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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment

To perform qualitative analysis of normal and abnormal constituent in given sample of urine.

Aim:

To perform qualitative analysis of normal and abnormal constituent in given sample of urine.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 15 – 19

Materials Required

Dilute HCl, dilute H,SO conc. HNO, AgNO, solution, ammonium molybdate solution, BaCl2, NaOH, phenolphthalein indicator, ammonium oxalate solution, 1% acetic acid, sodium hyprobromate solution (NaOBr), urea powder, benedict uric acid reagent, anhydrous Na_2CO., sodium nitropruside, picric acid, Fehling's reagent, sulphosalicyclic acid, chlorophenol red, benedict's reagent, solid (NH),SO, strong ammonia solution, conc, nitric acid, benzidine powder, red litmus paper beaker, glass rod, measuring cylinder, funnel, test tubes, test tube holder

Theory :

Urine is a sterile liquid, by-product of the body, formed by the kidneys by a process known as urination. It is excreted through the urethra by a process known as micturition. Analysis of urine is performed to

diagnose various pathological conditions. Urine analysis is based on either physical or chemical characteristics of urine which help in assessment of processes occurring within the body.

Normal Constituents of Urine: Urine is an aqueous solution, having more than 95% water and 5% of other constituents mentioned below, with their decreasing concentrations

1) Urea (9.3gm/lt),
2) Chloride 187gm/lt).
3) Sodium 117gm/11),
4) Potassium 0.750gm/11).
5) Creatinine 0.670gm/lt), and
6) Other dissolved ions, inorganic and organic compounds (proteins, hormones, and metabolites).

In the liver, ammonia and carbon dioxide undergo ornithine cycle to synthesise urea, which is non-toxic to mammals (unlike ammonia). The processed form of ammonia is urea which is non-toxic to mammals as ammonia is highly toxic in nature. Urine is sterile until it reaches the urethra, where epithelial cells lining the orethra have facultative anaerobic, gram-negative rods, and cocci.

Physical features of urine are its colour, turbidity or transparency, odour, pH (acidity or alkalinity), and density. Many of these features can be identifiable physically while for some laboratory testing is performed:

1) **Colour:** Normally yellow amber in colour, but colour may depend on recent diet and the concentration of urine Concentration of urine reduces due to drinking of more water and it causes lighter colour of urine, whereas dark coloured urine indicates dehydration. Red urine shows the presence of RBCs in urine due to kidney damage and disease.
2) **Odour:** Its smell indicates various pathological conditions, for example, urine of diabetic patients has sweet or fruity smell due to the

presence of ketones (organic molecules of a particular structure) or glucose. Commonly, fresh urine has a mild odour while aged urine smells stronger just like ammonia.

3) **pH:** The pH of normal urine ranges between 4.6-8 (average 6.0), which depends on the diet of an individual. For example, high protein diets make urine acidic while vegetarian diets result in alkaline urine.
4) **Density:** It is the ratio of the weight of a volume of a substance compared with the weight of the same volume of distilled water; and is also known as specific gravity. The density of normal urine ranges between 0.001-0.035.
5) **Turbidity:** The turbidity of the urine sample is evaluated from person to person and is reported as clear, slightly cloudy, cloudy, opaque, or flocculent. Generally, fresh urine is either clear or very slightly cloudy. Excess turbidity is observed in the presence of suspended particles in the urine that can be determined through the examination of microscopic urine sediment. Turbidity of the urine generally increases due to urinary tract infections or obstructions.

Abnormal Constituents of Urine

Urine has the following constituents, whose abnormality indicates a pathological condition.

