# Determination of A, B, O and Rh blood groups in human beings

## Aim:

The aim of this experiment is to determine the ABO and Rh blood groups in human subjects.

## Principle:

Blood groups are determined by the presence or absence of specific antigens on the surface of red blood cells (RBCs) and antibodies in the plasma. The ABO blood group system classifies blood into four main types: A, B, AB, and O, based on the presence or absence of A and B antigens. The Rh factor (Rhesus factor) determines whether the blood type is Rh-positive or Rh-negative.

## Materials and Methodology:

## Materials:

- Blood samples from human subjects
- Anti-A serum
- Anti-B serum
- Anti-Rh serum (Anti-D serum)
- Glass slides
- Droppers
- Alcohol swabs
- Lancets
- Gloves
- Safety goggles

**Methodology:**

1. **Sample Collection:**
    - Obtain blood samples from each subject using a lancet and collect a drop of blood.
2. **Slide Preparation:**
    - Label glass slides with the subject's identification.
    - Place a drop of blood on each slide.
3. **Testing for ABO Blood Group:**
    - Add anti-A serum to one blood drop and anti-B serum to another blood drop.
    - Mix the serum and blood gently using separate sticks or applicators.
    - Observe for agglutination (clumping) of RBCs:
        - If blood with anti-A serum clumps, the blood type is A.
        - If blood with anti-B serum clumps, the blood type is B.
        - If both anti-A and anti-B serums clump, the blood type is AB.
        - If neither serum clumps, the blood type is O.
4. **Testing for Rh (D) Factor:**
    - Add anti-Rh serum to a separate drop of blood.
    - Mix gently and observe for agglutination:
        - If agglutination occurs, the blood type is Rh-positive (Rh+).

- If no agglutination occurs, the blood type is Rh-negative (Rh-).

5. **Record Results:**
   - Record the blood type based on the observed reactions with the serums.

## 5. Detection of pH, sugar and protein in a sample of human urine.

### a) DETERMINATION OF pH OF URINE USING pH STRIPS:

**INTRODUCTION:**

The average pH of urine is, however, about 6.0 though it can range from acidic (pH4.6) to alkaline (pH8.0). The urine with pH below 5 is considered acidic and urine with pH higher than 8 is considered alkaline urine. The pH of human urine varies and depends on the diet. The acidic or alkaline pH of given sample (urine) can be determined by using pH strip, which is a special paper composed of different chemical compounds (dye) such as methyl red and bromothymol blue etc. The colour of the pH strip can be compared with a colour disc which shows the relevant pH value for the varying shades of colour.

**PRINCIPLE:**

To determine pH of the urine sample, a pH strip is dipped into given sample and the colour change is recorded. The change in colour is compared to the pH chart to determine approximate acidic or alkaline pH of the sample.

**MATERIALS REQUIRED:**

1. Urine sample
2. pH strip (Commercial available)
3. pH color chart

**pH strip or dipstick** is common diagnostic tool used to determine pH of urine sample (Fig. 7.1). A pH strip is already coloured and has about 10 different chemical pads or reagents which react to the sample when immersed in it.

**Fig. 7.1 : pH strips for urine test**

**PROCEDURE:**

1. Collect urine in a clean, dry container. Test the specimen as soon as possible. Do not add any preservatives.
2. Dip a pH strip into the container containing urine for few seconds (not more than 5 seconds).
3. Air dry the pH strip. Compare the colour of the strip with the colour chart given on the vial label under well-lit conditions.
4. While comparing, keep the strip horizontally to prevent possible mixing of chemicals if excessive urine is absorbed.
5. Discard the used pH strips.

**RESULT AND OBSERVATIONS:**

The change of the colour of pH strip to red colour indicates acidic pH of the urine while the appearance of blue indicates alkaline pH.

## b) To test the presence of sugar in the given sample of urine.

**Principle:** In normal urine, practically there is no glucose. Presence of glucose in urine is called glucosuria. To detect reducing sugars, such as glucose, fructose etc. in urine Benedict's or Fehling's tests are done.

$CuSO_4$ present in Benedict's solution or Fehling's solution is reduced on boiling by the reducing substances (glucose, fructose etc.) to form the coloured precipitate of cuprous oxide. The light green, green, yellow and brick red precipitates of cuprous oxides depend on the concentration of reducing substances present in urine.

