

# Determination of ABO Blood Group and Rh Factor

## Introduction

The test to determine an individual's blood group is called ABO typing. It helps identify the specific antigens present on the surface of a person's red blood cells. The presence or absence of these antigens determines whether a person belongs to blood group A, B, AB, or O. The Rh factor test determines if the person is Rh-positive ( $Rh^+$ ) or Rh-negative ( $Rh^-$ ).

## **Antisera Use**

The antibodies (also called antisera) used in blood grouping are:

- Anti-A serum (Antiserum A) – detects A antigen
- Anti-B serum (Antiserum B) – detects B antigen
- Anti-D serum (Antiserum D) – detects the Rh (D) antigen

These reagents are commercially prepared and are mixed with the blood sample to observe reactions.



Figure: Antiseras

## How the Test is Performed

### **Step 1 – ABO Typing**

1. A drop of the individual's blood sample is placed on a clean glass slide or tile in three separate spots.

2. Add one drop of Anti-A serum to the first spot, one drop of Anti-B serum to the second spot, and one drop of Anti-D serum (for Rh factor) to the third spot.
3. Mix each spot gently with a separate mixing stick or applicator.
4. Observe the reactions under good lighting for about 1–2 minutes.

### Observation and Interpretation

Reaction	Observation	Interpretation (Blood Group)	Possible Genotypes
Clumping (agglutination) occurs only with <b>Anti-A serum</b>	Visible clumps form in Anti-A area, smooth in others	Blood group A	AA or AO
Clumping occurs only with Anti-B serum	Visible clumps form in Anti-B area, smooth in others	Blood group B	BB or BO
Clumping occurs with both Anti-A or Anti-B	Visible clumps in both A and B areas	Blood group AB	AB
No clumping with either Anti-A or Anti-B	All spots remain smooth	Blood group O	OO
Clumping occurs with Anti-D serum	Clumps form Rh spot	Rh positive (Rh+)	DD or Dd
No clumping with Anti-D serum	Smooth suspension	Rh negative (Rh-)	dd

### Step 2 : Back Typing (Confirmatory Test)

The serum (liquid part of the blood) is separated and mixed with known red blood cells of type A and type B.

People with type A blood have anti-B antibodies, while people with type B blood have anti-A antibodies.

People with type O blood contain both anti-A and anti-B antibodies, while type AB contains none.

This second test helps confirm the blood group from the first typing.

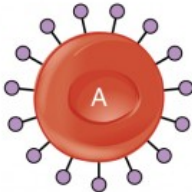
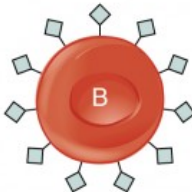
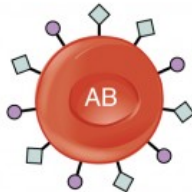







Blood Type				
	A	B	AB	O
Red Blood Cell Type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red blood Cell	 A antigen	 B antigen	 A and B antigens	None
Blood Types Compatible in an Emergency	A, O	B, O	A, B, AB, O (AB <sup>+</sup> is the universal recipient)	O (O is the universal donor)

Figure: This chart summarizes the characteristics of the blood types in the ABO blood group.

### ***Important Note***

There is no true colour change during this test.

The reaction is based on agglutination (clumping), not on a chemical colour change. The clumping may appear as small dark red clusters in the drop, while non-reactive areas remain smooth and evenly red.

The intensity of clumping may vary, but it is the presence or absence of clumping that determines the blood group, not any change in colour.

### **Conclusion**

The blood group and Rh factor of an individual are determined by observing agglutination reactions between the red blood cells and specific antisera. The pattern of reactions indicates whether the blood group is A, B, AB, or O, and whether the Rh factor is positive or negative.

## **Genotype Determination (AA, AS, AC, SS, CC, and others)**

Your genotype refers to the type of haemoglobin you inherited from your parents.

Haemoglobin is the protein in red blood cells that carries oxygen throughout your body.

There are different types of haemoglobin, such as A, S, and C, and the combination you have determines your genotype.

The most common genotypes are:

AA – Normal haemoglobin

AS – Sickle cell trait (carrier)

SS – Sickle cell anaemia

AC – Haemoglobin C trait (carrier)

CC – Haemoglobin C disease

### **How the Test is Performed**

A small blood sample is taken from the patient, usually into a purple-top (EDTA) tube.

The blood is then tested in the laboratory using one or more of the following methods:

- **1. Sickling Test**

This test checks if the blood cells contain sickle haemoglobin (HbS).

A chemical called sodium metabisulfite is added to a drop of blood on a glass slide.

This chemical removes oxygen from the blood. If HbS is present, the red blood cells lose their round shape and become sickle-shaped (crescent-like).

The slide is then covered with a coverslip and observed under a microscope.

