Experiment

Qualitative Analysis of Urine for Abnormal Constituents

Aim

To qualitatively analyze the given urine sample for the presence of abnormal constituents such as protein, glucose, ketone bodies, bile salts, bile pigments, and blood.

References

S.P Singh, Practical manual of Biochemistry. CBS Publication and Distributors Pvt. LTD, New Delhi, Fifth Edition, 65-119.

Requirements

- **Glassware Requirement:-** Test tubes, test tube holder, pipette/dropper, spirit lamp/burner.
- **Chemical Requirement:-** Fresh urine sample, Benedict's reagent, sulphosalicylic acid, sodium nitroprusside, ammonium sulphate, concentrated nitric acid, sulphur powder, benzidine reagent.

Principle

Normal urine contains water, urea, creatinine, and electrolytes. In certain diseases, abnormal constituents appear in urine. These can be detected using specific qualitative chemical tests based on color change, precipitation, or foam formation.

Tests for Abnormal Constituents

A. Test for Protein – Heat and Acetic Acid Test

Procedure

1. Take 5 ml urine in a test tube.
2. Heat the upper portion of the sample.
3. Add 2–3 drops of acetic acid.

Observation: Turbidity or white precipitate indicates protein present.

B. Test for Glucose – Benedict's Test

Procedure

1. Take 5 ml Benedict's reagent in a test tube.
2. Add 8–10 drops of urine.
3. Heat the mixture in a boiling water bath for 2 minutes.

Observation: Color change from blue to green/yellow/orange/brick red indicates glucose.

C. Test for Ketone Bodies – Rothera's Test

Procedure

1. Take 5 ml urine in a test tube.
2. Add solid ammonium sulphate.
3. Add sodium nitroprusside solution.
4. Add ammonia along the side of the test tube.

Observation: Purple ring formation indicates ketone bodies.

D. Test for Bile Salts – Hay's Sulphur Test

Procedure

1. Take urine in a test tube.
2. Sprinkle sulphur powder on the surface.

Observation: Sulphur powder sinks if bile salts are present.

E. Test for Bile Pigments – Gmelin's Test

Procedure

1. Take concentrated nitric acid in a test tube.
2. Carefully add urine along the side.

Observation: Formation of green/blue/violet rings indicates bile pigments.

F. Test for Blood – Benzidine Test

Procedure

1. Take urine sample in a test tube.
2. Add benzidine reagent and hydrogen peroxide.

Observation: Blue or green color indicates blood.

Experimental Data Sheet

Test	Reagent Used	Observation	Result
Protein	Heat + Acetic acid	No turbidity	Absent
Glucose	Benedict's reagent	Blue color	Absent
Ketone bodies	Rothera's test	No purple ring	Absent
Bile salts	Hay's test	Sulphur floats	Absent
Bile pigments	Gmelin's test	No color ring	Absent
Blood	Benzidine test	No blue color	Absent

Result

The given urine sample was qualitatively analyzed for abnormal constituents. Based on the observations, the abnormal constituents were absent.