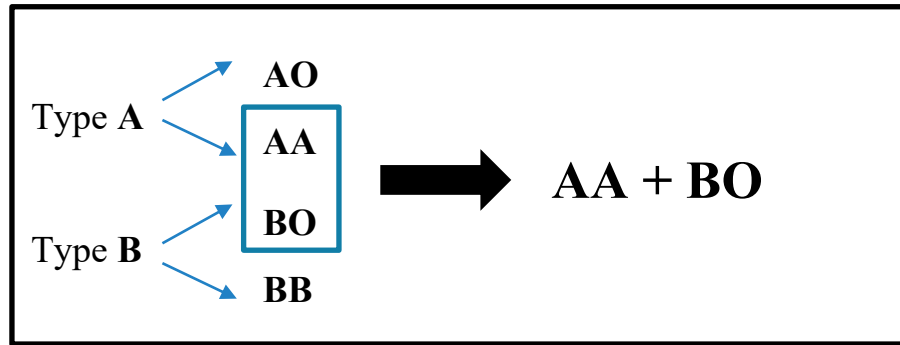
## Codominance (AB blood group)

Is a condition in which the alleles of a gene pair in a heterozygote are fully expressed thereby resulting in offspring with a phenotype that is neither dominant nor recessive.

Let's assume a female with blood type A married a male with blood type B.

What is the possible blood type combinations of their children?

1<sup>st</sup> Determine genotype:



2<sup>nd</sup> Determine combination using Punnett square:

	<b>A</b>	<b>A</b>
<b>B</b>	AB	AB
<b>O</b>	AO	AO

3<sup>rd</sup> Children's possible blood types:

