SECTION

A

Procedural Station

1. Heat Coagulation Test
2. Heller's Nitric Acid Test
3. Benedict's Test
4. O-Toluidine Test
5. Hay's Sulphur Test
6. Rothera's Test
7. Fouchet's Test
8. Measurement of Specific Gravity
9. Preparation of Buffer

1. TO PERFORM HEAT COAGULATION TEST AND DOCUMENT THE RESULT

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: This test is based on the principle of heat coagulation and precipitation of proteins. Appearance of turbidity which does not dissolve in glacial acetic acid or a white ppt indicates the presence of proteins. Albumin is the commonest and earliest excreted protein in many conditions.

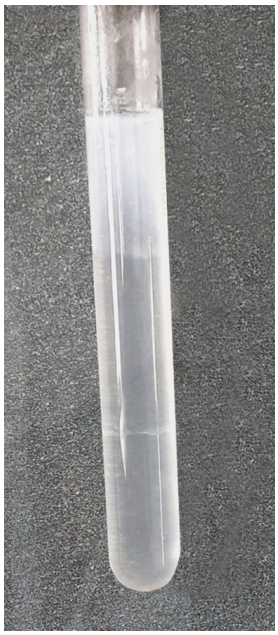

Fig. 4

Principle: Albumin is the major protein excreted in proteinuria. It is a heat coagulable protein and hence easily coagulated by heating the urine sample containing it. Formation or appearance of coagulum or turbidity is due to proteins or phosphates present in urine. Disappearance of coagulum upon addition of dilute acetic acid indicates the absence of proteins. Persistence of coagulum indicates the presence of protein.

Method

1. Fill part of test tube with urine. Add 8 drops of urine to the Benedict's reagent.
2. Heat the upper 1/3rd part of urine. Cool under tap water or by placing in a beaker containing tap water.
3. Add 1–2 drops of 1% acetic acid.
4. Heat again upper 1/3rd part of urine.
5. Document the test result (+ve/–ve).

Checklist: Detection of protein in given urine sample by heat coagulation test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Filled ¾ part of test tube with urine	1 M									
2.	Heated the upper 1/3rd part of urine	1 M									
3.	Added 1–2 drops of 1% acetic acid	1 M									
4.	Heated again upper 1/3rd part of urine	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- Presence of proteins in urine is called proteinuria. The smaller molecules of albumin pass through damaged glomeruli more readily than the heavier globulin and so, when the proteins appear in urine, the albumin fraction predominates. When glomeruli are damaged or diseased, they become more permeable and plasma proteins appear in urine.
- *Physiological proteinuria:* Less than 0.5 gm%.
- *Pathological proteinuria:* More than 0.5 gm%.

Types of proteinuria	*Causes*
1. Functional proteinuria	• Violent exercise • Cold bath • Pregnancy
2. Organic proteinuria a. Prerenal	• Cardiac diseases • Abdominal tumors • Fever • Anemia
b. Renal	• Acute and chronic glomerulonephritis • TB kidneys
c. Postrenal	• Inflammatory conditions of kidney, ureter, bladder, prostate • Bleeding in genitourinary tract

2. TO PERFORM HELLER'S NITRIC ACID TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Heller's test is a chemical test that shows that strong acids cause the denaturation of precipitated proteins. Heller's test is commonly used to test for the presence of proteins in urine.

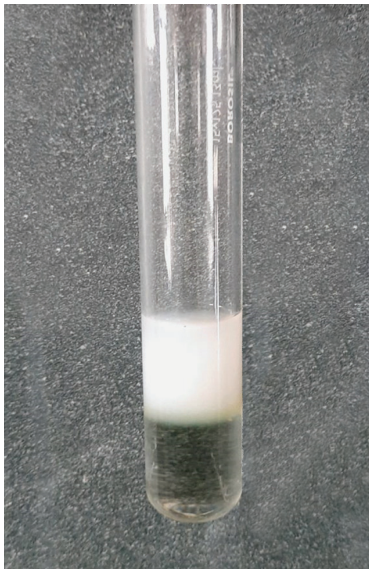

Fig. 5

Principle: Albumin is the major protein excreted in proteinurias. It is a heat coagulable protein and hence easily coagulated by heating the urine sample containing it. Formation or appearance of coagulum or turbidity is due to proteins/or phosphates present in urine. Disappearance of coagulum upon addition of dilute acetic acid indicates the absence of proteins. Persistence of coagulum indicates the presence of protein.

