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Practical Biochemistry

With Clinical Correlation

As per the Revised Competency-Based Medical Education Curriculum (NMC)



Nita Sahi
Disha Sahi

2nd Edition

JAYPEE

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3

CHAPTER

Qualitative Assessments: Analysis of Normal Urine

COMPETENCY

BC14.3: Describe the physical properties chemical constituents of normal urine and abnormal constituents of urine and perform urine analysis to determine normal and abnormal constituents including dipsticks method demonstrations.

LEARNING OBJECTIVES

After reading this chapter, the readers should be able to:

- Describe the normal urine chemical composition.
- Perform analysis of urine for estimation of normal constituents of urine.
- Describe the chemical components of normal urine.
- Perform urine analysis to estimate and determine normal compounds in the urine.
- Perform and interpret the common constituent analyte in a urine examination.

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Lesson Plan: Chemical Components—Normal Urine

Objective

By the end of the lecture, students should be able to:

1. Understand the composition of normal urine and its chemical components.
2. Describe the physiological significance of each chemical component in urine.
3. Discuss the factors affecting the composition of urine.

Duration: ***70 minutes***

Materials Required

1. Presentation slides outlining the chemical components of normal urine.
2. Samples of normal urine for demonstration and experiment.
3. Chemical reagents: (for urine testing) like dipsticks, reagent strips.
4. Laboratory glassware/equipment for urine analysis like test tubes, pipettes, etc.
5. Charts/diagrams illustrating the structure of urinary system.

Outline

Lecture, small group discussion, DOAP

I. **Introduction to urine composition** **(10 minutes)**
   - Define urine, its significance in the excretory system.
   - Explain the sources of urine formation along with role of the kidneys in regulation and its composition.
   - Discuss the factors affecting urine composition, hydration status, diet, metabolic processes and reactions.

II. **Major chemical components of normal urine** **(30 minutes)**
   - *Water*: Discuss the significance of water as the primary component of urine and its role in the solute transport/ waste excretion.

- *Electrolytes*: Describe the presence of electrolytes like sodium, potassium, chloride, and bicarbonate in urine along with their physiological importance.
- *Urea*: Explain the origin of urea in urine by protein metabolism and its role in nitrogen excretion.
- *Creatinine*: Discuss the formation of creatinine from muscle metabolism and its role as marker for renal function.
- *Uric acid*: Describe the synthesis of uric acid from purines metabolism and its excretion.
- *Other organic compounds*: Mention presence of other organic compounds in urine, including hormones, metabolites, and drugs.

III. **Physiological significance of urine components** **(10 minutes)**
- Explain the significance of maintaining electrolyte balance, osmolarity in urine for renal functions/body homeostasis.
- Discuss the importance of urea, creatinine, and uric acid in waste excretion and kidney function assessment.
- Highlight the diagnostic value of urine analysis in assessing the hydration status, renal functions and disorders.

IV. **Factors affecting urine composition** **(5 minutes)**
- Discuss how hydration status, dietary intake, physical activity, and hormonal factors have impact in the chemical composition of urine.
- Explain how certain medical conditions and medications may change the urine composition.

V. **Demonstration and hands-on activity** **(5 minutes)**
- Demonstrate use of urine test strips/reagents for analyzing the chemical composition of urine.
- Students to perform simple urine tests to identify like pH, protein, glucose, and ketones.

VI. **Review and summary** **(5 minutes)**
- Summarize the important points covered in the lecture.
- Emphasize the significance of urine analysis in clinical diagnosis and monitoring of renal and metabolic health in clinical scenarios.

VII. **Conclusion** **(5 minutes)**
- Conclude the lecture by encouraging students to focus on applying their knowledge in clinical practice and research settings.
- Invite questions from students and address any queries regarding interpretation.

Assessment: Written/viva voce, DOAP

INTRODUCTION

- Urine is the excretory waste product formed by the kidneys. It is the ultra filtrate of plasma which is formed when blood passes through both the glomeruli. Plasma is filtered by the glomerulus. The amount of glomerular filtrate for an adult is 180 liters per day.
- The final volume of urine in 24 hours is 1–2 liters because of reabsorption and secretion of water and solutes by kidney tubules. The normal glomerular filtration rate is 120 mL/minute. In this way, the kidneys excrete metabolic waste and retain important products and maintain acid-base balance.
- Routine urine examination is important to assess renal function tests and for the diagnosis and monitoring the severity of diseases, such as diabetes mellitus, nephrotic syndrome, jaundice, nephropathy, renal tuberculosis, nephrosclerosis and various aminoacidurias.

