BLOOD GROUPING and Rh TYPING PRACTICES

1. *Introduction*

ABO grouping is a test performed to determine an individual's blood type. It is based on the premise that individuals have antigens on their red blood cells (RBCs) that correspond to the four main blood groups: A, B, O, and AB. The ABO and Rh blood grouping system is based on agglutination reaction and is regarded as the most important blood-group system in transfusion medicine because of severe hemolytic transfusion reactions and, to a lesser degree, hemolytic disease of the newborn.

2. *General Component Required*

<table>
<tr>
<td colspan="2">Capillary blood from finger prick with lancet or venous blood collected in EDTA anticoagulant
  </td>
<td>Anti A Sera
  </td>
<td>Anti B Sera
  </td>
<td>Anti RhD Sera
  </td>
</tr>
<tr>
<td>Rohan tubes
  </td>
<td>Disposable Mixing Stick
  </td>
<td>Clean glass slide or Tile
  </td>
<td>70% Ethanol & Cotton wool
 
  </td>
<td>Pasteur pipette
  </td>
</tr>
</table>

Figure 1: The required components of blood grouping and Rh typing

3. *Procedure for Blood Grouping*

3.1. <u>Tile or Slide Method</u>

1) Place 1 drop of anti A and 1 drop of anti B reagent, separately on a labelled slide or tile.
2) Add 1 drop of blood to each drop of the type typing antiserum.
3) The cells and reagent are mixed using a clean stick. Spread each mixture evenly on the slide or tile over area of 10-15mm diameter.
4) Tilt the slide (or) mix with stick and leave the test for 2-3 min at room temperature.
5) Look for agglutination with naked eye. It is minimal confirmed by microscope.
6) Result interpretation:

Positive result (+)	**Negative results (–)**
Little clumps of red cells are seen floating in a clear liquid.	Red cells are floating homogeneously in a uniform suspension.

Figure 2: Result interpretation of Blood Grouping (Slide Method)

Anti A	Anti B	Blood Type
+	–	A
–	+	B
+	+	AB
–	–	O

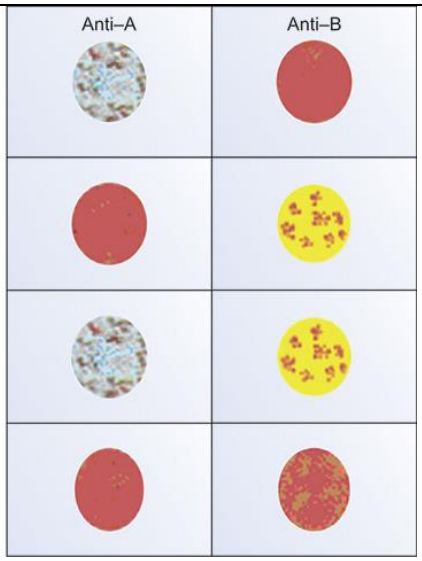


Figure 3: Interpretation of Blood Grouping (Slide Method)

3.2. Tube Method

In comparison to the slide method, the tube method is more sensitive and reliable; therefore, it can be used conveniently for blood transfusion. There are two types of tube method,

- Cell grouping (forward typing)
- Serum grouping (reverse typing)

3.2.1. Cell grouping: (forward typing)

1) Label properly the tubes.
2) Prepare a 2-5% cell suspension in saline from patient's unknown blood sample.
3) Take 3 test tubes 1, 2, 3 and put respectively a drop of anti-A, anti-B and anti-D serum to them.
4) Add one drop of red cell suspension in each test tube.
5) Centrifuge at approximate 1000- 1500rpm for 30-60 seconds.
6) Look for agglutination either with naked eye or under the microscope.
7) Result interpretation:

Positive result (+)
Little clumps of red cells are seen floating in a clear liquid.

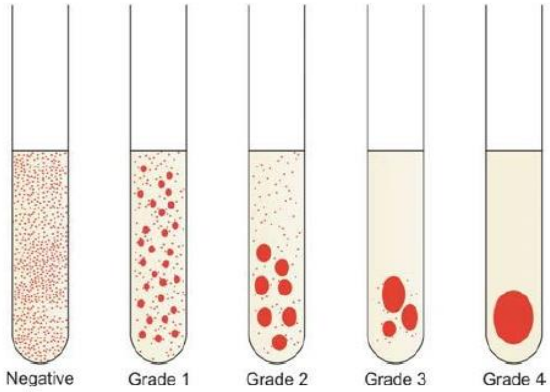
Negative results (–)
Red cells are floating homogeneously in a uniform suspension.