Method

1. Take 3 ml of concerted nitric acid in the test tube.
2. Then add 3 ml of sample slowly from the side of the test tube.
3. Do not mix.
4. White ring at junction of two layers.
5. Document the result (+ve/–ve).

Checklist: Detection of protein in given urine sample by Heller's nitric acid test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 3 ml of concerted nitric acid in the test tube	1 M									
2.	Added 3 ml of sample slowly from the side of the test tube	2 M									
3.	Does not mix	1 M									
4.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- This is a highly sensitive test and can be taken as confirmatory test for protein.
- If urine has a high concentration of urea, urea nitrate may be formed and it gives a false positive test for proteins.

- The amount of protein excreted normally in 24 hours urine is insignificant and it is less than 150 mg/day.
- When proteins appear in detectable quantities in urine, it is called proteinuria/albuminuria.
- The presence of detectable amount of protein is characteristic of kidney diseases.
- The normal glomeruli of kidneys are not permeable to substances with molecular weight of 70 kD. The plasma proteins of molecular weight of more than 70 kD, hence are absent in normal urine.
- When glomeruli are damaged or diseased, they become more permeable and plasma proteins appear in urine.
- The smaller molecules of albumin pass through damaged glomeruli more readily than the heavier globulin and so, when the proteins appear in urine, the albumin fraction predominates. Bence-Jones protein, an immunoglobulin appears in urine in cases of multiple myeloma. Protein precipitates between 40 and 60°C, disappears at 100°C and reappears on cooling.

Types of proteinuria	*Causes*
1. Functional proteinuria	• Violent exercise • Cold bath • Pregnancy
2. Organic proteinuria a. Prerenal	• Cardiac diseases • Abdominal tumors • Fever • Anemia
b. Renal	• Acute and chronic glomerulonephritis • TB kidneys
c. Postrenal	• Inflammatory conditions of kidney, ureter, bladder, prostate • Bleeding in genitourinary tract

3. TO PERFORM BENEDICT'S TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Benedict's test is used as a simple test for reducing sugars. A reducing sugar is a carbohydrate possessing either a free aldehyde or free ketone functional group as part of its molecular structure. This includes all monosaccharides (e.g. glucose, fructose, galactose) and many disaccharides, including lactose and maltose. Benedict's test is most commonly used to test for the presence of glucose in urine. Glucose found to be present in urine is an indication of diabetes mellitus.

Principle: Reducing sugars in alkaline medium are tautomerise to enediols, which are strong reducing agents. They reduce cupric ions to cuprous ions. Cuprous hydroxide which is formed is converted to red precipitate of cuprous oxide on heating.

Method

1. Take 5 ml of Benedict's reagent.
2. Add 8 drops of urine to it.
3. Mix and boil it for 2 minutes.
4. Document the test result (+ve/–ve).