1) **Proteins :** Presence of abnormal concentration of albumin and globulin in the urine is known as proteinuria (albuminuria), which may be of two types:
   i) Physiologic Proteinuria: Less than 0.5% protein is observed in urine due to severe exercise, after a high protein meal, and during pregnancy.
   ii) Pathologic Proteinuria: Amount of proteins increases in case of glomerulonephritis and nephrotic syndrome Proteinuria may also occur due to poisoning of renal tubules by heavy metals (like mercury, arsenic, or bismuth)

2) **Glucose :** Presence of glucose in urine is known as glycosuria which occurs due to diabetes mellitus and endocrinal disorders such as hyperpituitarism. hyperthyroidism, Cushing's syndrome, and pheochromocytoma Transient glycosuria may be observed rarely due to emotional stress (like in exciting athletic contest).
3) **Other Sugars :** Presence of fructose sugar in urine due to disturbed fructose metabolism is known as fructosuria. Similarly, gallactosuria and lactosuria occur rarely in infants, pregnant women, and in lactating mother Consumption of food rich in pentose sugars (like grapes, cherries and plums) causes pentosuria The condition of pentosuria also occurs in inherited diseases in which pentoses are not metabolised.
4) **Ketone Bodies :** Under normal conditions, less than Img of ketone bodies are excreted through urine in 24 hours. Excretion of ketone bodies increases in many conditions like starvation, diabetes mellitus, pregnancy, ether anaesthesia, and in certain types of alkalosis.
5) **Bilirubin and Bile Salts :** In case of obstructive or hepatic jaundice, bilirubin is excreted in urine. A condition in which bile salts are excreted in urine is known as bilirubinuria In certain stages of liver disease, bile salts are excreted without bile pigments in urine. Traces of bilirubin without bile saits are excreted in urine during excessive haemolysis. Bilirubin is absent in urine of an adult healthy person. It is a waste product, produced by the liver from the haemoglobin of RBCs that are broken down and removed from circulation. It is utilised in the synthesis of bile (a fluid involved in food digestion). In some liver diseases like biliary obstruction or hepatitis, excess amount of bilirubin is formed which is eliminated through urine. Presence of bilirubin in urine indicates liver disease (like development of jaundice).
6) **Blood:** Blood is excreted in urine due to lesions of kidney, urinary tract infection, and in case of nephritis Free haemoglobin molecules may

also be detected after quick haemolysis, e.g., black water fever (a consequence of malaria) and severe burns.

Procedure

Procedure

Table 1: Test for Normal Inorganic Constituents of Urine

	Test	Observation	Inference
1)	**Test for Bi-Carbonates:** 3ml urine should be treated with dilute HCl or dilute H_2SO_4.	Effervescence of CO_2 gas	bi-carbonate present
2)	**Test for Chlorides:** 5ml urine should be treated with 1ml. conc. HNO_3 (to prevent precipitation of other ions like phosphate). Then, 1ml $AgNO_3$ solution should be added to this solution.	White curdy precipitate of AgCl soluble in NH_4 OH solution.	Chlorides present
3)	**Test for Phosphates:** 3ml urine should be treated with 3ml conc. HNO_3. Then, 3ml or ammonium molybdate solution should be added to this solution and should be heated to boil.	Cannary yellow precipitate	Phosphate present
4)	**Test for Sulphates:** 5ml urine should be treated with 1ml conc. HCl (to avoid phosphate precipitation). Then, 2ml $BaCl_2$ solution should be added to this solution.	An opaque milkiness or a thick white precipitate of $BaSO_4$ is observed that is insoluble in cone. HCl.	Sulphate present
5)	**Test for Ammonia:**		
	i) 5 ml urine should be treated with 2ml of 40% NaOH and boiled. A red litmus paper should be then held in vapour.	The red litmus paper turns blue.	Ammonia present
	ii) The glass rod should be dipped in phenolphthalein indicator.	The colour becomes pink	Ammonia present
6)	**Test for Calcium:** 5ml urine should be treated with few drops of NaOH. To this solution, 1% acetic acid and 2-3ml of ammonium oxalate solution should be added.	White precipitate of calcium oxalate	Calcium present

