

**Aim:** Determination of Blood Group

**References:**

1. Hoffman R, et al., editors. Hematology E-Book: Basic Principles and Practice. Elsevier Health Sciences; 2022 Oct 27.
2. Westhoff CM. Blood group genotyping. Blood, The Journal of the American Society of Hematology. 2019 Apr 25;133(17):1814-20.

**Objective:** To determine the blood group of an individual using the agglutination test, based on the reaction between antigens on red blood cells and antibodies in the serum.

**Introduction:** Human blood groups are classified into four types: A, B, AB, and O, based on the presence or absence of antigens (A and B) on the surface of red blood cells (RBCs). The determination of blood groups is essential for safe blood transfusions and organ transplants.

**Type A:** Has A antigens on RBCs and anti-B antibodies in the plasma.

**Type B:** Has B antigens on RBCs and anti-A antibodies in the plasma.

**Type AB:** Has both A and B antigens on RBCs and no anti-A or anti-B antibodies.

**Type O:** Has no A or B antigens on RBCs and has both anti-A and anti-B antibodies.

The Rh factor (either positive or negative) is also important in blood grouping, determined by the presence or absence of the Rh antigen on RBCs.

**Materials Required:** Blood sample (from the subject), Blood grouping serum (Anti-A, Anti-B, Anti-D), Microscope slides, Droppers, Toothpicks or disposable pipettes, Clean water or saline solution, Lancet or needle, Alcohol swabs, Cotton, Gloves

**Procedure:**

**1. Preparation of the Slide:** To begin, take a clean microscope slide and place it on a flat, stable surface. Using a marker or pencil, label three distinct areas on the slide, each designated for testing with different sera: Anti-A, Anti-B, and Anti-D. This preparation ensures that each test can be conducted separately while maintaining organization and clarity in the testing procedure.

**2. Blood Collection:** Next, clean the fingertip or earlobe of the subject using an alcohol swab to ensure the area is free of any contaminants. Afterward, use a lancet to gently prick the finger,

obtaining a small drop of blood. Using a dropper, carefully place a drop of blood onto each of the labeled areas on the prepared slide—Anti-A, Anti-B, and Anti-D—ensuring the blood samples are deposited accurately for further testing.

**3. Adding Reagents:** After placing the blood samples on the slide, proceed by adding the appropriate reagents. To the area labeled "Anti-A," add one drop of Anti-A serum. Similarly, place one drop of Anti-B serum onto the area labeled "Anti-B." Finally, add one drop of Anti-D serum to the area labeled "Anti-D." Each reagent will react with the corresponding antigen in the blood sample, setting the stage for the next steps in the testing procedure.

**4. Mixing the Reagents:** Use a toothpick to mix the blood and serum in each of the three areas, ensuring they are well combined.

**5. Observing Agglutination:** Gently rock the slide to mix the contents.

Observe the slide for any agglutination (clumping of red blood cells):

- Agglutination in the presence of Anti-A serum indicates blood group A.
- Agglutination in the presence of Anti-B serum indicates blood group B.
- Agglutination in the presence of both Anti-A and Anti-B sera indicates blood group AB.
- No agglutination with both Anti-A and Anti-B sera indicates blood group O.

**6. Determining Rh Factor:** Observe the reaction with Anti-D serum.

- Agglutination with Anti-D serum indicates Rh-positive (Rh+).
- No agglutination with Anti-D serum indicates Rh-negative (Rh-).

Based on the agglutination results with Anti-A, Anti-B, and Anti-D sera, the blood group (A, B, AB, or O) and Rh status (+ or -) of the individual can be determined.

**Example Data Table:**

Blood Sample	Anti-A Reaction	Anti-B Reaction	Anti-D Reaction	Blood Group
Sample 1	Agglutination	No Agglutination	Agglutination	A+
Sample 2	No Agglutination	Agglutination	No Agglutination	B-
Sample 3	Agglutination	Agglutination	No Agglutination	AB-
Sample 4	No Agglutination	No Agglutination	No Agglutination	O+

**Safety Precautions:**

1. Always wear gloves and protective eyewear while handling blood samples.
2. Dispose of lancets, needles, and other sharp instruments in a puncture-resistant container.
3. Sterilize the work surface with alcohol before and after the experiment.
4. Ensure that reagents are properly labeled and stored.

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