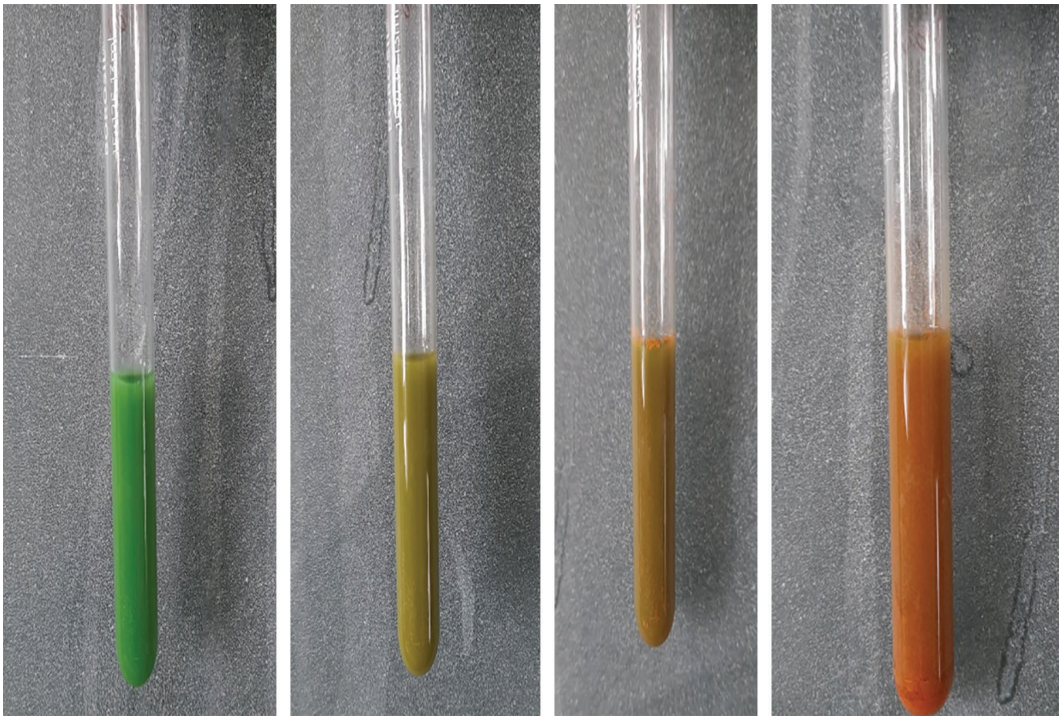

Fig. 6

Checklist: Detection of reducing sugar in given urine sample by Benedict's test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 5 ml of Benedict's reagent	1 M									
2.	Added 8 drops of urine to it	2 M									
3.	Mixed and boiled it for 2 minutes	1 M									
4.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation: Presence of glucose in urine is called glycosuria colour of the precipitate indicates severity of glycosuria as follows:

Colour of the precipitate	*Degree of glycosuria*	*Approx. glucose conc. (gm%)*
Green precipitate	+	0.5
Yellow precipitate	++	1.0
Orange precipitate	+++	1.5
Brick red precipitate	++++	or more

- Presence of glucose in urine is found in:
  a. Diabetes mellitus
  b. Hyperadrenalism
  c. Renal glycosuria
  d. Alimentary glycosuria: It is a benign condition which is seen after excessive intake of carbohydrate or patient is on glucose infusion.
- False positive results are obtained by the presence of excessive non-reducing substances like creatinine, ascorbic acid, urates, salicylates, glucuronides, etc.

Reducing sugar	*Condition*
Glucose	Diabetes mellitus, renal glycosuria
Fructose	Disorders of fructose metabolism, essential fructosuria, hereditary fructose intolerance
Galactose	Galactosemia
Lactose	Pregnancy, lactating woman
Pentose	Disorder of uronic acid pathway (essential pentosuria)

4. TO PERFORM O-TOLUIDINE TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Blood pigments, hemoglobin, present in the urine may be either in the form of intact cells, hematuria, or free in solution, hemoglobinuria. In certain pathological conditions blood appears in urine. The presence of blood can be recognized by reddish color of urine and by microscopic examination of red blood cells, if it has not been hemolyzed.

O-Toluidine test is used to detect blood in urine sample.

Principle: Heam of hemoglobin acts as chemical catalyses to breakdown H_2O_2 to $H_2O + [O]$. The nascent oxygen produced; oxidize O-Toluidine to its oxidized product which is greenish blue in colour.

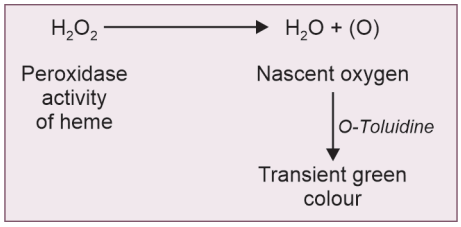

Fig. 7

Method

1. Take 2 ml of urine in a test tube.
2. Boil it for 30 sec and cool it.
3. Add 1 ml of O-toluidine reagent.
4. Add 1 ml of H_2O_2 and mixed gently.
5. Document the result (+ve/–ve).