Urine Analysis

Bacteriological	Microscopic	Biochemical
For identification of infecting organism by doing culture	For detecting the presence of pus cells, RBCs casts, etc.	• Qualitative estimations • Quantitative estimations

Sample type	Sampling	Purpose
Random specimen	• No specific time • Very common • Taken anytime in the day	• Routine screening • Chemical and FEME
Morning sample	• First urine in the morning • Most concentrated	• Pregnancy test • Microscopic test

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Sample type	Sampling	Purpose
Clean catch midstream	• Discard first few milliliters • Collect the rest of urine	Culture
24 hours	All the urine samples passed during the day, night and next day 1st sample is collected	• Qualitative analysis • Quantitative analysis
Postprandial	Two hours after meal	Determine glucose for monitoring of diabetes
Suprapubic aspired	Needle aspiration	Obtaining sterile urine

Specimen Collection

- For most of the urine investigations, fresh, midstream specimen of 10–20 mL urine is collected in a dry vial.
- In some cases, 24 hour urine sample is also collected.
- It should be done in clean sterile containers.
- The lid should be tight.
- It should be properly labeled.

Sample Preservation

- There is no single all-purpose preservative.
- Toluene is a very good and commonly used preservative for urine.

Type of urine investigation	Preservative used
Urea, ammonia, nitrogen, calcium	2N/concentrated HCl
Sodium, potassium, chloride, bicarbonate, calcium, phosphorus, urea, ammonia, amino acids, creatinine, proteins, reducing substances, ketone bodies	Thymol—5 mL of 100 g/L solution in iso-propanol (It is not used for 17-ketosteroids)
Ascorbic acid	Acetic acid

Organic Constituents of Urine

Sl. No.	Constituent	Concentration (G/day)
1.	Urea	25–30
2.	Uric acid	0.5–0.8
3.	Creatinine	1–1.8
4.	Hippuric acid	0.7–0.8

Inorganic Constituents of Urine

Sl. No.	Constituent	Concentration (G/day)
1.	Chlorides	10–15
2.	Sodium	3–5
3.	Potassium	2–2.5
4.	Calcium	0.1–0.3
5.	Phosphates	0.8–1.3
6.	Sulfates	1.0–1.2
7.	Ammonia	0.7–0.8

Characteristic	*Description*
Appearance	Clear, transparent, without turbidity and no sediments present
Amount	1–2 liters, depends on intake
Color	Straw/amber color (darker—more concentrated)

Contd....

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Characteristic	*Description*
Specific gravity	1.010–1.025, measure of dissolved material in urine, lower value = more diluted urine, depends on diet, fluid intake and renal functions, it is inversely related to urinary output except in diabetes mellitus where it is increased due to glucose excretion
pH	Diet has major effect on urine pH, varies between 4 to 8, usually 6.0
Odor	Slightly ammonical
Composition	95% water, 5% organic and inorganic solids
Nitrogenous wastes	• Urea—amino acid metabolism • Creatine—from muscle metabolism • Uric acid—from nucleic acid metabolism

PHYSICAL EXAMINATION OF URINE

Normal Color

It is mainly due to the presence of pigment urochrome, traces of uroerythrin, urobilin, uroporphyrin and coproporphyrin also contribute to the normal amber color of urine.

Physiological Variations in the Urine Color

S. No.	Color	Interpretation
1.	Dark yellow	• Concentrated urine • Mild dehydration • Vitamin B complex therapy specially riboflavin
2.	Orange	Drug induced
3.	Pinkish	Excess intake of beetroot, drug called pyridium
4.	Red	Intake of rifampicin
5.	Brownish yellow	Laxatives, such as rhubarb, senna

Pathological Variations in the Urine Color

S. No.	Color	Interpretation
1.	Deep yellow	Jaundice (presence of bile pigments in hepatic and obstructive cases)
2.	Reddish	Hematuria
3.	Brownish	Hemoglobinuria, myoglobinuria and porphyrias (porphyrins)
4.	Brown to black	Alkaptonuria—presence of homogentisic acid, (first inborn error of metabolism)
5.	Cloudy	Pus cells and bacteria in infected cells
6.	Smoky	Red blood cells
7.	Black	Iron therapy, melanotic sarcoma due to presence of melanin
8.	Pinkish brown	Presence of urobilin–hemolytic anemia
9.	Milky white	Chyluria (fat globules)

Normal Volume of Urine

- Around 800–2500 mL/day, average—1500 mL/day
- Around 500 mL/day is the minimum volume of urine needed in normal health to remove the waste products.