Genotypes	Blood type
A+A	A
A+O	A
A+B	AB
B+B	B
B+O	B
O+O	O

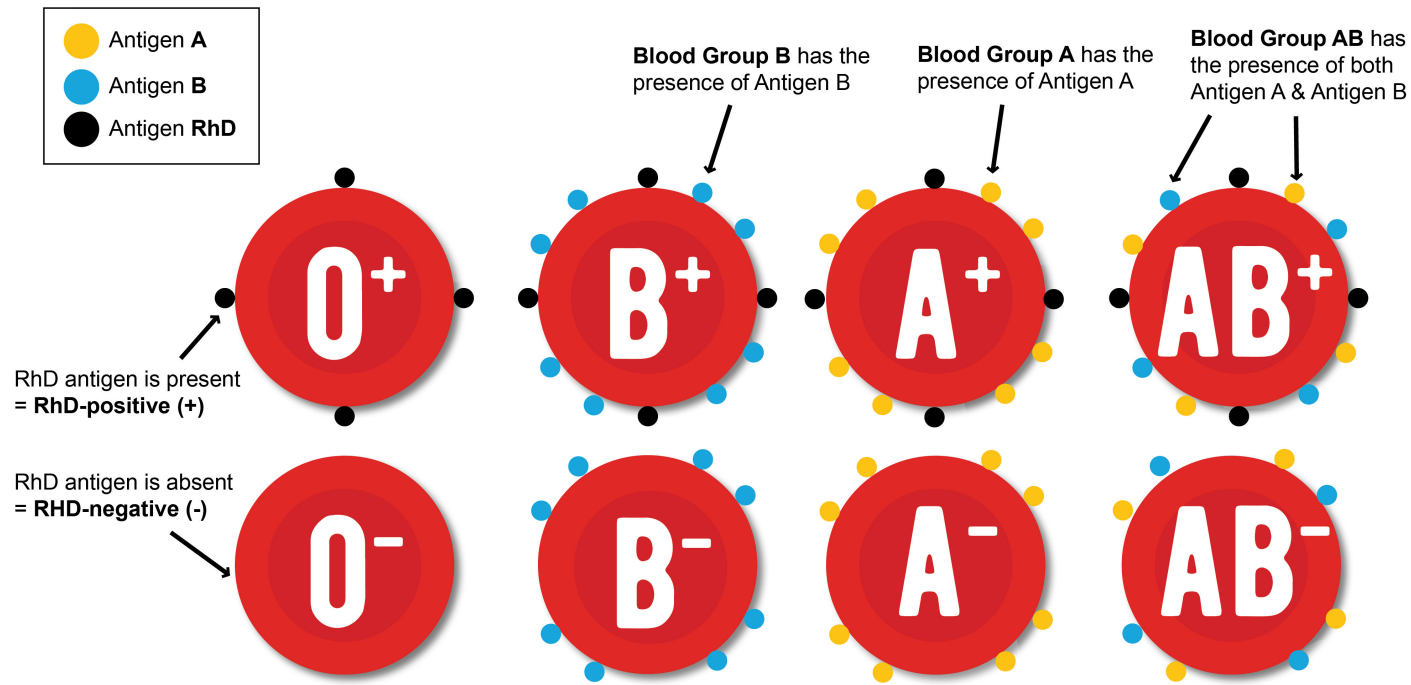
**“AB and A”**



# Rhesus Blood Group



- First studied in *rhesus* monkeys.
- Is the **second most significant** blood group system in human transfusion.
- The **D** antigen (**RhD**) is the most important.
- If it is present on RBCs' surface, the blood is RhD positive (~80% of the population), if not it's RhD negative.



If people with **group A** have it, and will therefore be classed as **A+** (or **A positive**), while the ones that don't, are **A-** (or **A negative**) and so it goes for groups B, AB and O.

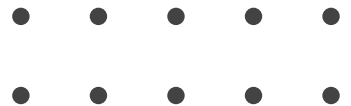


# Rh Blood Group Transfusion

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- **A person with Rh<sup>+</sup>** blood can receive blood from a person with Rh<sup>-</sup> blood without any problems.
- **A person with Rh<sup>-</sup>** blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh<sup>+</sup> blood, whose Rh antigens can trigger the production of Rh antibodies.

Blood Type	Surface antigens	Plasma antibodies
Positive	D antigen	No antibodies
Negative	No surface antigens	Anti-D



# Blood Types Compatibility

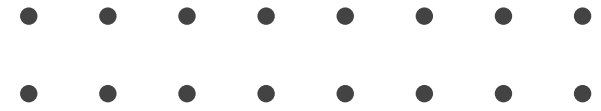
Red Blood Cells Compatibility Table

Recipient	Donor							
	O-	O+	A-	A+	B-	B+	AB-	AB+
O-	✓	✗	✗	✗	✗	✗	✗	✗
O+	✓	✓	✗	✗	✗	✗	✗	✗
A-	✓	✗	✓	✗	✗	✗	✗	✗
A+	✓	✓	✓	✓	✗	✗	✗	✗
B-	✓	✗	✗	✗	✓	✗	✗	✗
B+	✓	✓	✗	✗	✓	✓	✗	✗
AB-	✓	✗	✓	✗	✓	✗	✓	✗
AB+	✓	✓	✓	✓	✓	✓	✓	✓