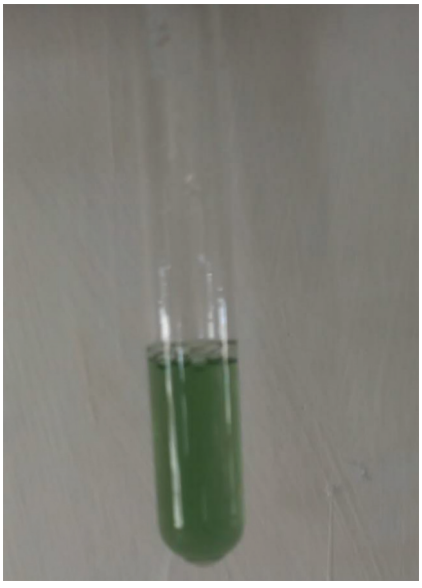

Fig. 8

Checklist: Detection of blood pigment in given urine sample by O-toluidine test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 2 ml of urine in a test tube	1 M									
2.	Boiled it for 30 sec and cooled it	1 M									
3.	Added 1 ml of O-touidine reagent	1 M									
4.	Added 1 ml of H_2O_2 and mixed gently	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- This is a very sensitive test but not specific for blood.
- Presence of blood in urine is called hematuria.
- Causes:
  a. Injury to urinary tract or kidney.
  b. Infection of urinary tract.
  c. Benign or malignant carcinoma of kidney or urinary tract.
  d. Enlargement of prostate due to rupture of engorged venous plexus.
  e. Obstruction due to urinary stones.
  f. Nephritis.
  g. Nephrotic syndrome.
  h. Due to trauma, caused by introduction of catheter through the urethra.
  i. Acute glomerulonephritis.
- Hematuria can be frank when urine appears red (due to blood) or it can be microscopic when it is not visible to naked eye (occult blood).
- Microscopic hematuria may be seen in:
  a. Malignant hypertension
  b. Sickle cell anemia
  c. Coagulation abnormalities
  d. Polycystic kidney diseases.
- Excretion of free hemoglobin in urine is called hemoglobinuria.
  This occurs in severe burns, chemical poisoning, incompatible blood transfusion, malaria, typhoid and hemolytic jaundice.
- Other tests by which blood pigments in urine samples can be detected are:
  a. Benzidine test
  b. Guaiac test

5. TO PERFORM HAY'S SULPHUR TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Hay's test for bile salts is a specific test used for the qualitative detection of bile salts in urine. Bile salt appears in the urine of patients suffering from jaundice. It consists of a watery mixture of organic and inorganic compounds. Bile acids are cholic acid and chenodeoxycholic acid which form conjugation with glycine and taurine. These two bile acids combine with sodium and potassium to form bile salts. Bile salts are sodium or potassium taurocholate, and sodium or potassium glycocholate. Hay's test is also called sulphur powder test.

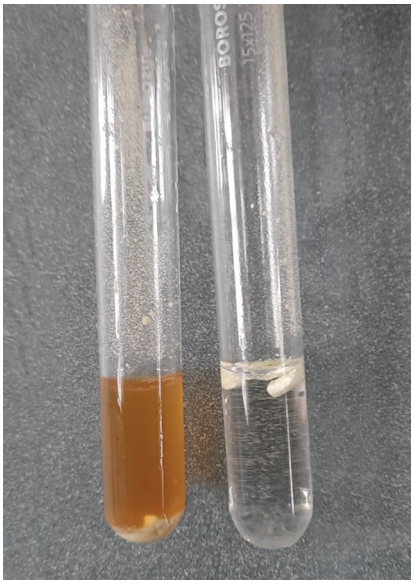

Fig. 9

Principle: Bile salts have a property of lowering the surface tension of the fluid. If bile salts present in urine and sulphur powder is added to the urine in the test tube, the sulphur particles will sink. In normal cases it does not sink rather, it floats on the surface of the fluids.

Method

1. Take 3 ml of urine in a test tube
2. Add a pinch of sulphur powder to it
3. Do not shake
4. Control is run with distilled water
5. Documented the result (+ve/–ve).

Checklist: Detection of bile salts in urine by Hay's sulphur test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 3 ml of urine in a test tube	1 M									
2.	Added a pinch of sulphur powder to it	1 M									
3.	Does not shake	1 M									
4.	Control is run with distilled water	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- Bile salts are sodium and potassium salts of glycocholates and taurocholates.
- Normally, bile salts and bile pigments do not enter the general circulation and therefore, they are absent in the normal urine.
- But, if there is intrahepatic or posthepatic obstruction to the flow of bile, regurgitation occurs in the general circulation and bile salts appear in urine.
- Bile salts are present in urine along with bile pigments in obstructive jaundice.
- This is not a specific test for bile salts but is usually done to detect bile salts.
- Alcohol and salicylates give a false-positive test.