Table 2: Test for Normal Organic Constituents of Urine		
Test	**Observation**	**Inference**
1) **Test for Urea:** i) 3ml of urine should be treated with few drops of alkaline sodium hyprobromate solution (NaOBr). ii) 5ml urine should be taken and 4 drops of phenolphthalein should be added. Then to the above mixture pinchfull of urea powder (jack bean/soybean meal) should be added and then mixed. It should be allowed to stand for 5 minutes.	Effervescence of nitrogen Solution becomes pink (If solution is already pink before adding urea then 10% acetic acid to should be added to it to make the solution colourless).	Urea present Urea present
2) **Test for Uric Acid:** i) **Schiffs Test:** A strip of filter paper should be moisten with $AgNO_3$ solution and a drop of urine should be added to it. This test is known as. ii) 5 ml urine should be treated with 5 drops of benedict uric acid reagent. To this solution, 3gm of anhydrous Na_2CO_3 should be added and mixed by shaking.	Black or yellow brown stain formed. A deep blue colour develops	Uric acid present Uric acid present
3) **Test for Creatinine** i) **Weyl's Test:** 5ml of urine should be treated with 5 drops of sodium nitropruside. To this solution, 2ml of 10% NaOH should be added. ii) **Jaffes Test:** 5ml urine should be treated with 1 ml of saturated solution of picric acid. To this solution, 3gm of anhydrous Na_2CO_3 should be added and mixed well by shaking.	Rubby red colour is formed and soon changes to yellow A deep orange colour is formed.	Creatinine present Creatinine present

Table 3: Analysis of Urine for Abnormal Constituents

Test	Observation	Inference
1) **Test for Proteins (Albumin and globulin):**		
i) **Salphosalicyic Acid Test:** 3 ml clear urine should be treated drop by drop with sulphosalicyclic acid.	White precipitate appears	Albumin present
ii) **Hellers Nitric Acid Ring Test:** 3 ml conc. HNO_3 should be treated with urine drop wise from the side of the test tube.	White ring at the Junction of two fluids.	Albumin confirmed
iii) **Heat Coagulation Test:** 5 ml urine should be treated with 2 drop of chlorophenol red. Then, 1% acetic acid or 2% Na_2CO_3 should be added to adjust the PH faint pink colour and then it should be boiled for 2 minutes. At last few drops of acetic acid should be added to it.	Turbidity or precipitates	Albumin confirmed
2) **Test for Sugar (Glucose):**		
i) **Benedicts Test:** 5 ml urine should be treated with 5 ml of Benedict's reagent. This mixture should be boiled for 2 minutes and then cooled.	i) Green precipitate ii) Yellow precipitate iii) Red precipitate	Glucose present – 1% Glucose present – 2% Glucose present more than 2%
ii) **Fehlings Test:** In a test tube, 2 ml of Fehling's reagent (Fehling I and Fehling II in a 1:1 ratio) should be heated until it boils. An equal volume of examined urine is should be added and the reaction mixture should be boiled for 2 minutes. If a yellow-red to red cuprous oxide precipitate appears then the reaction is positive. A fresh urine sample that is not cloudy should be used for reducing test since the sensitivity is around 10 mmol. Urine should be de- deproteinated before testing if it contains proteins.	Red/yellow precipitate appears	Glucose confirmed.

3)	**Test for Ketones (Rothera's test):** 5 ml urine should be treated with solid $(NH_4)_2SO_4$ to saturate it completely. To this solution, 2 drop of sodium nitropruside solution and 2 ml of strong ammonia solution should be added from the sides of the test tube and wait for 10 minutes.	Permanganate colour develops	Ketones, like acetone present.
4)	**Test for Bile Salts:** 5ml urine should be taken in a beaker and sublimed sulphur powder should be sprinkled on the surface.	i) Powder sinks to bottom ii) Powder floats on the surface	Bile salts present Bile salts absent.
5)	**Test for Bile Pigments:** i) **Gmelins Test (Modified):** 10ml urine should be treated with 2-3 drops of dilute HCl and then filtered. It should be then filtered and allowed to dry. A drop of conc. HNO_3 should be added at the apex of the paper. ii) **Nitric Acid Test:** 3ml conc. nitric acid should be treated with urine slowly from side of the test tube.	Coloration on paper in following order green, blue, violet, red and yellowish red is observed. Fine play of colours.	Bile pigments present. Bile pigments present
6)	**Test for Blood (Benzidine Test):** Pinch of benzidine powder should be treated with 1ml of glacial acetic acid and shaken for 1 minute. To this solution, 2ml urine and few drops of H_2O_2 should be added.	Green/blue colour due to iron-benzidine formation.	Blood present

Result :

Qualitative analysis of normal and abnormal constituent in given sample of urine was performed.

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