Glucose reduces the blue cupric sulphate of Benedict's reagent or Fehling's reagent to a coloured insoluble precipitate.

$$\underset{\text{Glucose (reducing sugar)}}{CH_2OH(CHOH)_4\,CHO} + \underset{\text{Cupric sulphate (blue solution)}}{2Cu^{++}\,SO_4^{--}} + 2H_2O \xrightarrow{\text{heating}}$$

$$\underset{\text{Gluconic acid (oxidised sugar)}}{CH_2OH(CHOH)_4\,COOH} + \underset{\text{cuprous oxide (red precipitate)}}{Cu_2O} + 4H^+ + SO_4^{--}$$

**Requirement:** Glasswares: Test tubes, beakers, spirit lamp, pipette; Chemicals: Benedict's solution, Fehling's solution A and B, Seliwanoff's reagent, Miscellaneous: Test tube holder, test tube stand, urine sample.

### Preparation of Reagents

(i) Benedict's reagent Mix: 173 g of sodium citrate and 100 g of anhydrous sodium carbonate in 600 mL of water in a beaker and warm gently (solution A). Dissolve 17.3 g of hydrated $CuSO_4$ in 100 mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make upto the mark with water.

(ii) Fehling's reagent A: Dissolve 6.93 g of copper sulphate in 100 mL of distilled water.

(iii) Fehling's reagent B: 20 g of KOH and 34.6 g of sodium-potassium tartarate (Rochelle's salt) dissolved in 100 mL of distilled water.

## Procedure

**(a) Benedict's test**

- Take 5 mL of Benedict's reagent in a test tube. Add 0.5 mL (8 drops) of freshly passed urine to it.
- Boil for 2 minutes holding the test tube firmly with a test tube holder (during boiling, the contents of the test tube get a tendency to spurt out. Hence, it is wise to keep shaking the test tube after holding it in the inclined position near the flame to avoid overboiling).
- A light green, green, yellow and brick red precipitate indicates the presence of reducing substances in urine.
- The various coloured precipitates depend on the concentration of reducing sugars in urine which gives a rough estimate of the concentration given below:

| Colour of precipitate | % of reducing suger present |
|---|---|
| Light green ----- | 0.1 to 0.5 |
| Green ------ | 0.5 to 1.0 |
| Yellow ------ | 1.0 to 2.0 |
| Brick red ------ | above 2 |

**(b) Fehling's test**

- Take equal volumes (2 mL) of Fehling's solution A and B in a test tube. Mix them well.
- Add the above solution drop wise to 1 mL of urine sample taken in a test tube. Heat the test tube after each drop is added.
- A yellow or orange or brick red precipitate is formed which indicates the presence of reducing sugar in urine.

**RESULT AND OBSERVATIONS:**

c) To detect the presence of albumin protein in the given sample of urine.

**Principle:** Nitric acid causes the precipitation of albumin. When heated or treated with sulphosalicylic acid, albumin undergoes coagulation.

**Requirement:** Glasswares: Test tubes, graduated pipette (5 mL capacity), spirit lamp; Chemicals: Concentrated nitric acid, acetic acid, Robert's solution, sulphosalicylic acid or a solution containing 13% salicylic acid and 20% sulphuric acid; Miscellaneous: test tube stand, test tube holder.

## Procedure

**(a) Nitric acid ring test**

- Take 5 mL of concentrated nitric acid in a test tube.
- Incline the tube and add the urine sample with a dropper, so that the latter flows down slowly along the side of the test tube to form a separate layer.
- A white ring develops at the junction of the two liquids which indicates the presence of albumin in the urine sample.

**OR**

- Take about 5 mL of Robert's solution in a test tube.
- Now incline the test tube and add 2 to 3 mL of the given sample of urine by means of a dropper along the inner side of the test tube so that it forms a layer over the Robert's solution.
- The presence of white ring at the junction of two layers indicates the presence of albumin in the sample.

**(b) Heat coagulation test**

- Take about 6 to 8 mL of urine in a test tube.
- Incline the test tube at an angle and heat the upper one-third of the test tube by a low flame.
- Turbidity develops in the heated portion of the urine.
- Add 1% acetic acid drop by drop and boil or simply add a drop of 33% acetic acid.
- If the turbidity persists it confirms the presence of albumin in the urine sample (disappearance of turbidity, confirms the presence of phosphates).

**(c) Sulphosalicylic acid test**

- Take 3 mL of urine in a test tube.
- Add a few drops of sulphosalicylic acid and heat it gently.
- A whitish or cloudy turbid solution or precipitate (coagulation) in the solution indicates the presence of albumin in the urine sample.