6. TO PERFORM ROTHERA'S TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Rothera's test is well known in human as a method of detecting acetone and acetic acid in urine.

Principle: Sodium nitroprusside in alkaline medium reacts with ketone groups of acetone and acetoacetic acid to form permanganate ring at the junction of the two liquids. This method can detect above 1–5 mg/dl of acetoacetic acid and 10–20 mg/dl of acetone. β-Hydroxybutyrate is not detected.

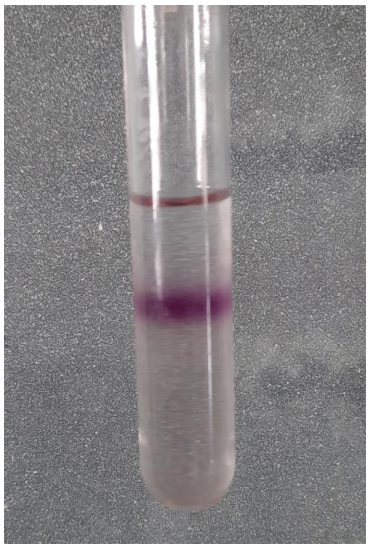

Fig. 10

Method

1. Take 3 ml of urine in the test tube.
2. Saturate with ammonium sulphate powder.
3. Add a drop of freshly prepared sodium nitroprusside and mix it.
4. Add 3 ml of strong ammonia carefully by the side of the test tube.
5. Document the result (+ve/–ve).

Checklist: Detection of ketone bodies in given urine sample by Rothera's test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 3 ml of urine in a test tube	1 M									
2.	Saturated with ammonium sulphate powder	1 M									
3.	Added a drop of freshly prepared sodium nitroprusside and mixed it	1 M									
4.	Added 3 ml of strong ammonia carefully by the side of the test tube	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- Ketone bodies are acetone, acetoacetic acid and β-hydroxybutyric acid.
- Ketone bodies do not appear in urine because acetoacetic acid, which is produced normally in the liver, is completely oxidized in tissues. Ketone bodies are formed in

excess when the glucose metabolism is impaired as in diabetes mellitus or when fat is used exclusively to give energy as in starvation (starvation ketosis). This condition is called ketosis.

- The tissues are unable to oxidize the excess amount of acetoacetic acid with the limited supply of oxygen. A part of excess acetoacetic is decarboxylated to acetone and remaining circulates in blood as acetoacetic acid and β-hydroxybutyric acid.
- Rothera's test is very sensitive. It is answered even by small amounts of acetone and acetoacetic acid.
- β-Hydroxybutyrate does not answer Rothera's or Gerhardt's test because it does not have a ketone group. It gives positive when converted to acetoacetic acid and then to acetone by oxidation.
- The excretion of ketone bodies in urine is called ketonuria. This occurs in ketosis where there will be ketonemia and ketonuria.
- Total ketone bodies are found in normal urine to the extent of about 20 mg/day.
- Ketonuria may also be seen in conditions like intake of high fat and low carbohydrates diet and toxemia of pregnancy.
- Whenever glucosuria is more than 0.5 mg% (++) the patient should be tested for ketone bodies also.
- If Gerhardt's test is negative and Rothera's test is positive, acetone is present.
- Gerhardt's test or ferric chloride test is useful in detecting a large number of abnormal constituents in urine, in rare disorders. In addition to metabolites, drugs excreted can be detected by this test. Some of the compounds detected are listed as follows.

7. TO PERFORM FOUCHET'S TEST

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

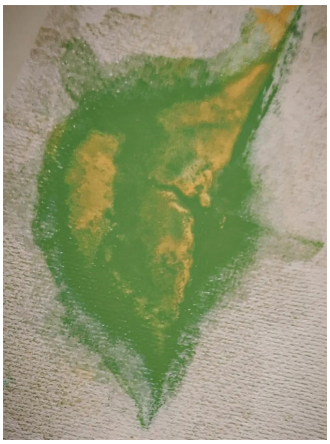

Fig. 11

Introduction: For testing bilirubin in urine, two types of tests are available, namely (1) oxidation test—where bilirubin is oxidized to green biliverdin and (2) diazotization test—where bilirubin is diazotized to a highly colored compound.