Factors Affecting Volume of Urine

- Fluid intake
- Fluid loss (output of urine may decrease due to heavy perspiration in summers and after exercise)
- Type of diet (salty, spicy foods, beverages, such as tea, coffee and alcohol increases the output of urine
- Cardiovascular status
- Renal functions

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Variations in the Volume of Urine Excreted

Polyuria	Oliguria	Anuria
Increased volume of urine is excreted per day, exceeding 2500 mL/day	Volume of urine is less than 500 mL/day	Complete cessation of urine or volume of excreted urine is less than 100 mL/day
Conditions causing polyuria	Conditions causing oliguria	Conditions causing anuria
Diabetes mellitus	Fever	Acute tubular necrosis
Diabetes insipidus	Diarrhea (loss of fluid from extrarenal sites)	Blood transfusion reaction
Late stage of chronic glomerulonephritis	Severe edema	Severe shock/Surgical shock
Drug-induced diuretics	Acute nephritis	Bilateral renal stones
Alcohol	Early stage of acute glomerulonephritis	Sulfonamide therapy
Compulsive polydipsia	Cardiac failure and hypertension (decreased circulatory volume)	

Normal Specific Gravity of Urine

- The specific gravity indicates the concentrating ability of the kidneys; it is the crude measure of total solutes excreted in the urine.
- In normal health, the urinary specific gravity ranges between 1.016–1.025, the average being 1.020.

Factors Affecting Specific Gravity

- Volume of urine excreted
- The amount of solids present in the urine

Variations in the Specific Gravity of Urine

Low SG—1.016 or less	High SG—1.025 or more	Fixed SG (Isosthenuria): SG of urine is based on tubular function
Conditions causing decreased specific gravity	Conditions causing increased specific gravity	Conditions causing fixed specific gravity
Diabetes insipidus	Diabetes mellitus (due to glycosuria)	Fixed SG is seen in chronic renal failure, in the later stages of chronic renal failure, the kidneys fail to concentrate or dilute the urine which has a constant SG, ranging between 1.008–1.012, average–1.010, same as that of plasma
Polyuria	Severe dehydration	
Compulsive polydipsia	Nephrotic syndrome (due to proteinuria)	
Diuretics	Adrenal insufficiency (excess of sodium in urine)	
Glomerulonephritis	Congestive heart failure	
Pyelonephritis	Hepatic diseases	
	Extrarenal water loss (fever, vomiting, diarrhea)	
	Less water intake (passing concentrated urine)	

Measurement of Specific Gravity of Urine

Instrument—Urinometer

1. It is made of glass.
2. It has a bulb and a stem.
3. Bulb is filled with mercury and the stem has marks ranging from 1.000–1.060.
4. Because of the mercury weight, it dips into the urine to an extent depending on the SG of the urine sample.
5. The level of urine corresponding to the markings on the neck gives us the specific gravity measurement.
6. The instrument is calibrated at 15°C so the temperature correction is made based on at what RT you are doing the measurement.
7. For every 3°C of temperature more than 15°C, 0.001 is added in the measured value and for every 3°C temperature below 15°C, 0.001 is deducted from the measured value.

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Operation:

- Fill 3/4th of a 100 mL cylinder with urine sample and gently dip the urinometer in the cylinder.
- Note the reading corresponding to the level of urine and calculate the SG.
- *Precaution*: The urinometer should not touch the walls of the cylinder.

8. **Examples:**
   - RT = 24°C
     - Calibration temperature = 15°C
     - Difference is plus 9°C
     - For every 3°C increase of temperature, correction factor is 0.001 therefore for 9°C increase, the correction factor will be +0.003
   - RT = 12°C
     - Calibration temperature = 15°C
     - Difference is minus 3°C
     - For every 3° decrease in temperature below 15°C, the correction factor to be deducted is 0.001.

