



EXPERIMENT

13

Aim

To detect the presence of bile salts in urine.

THEORY

Liver, the largest gland of the body produces bile. Bile salts are formed by conjugation of glycolic acid and taurocholic acid with sodium or potassium salts. The colour of urine appears dark yellow or reddish due to the abnormal presence of bile salts in it when a person suffers from jaundice.

URINE ANALYSIS FOR BILE SALTS

Various tests can be performed for detection of bile salts in urine sample, but among all these, Fouchet's test is considered as a confirmatory test. In this test, the colourless bilirubin is oxidised by the ferric ion of ferric chloride (present in Fouchet reagent) to green biliverdin. This can be shown as follows:

MATERIAL REQUIRED

Test tubes, 10 ml measuring cylinders, funnel, dropping pipette or drop bottle, test tube stand, test tube holder, cotton, filter paper, distilled water, urine sample, Lugol's iodine solution, barium chloride solution (10%), Fouchet reagent, Tincture iodine, trichloroacetic acid, ferric chloride, ethyl alcohol, concentrated nitric acid, conc. H_2SO_4 and sucrose.

PROCEDURE AND OBSERVATIONS

1. Lugol's Iodine Test

- (i) Take 4 ml of urine sample in a test tube.
- (ii) Add 4 drops of Lugol's iodine solution in it with the help of a dropper.
- (iii) Shake the tube well.
- (iv) Light to dark green colour ring indicates the presence of bile pigments, while faint yellow to brown colour indicates the absence of bile pigments.

2. Smith's Reagent Test

- (i) Take 1-3 ml of Smith's reagent in a test tube. Tilt the test tube and add urine along the inner sides of the test tube.
- (ii) A green-coloured ring appears at the junction of the two layers. This indicates the presence of bile pigments in the urine sample.

3. Gmelin's Test

- (i) Take 5 ml of conc. HNO_3 in a test tube.
- (ii) Add an equal volume of urine to it slowly along the sides of the test tube.
- (iii) The presence of bile pigments in the given urine sample is indicated by the formation of a green, blue, yellow or red ring at the junction of the two solutions.

Note Bile pigments present in urine sample react with conc. nitric acid to form a coloured ring at the junction between urine and acid layer.

4. Fouchet's Test

- (i) Take 5 ml of urine sample.
- (ii) Add 2-5 ml of BaCl_2 in the test tube and shake it well to mix the two solutions.
- (iii) A precipitate is formed. Filter the mixture with the help of filter paper.
- (iv) Add 2 drops of Fouchet reagent to the precipitate present on filter paper.
- (v) The precipitate turns green which shows the presence of bile pigments.

5. Pattern Raffo's Test

- (i) Take 2 ml of urine sample and little amount of sucrose in a test tube.
- (ii) Pour 2 ml of conc. H_2SO_4 (sulphuric acid) along the inner side of the test tube.
- (iii) A red colour appears which shows the presence of bile salts in the solution.

RESULT

The above tests show the presence of bile salts in the urine sample.

PRECAUTIONS

1. Urine sample taken must be fresh.
2. The reagents must be prepared fresh.
3. Handle conc. H_2SO_4 very carefully.
4. Don't mix droppers used for different chemicals

VIVA VOCE

Q1. Name the two pigments found in bile.

Ans. Bilirubin and biliverdin.

Q2. Which pigment provides colouration to bile?

Ans. Bilirubin (yellow).

Q3. How are the bile pigments produced?

Ans. Iron-free portion of haemoglobin is converted to biliverdin. It is then converted into bilirubin. These are contained in the bile juice secreted by liver and stored in gall bladder.

Q4. How is urochrome formed?

Ans. Urochrome is formed by the breakdown of bilirubin.

Q5. What is urobilin?

Ans. It is the other name of the urochrome.

Q6. What are bile salts?

Ans. Bile salts are glycolic acid and taurocholic acid in combination with sodium or potassium salts.

Q7. What is the function of bile pigments?

Ans. Bile pigments help in breakdown of haemoglobin (RBC) into bile salts such as urobilin.

Q8. What is the function of bile salts?

Ans. Bile salts form an emulsion with fat, thus helping in the digestion of fat.

Q9. Give a very primary symptom of jaundice.

Ans. The primary symptom of jaundice is yellowing of eyes and skin.