Fouchet's reagent used in Fouchet's test comes under oxidizing reagent or test. Barium chloride precipitates the sulphate radicals present in urine to form precipitate of barium sulphate. If bile pigments are present in urine, they adhere to these molecules. Ferric chloride present in Fouchet's reagent then oxidizes yellow bilirubin, in the presence of trichloroacetic acid to green biliverdin. Therefore, the development of green colour due to the formation of biliverdin indicates the presence of bilirubin (bile) in urine.

Principle: The bile pigment present in the urine gets adsorbed onto the precipitate of barium sulphate which is separated by filtration. The dried precipitate is then treated with Fouchet's reagent which contains ferric chloride. Ferric chloride is an oxidizing agent and it oxidizes bilirubin to biliverdin to give green colour.

Method

1. Take 5 ml of urine sample in the test tube.
2. Then add 1 ml of magnesium sulphate.
3. Add 2 ml 10% $BaCl_2$.
4. Filter the precipitate and add few drops of Fouchet's reagents on filter paper and observe.
5. Document the result (+ve/–ve).

Checklist: Detection of bile pigments by Fouchet's test.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Took 5 ml of urine sample in the test tube	1 M									
2.	Added 1 ml of magnesium sulphate	1 M									
3.	Added 2 ml 10% $BaCl_2$	1 M									
4.	Filtered the precipitate and added few drops of Fouchet's reagents on filter paper and observe	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- Bile pigments are bilirubin and biliverdin.
- They are produced by the breakdown of heme in the reticuloendothelial system.
- Bilirubin is in unconjugated form soon after it is produced from heme. It gets conjugated with UDP glucuronic acid in liver to form mono-/diglucuronide. Bile contains conjugated bilirubin which is excreted into the intestine.
- In normal persons bile pigments are not present in urine.
- Fouchet's test is a highly sensitive test for bilirubin.
- Ferric chloride, present in the Fouchet's reagent acts as an oxidizing agent. It oxidizes bilirubin to biliverdin (green) or bilicyanin (blue).
- Causes of bilirubinuria are:
  1. Moderate to severe hepatocellular damage (infective hepatitis).
  2. Obstruction to bile duct—intra- or extrahepatic course (obstructive jaundice).

  In prehepatic jaundice, bilirubin is absent in urine.

8. TO MEASURE SPECIFIC GRAVITY OF THE GIVEN URINE SAMPLE

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Specific gravity of urine serves to assess the concentration ability of the kidneys. It can vary widely depending on diet, fluid intake and renal function. It is inversely related to urinary output except in diabetes mellitus where it is increased due to glucose excretion.

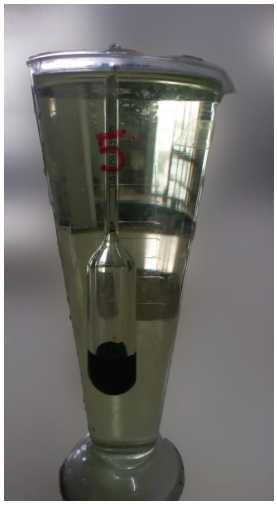

Fig. 12

Method

1. Fill the jar with the urine sample up to the mark.
2. Dip the urinometer in the jar without touching the walls of the jar.
3. Note the observed specific gravity.
4. Calculate the corrected specific gravity with the help of formula.
5. Document the result (+ve/–ve).

Checklist: Measurement of specific gravity of urine sample.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Filled the jar with the urine sample up to the mark	1 M									
2.	Dipped the urinometer in the jar without touching the walls of the jar	1 M									
3.	Noted the observed specific gravity	1 M									
4.	Calculated the corrected specific gravity with the help of formula	1 M									
5.	Documented the result (+ve/–ve)	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Name of observer **Signature**

Interpretation

- It depends upon the concentration of various solutes.
- Specific gravity usually lies between 1.015 and 1.025 (specific gravity of water is taken as 1000).
- Increased specific gravity more than 1.030 seen in:
  a. Dehydration
  b. Diabetes mellitus

c. Congestive heart failure
d. Proteinuria
e. Adrenal insufficiency

- Decreased specific gravity seen in:
  a. Hypothermia
  b. Diuretic therapy
- Fixed specific gravity: The specific gravity of urine is identical to the glomerular filtrate around 1.010. It is seen in patients with chronic kidney disease (CKD).
- Presence of substances with high molecular weight substances like proteins and glucose in the urine impart much higher specific gravity than due to the excessive excretion of crystalloids.
- Measurement of specific gravity is done by:
  i. Urinometer
  ii. Refractometer used in higher laboratories.