Normal Odor of the Urine

Fresh Urine–Aromatic

Variations in the Odor of Urine

Odor	Characteristic
Ammonical	On keeping sample for long time due to decomposition of urea
Acetone	Ketonuria, such as diabetic ketoacidosis or starvation
Foul smell	Bacterial infections
Mousy/musty	Phenylketonuria
Cheesy	Isovaleric aciduria
Burnt sugar	Branched chain ketonuria (MSUD)
Cabbage	Tyrosinosis (tyrosinemia type 1)

Normal pH of Urine

Normal urine is acidic; pH range between 4.5–8.0 with a mean of 6.0 in 24 hours.

Variations in the pH of Urine

Acidic urine	Alkaline pH
Physiological factors: • Large intake of proteins in diet • Presence of sulfates, phosphates and chlorides in the diet	**Physiological factors:** • Fruits and vegetables/citrus fruits (alkaline tide is seen soon after meals) • Excess intake of milk and antacids • Ingestion of alkali, such as sodium bicarbonate • On standing it becomes alkaline due to decomposition of urea which forms ammonia
Pathological factors: • Acidosis • Diabetes mellitus • Starvation • Diarrhea	**Pathological factors:** • Severe vomiting • Infections of the urinary tract • Conditions of alkalosis

Measurement of Urinary pH

- pH papers
- Litmus papers

Normal Appearance of Urine

Fresh void urine is clear and transparent.

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Variations in the Appearance of Urine

Appearance	Characteristic
Turbidity on standing	• Due to precipitation of mucoproteins and nucleoproteins • Ammonium urate precipitates in alkaline urine • Other urates precipitate in acidic urine
Fresh urine turbidity	Presence of cells, casts, crystals, chyle and pus

Chemical Composition of Normal Urine

Organic Constituents

Urea

- Exogenous and endogenous proteins catabolize to form waste product called urea.
- More than 75% of nonprotein nitrogen is excreted from our body in form of urea (blood urea is called BUN that is blood urea nitrogen).
- More than 90% of urea is excreted by the kidneys and the rest is excreted by skin and gastrointestinal tract.
- Urea is freely filtered by the glomerulus, and is not secreted by the renal tubules.
- Approximately 50–70% of filtered urea passively diffuses through the renal tubules and re-enters plasma.
- If the rate of urine flow is slow, this passive diffusion increases.
- Normally excretion of urea in urine is 25–30 g/day.
- Amount of protein intake influences the amount of urea in urine
- In renal disorders, the plasma urea becomes high than normal and urine urea is low than normal
- If the concentration power of the kidney is decreased, the urinary urea is higher than normal
- **General overview:** Tissue/diet → amino acids → transamination → amino nitrogen glutamate → deamination → toxic ammonia to less toxic urea

Creatinine (End Product of Creatine Metabolism)

- Nonenzymatic irreversible dehydration of creatine in muscles results in the formation of creatinine which is the waste end product of tissue proteins that is excreted by the kidneys.
- Creatine made in liver and kidneys with the help of three amino acids—glycine, arginine and methionine go to the muscles where creatine gets converted to creatine phosphate which acts as stored form of energy in skeletal muscles.
- Since creatine is involved in all processes that need energy, muscles, brain and nerve cell receive correspondingly larger amounts.
- Per day conversion of creatine to creatinine is around 2%.
- The amount of creatinine formation depends on the total muscle mass.
- Serum creatinine level is a very sensitive test for assessing renal functions as it depends on mass of the muscles and renal excretion.
- Its level does not change much with intake of dietary proteins.
- Normally excretion of creatinine in urine is 1–1.5 g in 24 hours.
- Its estimation in urine is preferred over urea estimation as it is constant on a day-to-day basis since the muscle mass does not change.
- Urinary creatinine is used as earliest test of glomerular dysfunctions.
- Creatinine excretion can be used to measure glomerular filtration rate.