### **Interpretation:**

If sickle-shaped cells are seen → HbS is present (AS or SS).

If all cells remain round → No HbS (AA, AC, or CC).

*There is no real colour change in this test, the result is based on the shape of the cells, not colour.*

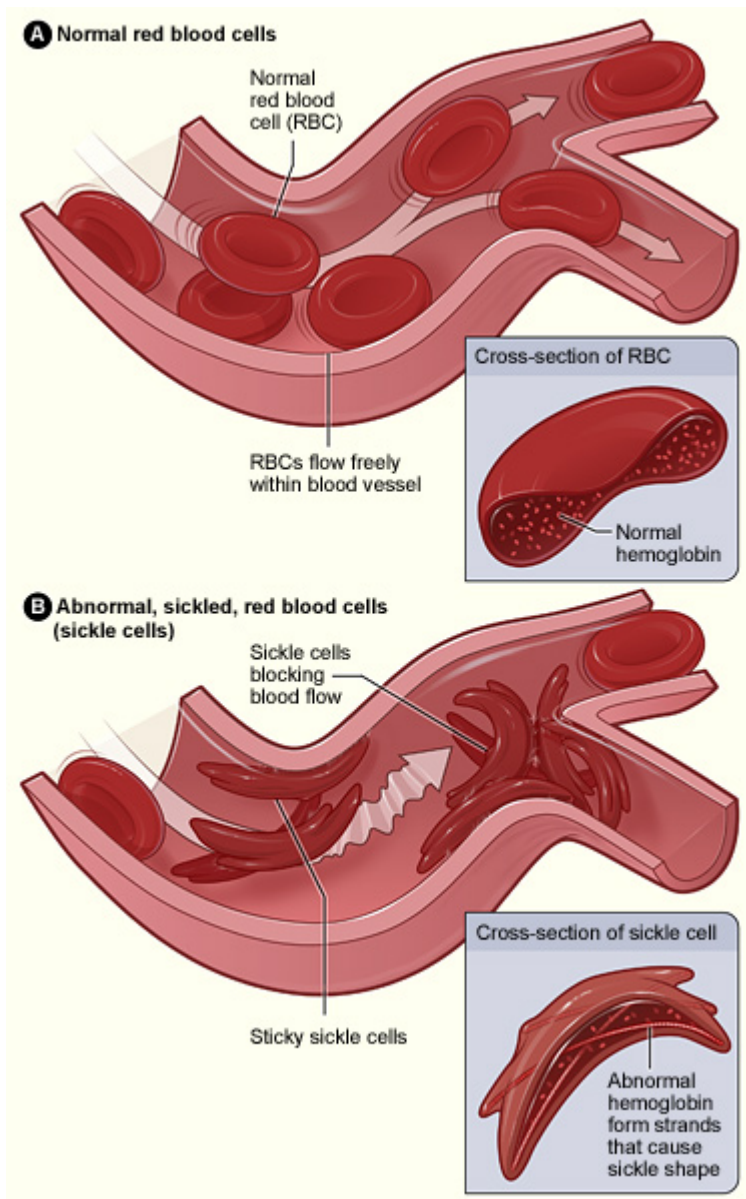


Figure: (A) Shows normal red

blood cells flowing freely through a blood vessel. The inset shows a cross section of a normal red blood cell with normal haemoglobin. Figure (B) shows abnormal, sickled red blood cells sticking at the branching point in a blood vessel. The inset image shows a cross section of a sickle cell with long polymerized sickle haemoglobin strands stretching and distorting the cell shape to look like a crescent moon.

- ## 2. Haemoglobin Solubility Test

This test also screens for the presence of HbS.

A special reagent (usually containing sodium dithionite) is added to a small amount of blood.

If HbS is present, it becomes insoluble and makes the mixture cloudy or turbid.

If HbS is not present, the solution remains clear.

### Interpretation:

Cloudy/Turbid → HbS present (AS or SS)

Clear → No HbS (AA, AC, or CC)