9. PREPARATION OF BUFFER

Domain: Psychomotor, cognitive.

Time allotted: 5 minutes/***Marks:*** 5

Introduction: Acids are protons and bases are proton acceptors. Strength of an acid is indicated by its pK value which is low for strong acid and vice versa.

Buffer is a mixture of weak acid and its salt with strong base or a mixture of weak base and its salt with strong acid.

Method

1. Identify and take instruments required for this practical measuring cylinder, beaker, weighing balance and pH indicators.
2. Add 2.96 gm of sodium phosphate dibasic (Na_2HPO_4).
3. Add 1.30 gm of sodium dihydrogen orthophosphate (NaH_2PO_4).
4. Dissolve above reagents to 100 ml of double distilled water.
5. Measure the pH of this solution on pH indicator.

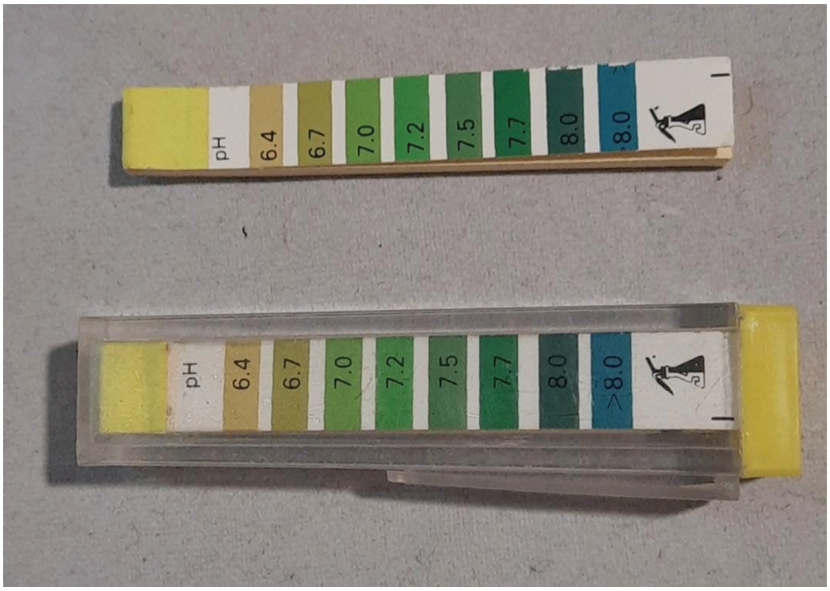

Fig.13

Checklist: Preparation of phosphate buffer.

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
1.	Identified and took instruments required for this practical measuring cylinder, beaker, weighing balance and pH indicators	1 M									

(*Contd...*)

(*Contd...*)

Sr. No.	*Checklist*	*Marks (5)*	*Roll No.*								
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9 and so on*
2.	Added 2.967 gm of sodium phosphate dibasic (Na_2HPO_4)	1 M									
3.	Added 1.30 gm of sodium dihydrogen orthophosphate (NaH_2PO_4)	1 M									
4.	Dissolved above reagents to 100 ml of double distilled water	1 M									
5.	Measured the pH of this solution on pH indicator	1 M									
		Total: (5)									

Instruction to observer—Mark (✓) if yes/done, Mark (✗) if no or not done

Interpretation

- The buffer systems of blood are:
  i. Bicarbonate–carbonic acid ($BHCO_3$:H_2CO_3)
  ii. Hemoglobin–hemoglobin (BHb:HHb)
  iii. Oxyhemoglobinate–oxyhemoglobin ($BHbO_2$:$HHbO_2$)
  iv. Phosphate buffer (B_2HPO_4:BH_2PO_4)
  v. Protein buffer (B protein:H protein)
- The most important buffer of plasma is bicarbonate–carbonic acid system. It is of great importance in the acid–base balance of the extracellular fluid and in the maintenance of the blood pH within normal limits.