Uric Acid (2, 6, 8 Trihydroxy Purine)

- It is the end product of catabolism of purine bases present in nucleoproteins, thus tissue nucleoprotein catabolism results in formation of most of the uric acid.
- It can act like a dibasic acid and can form mono sodium salts as well as disodium salts depending on the pH, which get deposited in the joints in our body to cause arthritis or gout.
- pKa of uric acid = 5.75, at pH >5.75 it act as monosodium urate ion whose solubility in water is 10 times more than uric acid, and at pH <5.75, it exists as uric acid.
- The four steps of renal picture of uric acid:
  1. Glomerular filtration of most of the plasma uric acid
  2. About 98% of filtered uric acid gets reabsorbed in the proximal convoluted tubules

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3. Secretion of half of the reabsorbed uric acid into the lumen in the distal part of the proximal tubule
4. Reabsorption of 40% in the distal tubule

- ❖ Thus, the final excretion of uric acid in urine is about 12% of the amount filtered.
- ❖ The uric acid/urate pool in our body is around 1–2 g in men and 600 mg in women.
- ❖ Normally uric acid excretion in urine is 0.5–0.8 g in 24 hours.
- ❖ Its excretion is increased in basic urine (at pH more than 5.75, uric acid is soluble so taking base forming foods which increase the pH of urine, prevent calculi formation by increasing solubility of uric acid).
- ❖ **General overview:** Exogenous by diet, endogenous by tissue breakdown forms adenosine and guanosine which form hypoxanthine and xanthine, which finally forms uric acid.

Urobilinogen

- ❖ **General overview:** Reticuloendothelial cells: → RBC's lysis after 120 days → Hemoglobin released and splits to form heme and globin, globin gets degraded and heme in presence of heme oxygenase system forms bilirubin.
- ❖ **Blood:** Bilirubin is insoluble in blood so transported to liver by binding with albumin.
- ❖ **Liver:** Conjugation of bilirubin takes place by glucuronyl transferase enzyme to form bilirubin diglucuronide (conjugated bilirubin) which goes through bile duct to intestine.
- ❖ **Intestine:** Conjugated bilirubin gets reduced by intestinal bacteria to form urobilinogen.
- ❖ Most part of UBG is excreted in feces and the rest is reabsorbed, greater part of which is excreted in bile and small part goes to systemic circulation, filtered at glomerulus and excreted in urine.

Tests for Organic Constituents of Normal Urine

Tests for Urea

Experiment	Observation	Principle	Conclusion/Interpretation
Sodium hypobromite test: Urine sample = 2 mL + sodium hypobromite = 4 drops = see the effervescence of nitrogen	Brisk effervescence is observed	When urea is treated with alkaline hypobromite urea breaks to form nitrogen which is observed as brisk effervescence • $NH_2CONH_2 + 3NaOBr \rightarrow 3NaBr + N_2 + CO_2 + 2H$ • $CO_2 + 2NaOH \rightarrow Na_2CO_3 + 2H_2O$	• Urea is present in the urine • *BUN in blood*: 5–17 mg/dL • Normal urea in urine: 7–16 gm/day • *Increased urinary urea is seen in*: – Fever – Diabetes mellitus – Hyper functioning of adrenal cortical activity • *Decreased urinary urea is seen in*: – Liver disorders – Respiratory acidosis – Nephritis
Specific urease test: Urine sample = 2 mL + Phenol red = 2 drops → Phenol red turns red in basic medium + Drop wise add 2% acetic acid till the pink color disappears + Urease enzyme (soybean powder) = 1 mL	Solution turns pink	• Soybean powder has enzyme urease • This enzyme urease can break urea at pH 7–8 and temperature of 55°C to form ammonia and carbon dioxide $CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$ The evolution of ammonia is detected by indicator—phenol red • The above substrates join together to form alkaline ammonium carbonate which changes the slightly acidic reaction (yellow) to basic (pink)	
Biuret test: Urine sample = 2 mL + 10% sodium hydroxide = 2 mL, mix, heat + 0.5% solution of copper sulfate = 2 mL drop wise	Presence of purplish violet color indicate the presence of urea	• Urea decomposes to form ammonia and Biuret after heating $2CO(NH_2) \rightarrow NH_3 + \text{Biuret}$ • Biuret forms a violet color complex with alkaline copper sulfate solution	

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Test for Creatinine

Experiment	Observation	Principle	Conclusion/Interpretation
Jaffe's test: Saturated picric acid = 5 mL + 5% sodium hydroxide = 5 mL Total solution = 10 mL Divide the solution in two equal halves in 2 test tubes **In test tube 1:** *Control tube* Add distilled water = 5 mL **In test tube 2:** *Test* Add urine solution = 5 mL	No change in color is seen Orange red color is seen	Creatinine reacts with alkaline picrate to form creatinine picrate which forms orange red color	• Creatinine is present in urine • *Normal creatinine level in blood*: – In males: 0.6–1.2 mg/dL – In females: 0.5–1.1 mg/dL • *Normal urinary creatinine is*: 1–2 gm/day • *Increased urinary creatinine is seen in*: – Muscular disorders – Myopathies – Muscle trauma and injury – Fever • *Decreased urinary creatinine is seen in*: Renal failure • *Creatinuria is generally seen in*: – Uncontrolled diabetes mellitus – Thyrotoxicosis – Myasthenia gravis – Starvation – Infancy – Pregnancy – Growing phase