Universal Donor

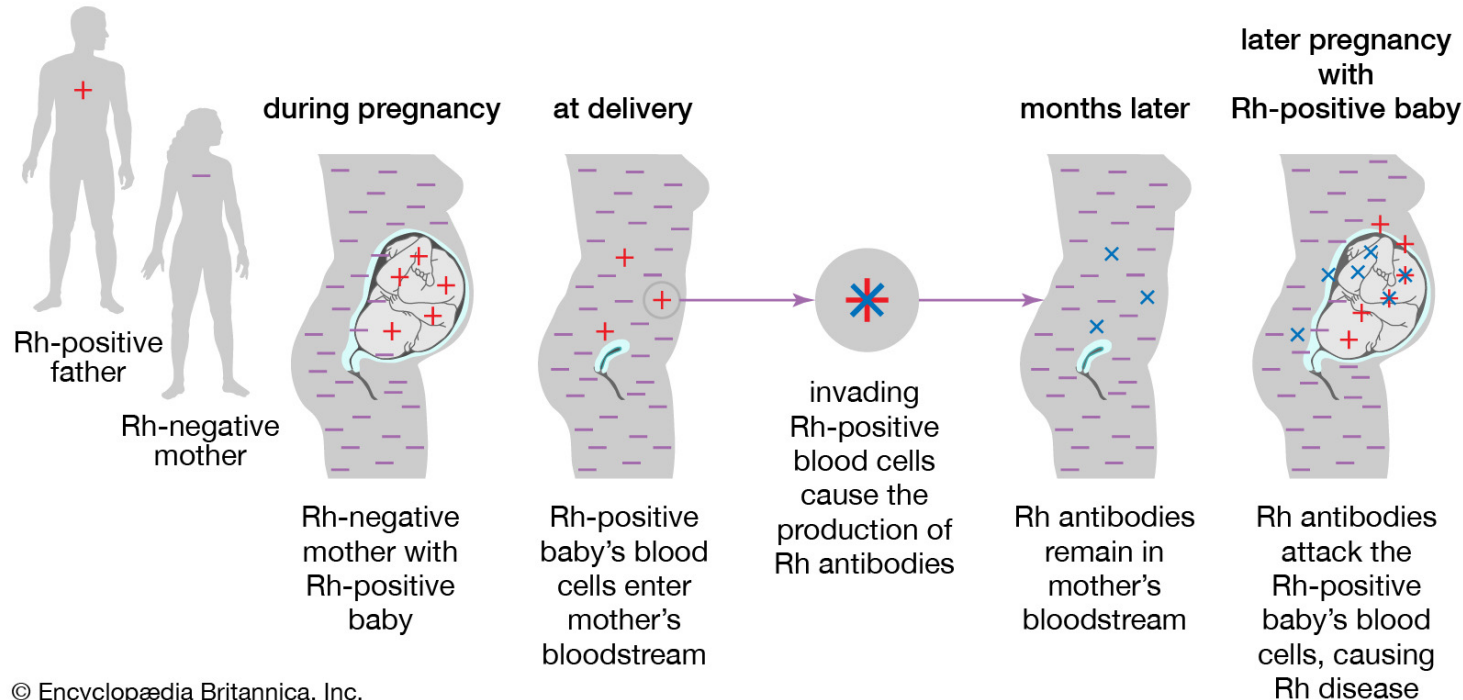
Universal Recipient

# Hemolytic Disease of The Newborn (HDN)



- Also called, **Erythroblastosis Fetalis** a **hemolytic anemia** in the fetus or neonate, caused by trans-placental transmission of maternal antibodies to fetal RBCs.
- Mother is Blood type **Rh<sup>-</sup>**, Father and fetus are **Rh<sup>+</sup>**.
- **First pregnancy** = Sensitization at delivery due to hemorrhage.
- **Second pregnancy** = Mother produce anti-Rh IgG antibodies that cross placenta to attack fetal RBCs leading to

hemolysis.