3.2.2. Serum grouping: (reverse typing)

1) Allow patient's unknown blood sample stand for some time and separate the serum.
2) Add 2 drops of unknown serum in 3 separate test tubes.
3) Add 1 drop of 2-5% cell suspension of known blood of A, B and O group into these test tubes.
4) Centrifuge at approximate 1000- 1500rpm for 30-60 seconds.
5) Look for agglutination either with naked eye or under the microscope.

3.2.3. Tube methods result interpretation

- Negative results (–) Test: Uniform suspension of red cells.
- Positive results (+) Test: Clumps of red cells suspended in a clear fluid. Agglutination in tube test is graded from 1+ to 4+ and read macroscopically.



Forward (Cell) Grouping		Reverse (Serum) Grouping		Interpretation
Anti A serum	Anti B serum	A1 Cells	B Cells	Blood Group
+	-	-	+	A
-	+	+	-	B
+	+	-	-	AB
-	-	+	+	O

Figure 3: Interpretation of Blood Grouping (Tube Method)

4. Procedure for Rh Typing

The Rhesus (Rh) group is the second most important blood group system, after the ABO blood group system. It is used to find out if you have a certain protein called Rh factor on the outer layer of your red blood cells. If you don't have Rh factor in your blood, you are Rh negative. If you do have Rh factor in your blood, you are Rh positive. Most people are Rh positive.

4.1. Slide Method

1) Place one drop of anti D serum on a pre-warmed glass slide.
2) Add one drop of 10% suspension of red blood cells (in case of anemic patients, use one drop of sedimented red cells) using a pasture pipette.
3) With the clean stick, mix cell-serum mixture well.
4) Tilt the slide back and forth and observe for agglutination.
5) Tests that show no agglutination within three to five minutes are considered negative.

4.2. Tube Method

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.

1) Prepare 5% washed red cell suspension of test sample.
2) Take three clean test tubes and label tubes 1 & 2 as “test” and tube 3 as “control”.

3) Place 1 drop of anti-D (D1) in tube 1 and 1 drop of anti-D (D2) in tube 2.
4) Place 1 drop of 22% bovine albumin / control in tube 3.
5) Add 1 drop of 5% test cell suspension to each tube.
6) Mix well, centrifuge at 1000 rpm for 1 min.
7) Resuspend cell button & look for agglutination.
8) Control tube should show no agglutination.

5. Result interpretation

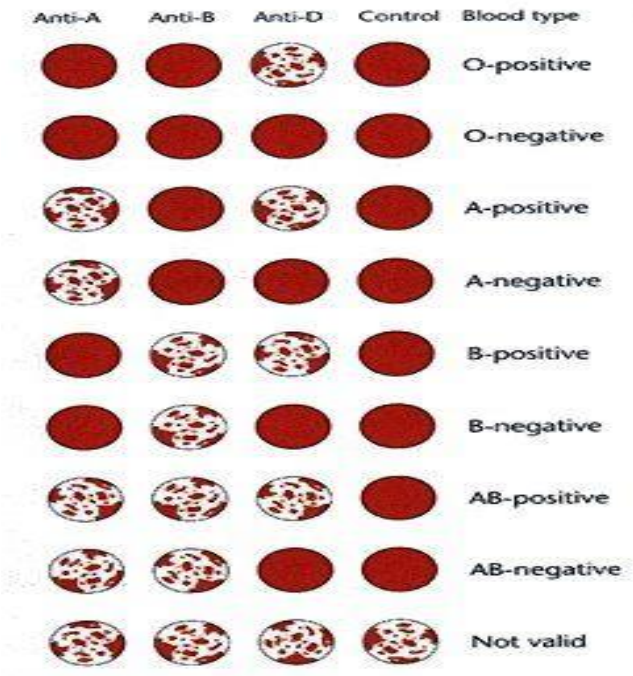


Figure 4 : Reading blood grouping results

Reference

- https://www.todaypk.video/youtube/tCzZ3Z-NI5o/amp
- University of Medical Technology University: Hematology General Practical Guidance on Blood Bank Handbook
- http://nbtc.naco.gov.in/assets/resources/training/5.pdf