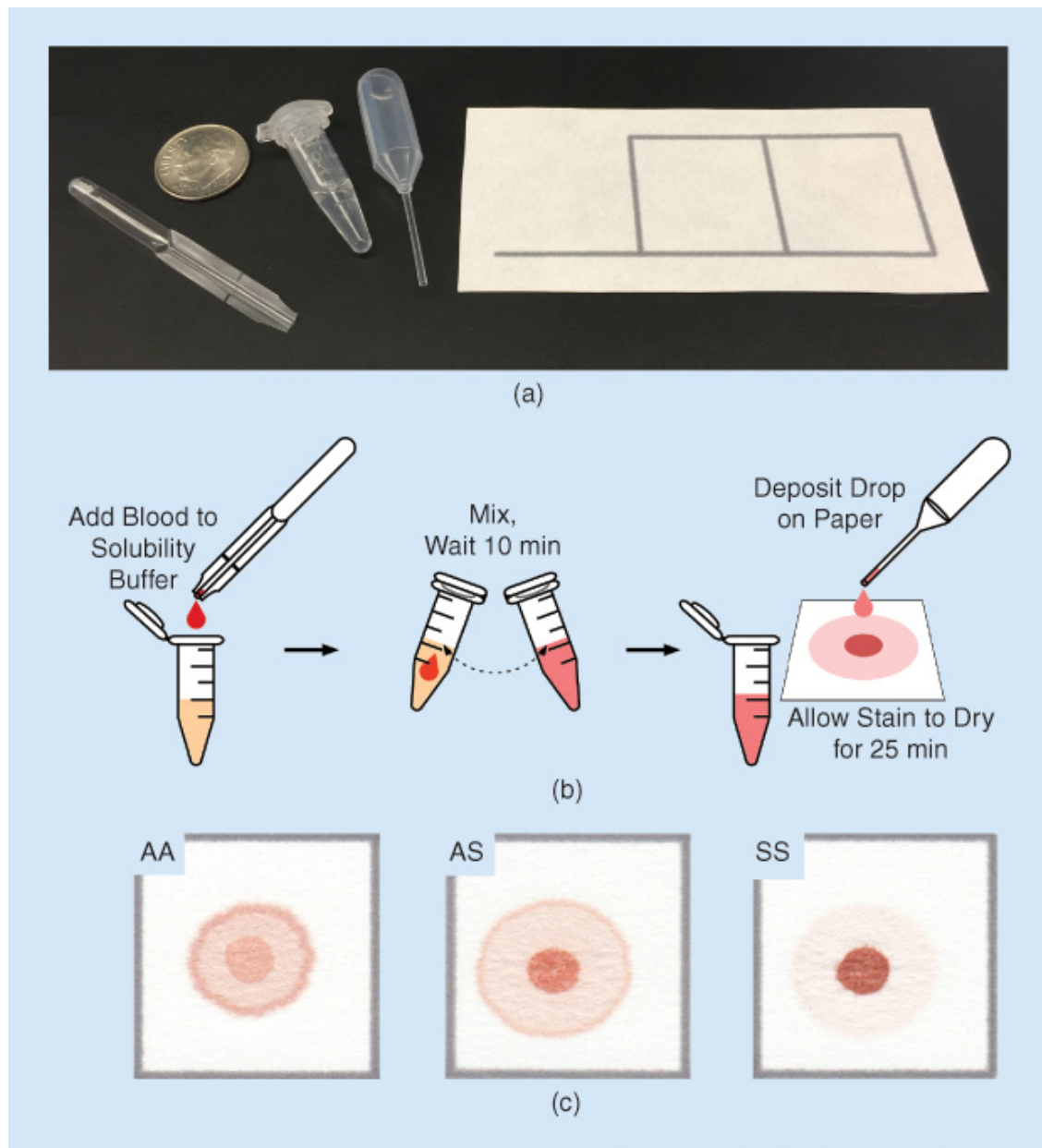


Figure: Paper based sickle cell disease test: (A) A photo of the necessary components, (B) An outline of the procedure, and (C) The results for typical adult blood (AA), sickle cell trait (AS)

*And Sickle cell disease (SS). The coin in (A) is shown for reference.*

*Again, there is no distinct colour change, only a difference in clarity of the mixture.*

- **3. Haemoglobin Electrophoresis**

This is the main confirmatory test for genotype.

A small portion of the blood is treated to release the haemoglobin.

The haemoglobin is then placed on a special gel (usually cellulose acetate) and an electric current is passed through it.

Different types of haemoglobin (A, S, C) move at different speeds on the gel, forming separate bands or lines.

*After staining the gel, the pattern of the bands is observed and compared to control samples.*

**Interpretation:**

AA: One band at A position

AS: Two bands (A and S)

SS: One band at S position

AC: Two bands (A and C)

CC: One band at C position

*There is no actual colour change in this test. The difference is seen in band positions after staining not in the colour of the blood itself.*

- **4. Other Methods**

In some laboratories, more advanced techniques such as Isoelectric Focusing (IEF) or High-Performance Liquid Chromatography (HPLC) are used.

These methods give a clearer separation and more accurate identification of the haemoglobin types.

## Summary

Genotype	Hb Type Present	Observation in Tests
AA	HbA only	Clear solubility, round cells, A band only.
AS	HbA + HbS	Cloudy solubility, few sickled cells, A and S bands
SS	HbS only	Cloudy solubility, many sickled cells S band only
AC	HbA + HbC	Clear solubility, round cells, A and C bands
CC	HbC only	Clear solubility, round cells, C band only