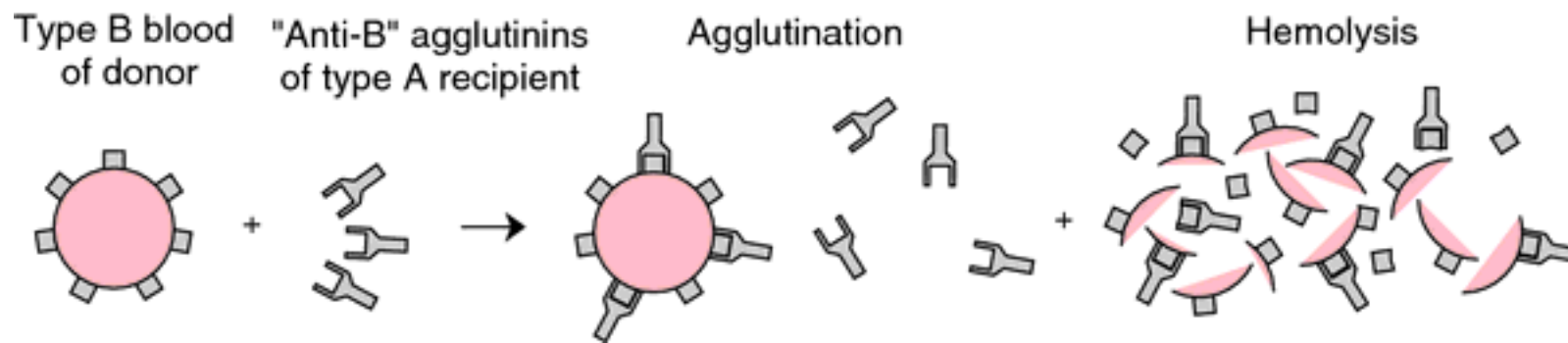


# Practical Part

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# Principle of Test

- The **ABO** and **Rh blood** grouping system is based on **agglutination reaction**.
- **Agglutination** is the reaction **between antigens** present on red blood cells and **antibodies** present in serum **resulting in visible clumping**.
- **Agglutination** occurs if an antigen is mixed with its corresponding antibody, i.e. occurs when A antigen is mixed with anti-A or when B antigen is mixed with anti-B.

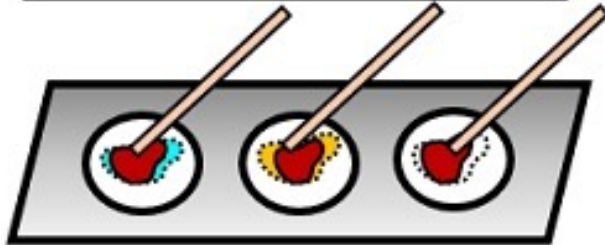


Blood grouping solutions



# Procedure

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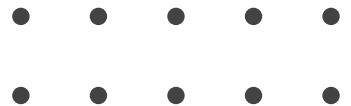


1. Add three drops of blood in a clean glass slide

2. Add antisera A, B and D sequentially to the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> drop of blood

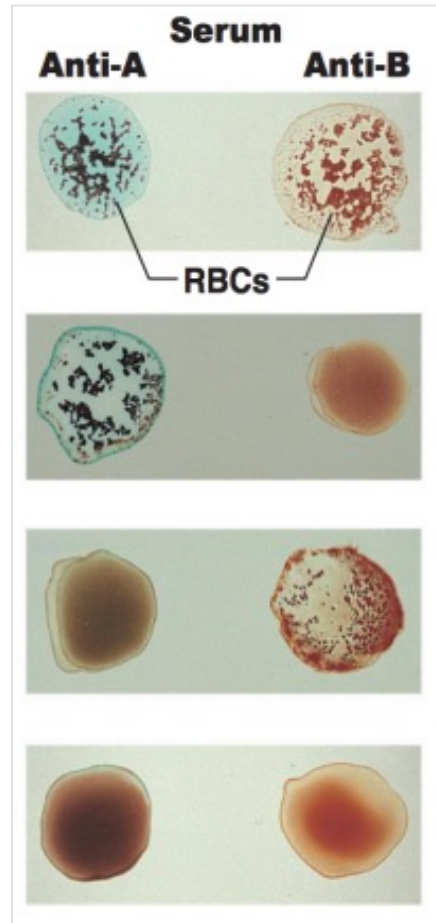
3. Properly mix the antisera with the blood by separate toothpicks

4. Allow to stand for 2-3 minutes and note down the result on the basis of clump formation



# Results

BLOOD TYPE	ANTI-A	ANTI-B	ANTI-D
A	Agglutination	No agglutination	No agglutination
B	No agglutination	Agglutination	No agglutination
AB	Agglutination	Agglutination	No agglutination
O	No agglutination	No agglutination	Agglutination



- If the agglutination occurs in the RBCs to which **anti-A** is added, then the **blood group is 'A'**.
- If agglutination occurs in the RBCs to which **anti-B** is added, then the **blood group is 'B'**.
- If the agglutination occurs in the RBCs to which **both anti-A and B** is added, then the **blood group is 'AB'**.
- If there is **no agglutination** occurs in the RBCs, then the **blood group is 'O'**.

- If the **agglutination** occurs in the RBCs to which **anti-D** is added, then the blood type is **positive (+)** whereas if **no agglutination** occurs in the RBCs to which anti-D is added, then the blood type is **negative (-)**.

# Homework

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- Does blood type change with a **bone marrow transplant** in case of **leukemia patients**?  
If yes, why?
- How many human **blood group systems** are there? *Name five.*