Test for Uric Acid

Experiment	Observation	Principle	Conclusion/Interpretation
Shiff's test: A strip of filter paper is moistened with 3% ammonical silver nitrate + Urine drops	Filter paper turns black	This happens because of reduction of silver nitrate to free silver in basic medium in presence of uric acid	• Uric acid is present in the urine • *Uric acid in blood*: In males—3.6–7.8 mg% and in females—2.5–6.8 mg% • *Uric acid in urine*: 300–800 mg/day • *Increased urinary uric acid is seen in*: – Cancers – Leukemia – ACTH administration – Wilson's disease – High protein diet • *Decreased urinary uric acid id seen in*: – Purine free diet – Gout
Benedict's uric acid test: Urine = 5 mL + Anhydrous sodium carbonate = 2 gm + Benedict's uric acid reagent = 5 drops	Intense blue color is obtained	• Benedict's uric acid reagent contains arseno-phosphotungstate • Sodium carbonates provides alkaline medium • Arseno-phosphotungstate gets reduced to Arseno-phosphotungstite by uric acid in basic medium • Arseno-phosphotungstite is blue in color	
Murexide test for uric acid (generally done for stone analysis/solid uric acid) Pinch of uric acid in a petri dish + Concentrated nitric acid = 3 drops + Heat it on water bath till it becomes dry + Add a drop of ammonia	Purplish red color is seen	• Concentrated nitric acid oxidizes uric acid to produce dialuric acid and alloxan • These two condense to form alloxanthine • Alloxanthine reacts with dilute ammonia to form ammonium purpurate/murexide which forms a purplish red color	

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Test for Urobilinogen (UBG)

Experiment	Observation	Principle	Conclusion/Interpretation
Ehrlich's test: Urine = 5 mL + Ehrlich reagent = 1 mL Let it stand for 5 minutes at room temperature Observe the change in color and compare with negative test	• Red color is seen for a positive UBG test • Fresh urine gives this test positive as urobilinogen is oxidized to urobilin in stored urine	• It confirms the presence of urobilinogen in urine • UBG reacts with para dimethylaminobenz-aldehyde and strong acid medium of Ehrlich's reagent to form a red color complex	• *Increase in urine urobilinogen is seen in*: – Viral hepatitis – Cirrhosis of liver – Obstruction of the gallbladder by gallstones (liver disorders that disturb normal passage of urobilinogen through the liver and gall bladder) – Hemolytic anemia – Pernicious anemia (destruction of many RBC's) • *Decreased levels of urine UBG is seen in*: – Obstructive jaundice – Broad spectrum antibiotics which destroy the bacterial flora (obstruction of bilirubin passage in the gut or failure of urobilinogen formation in the gut)

Tests for Inorganic Constituents of Normal Urine

Test for Urinary Chlorides

Experiment	Observation	Principle	Conclusion/Interpretation
Silver nitrate test: Urine = 2 mL + Concentrated nitric acid = 2 drops + 3% silver nitrate = 1–2 mL	White precipitate of silver chloride is seen	• Chlorides in urine react with silver nitrate to produce a white precipitate of silver chloride • Nitric acid prevents the precipitation of salts other than chlorides	• Chlorides are present in the urine sample • Dietary salts are sources of chlorides • Normal excretion of chlorides per day is 10–15 g • When reabsorption of sodium and chloride is impaired, the excretion of chloride is increased • *Increased chlorides in urine*: – Polydipsia – Use of diuretics – Addison's disease (aldosterone deficiency) • *Decreased chlorides in urine*: – Excess sweating – Fasting – Diarrhea – Excessive vomiting – Edema – Diabetes insipidus – Infections – Adrenocortical hyper functions (Cushing's syndrome)

Test for Urinary Sulfates

Experiment	Observation	Principle	Conclusion/Interpretation
Barium chloride test: Urine sample = 3 mL + Concentrated hydrochloric acid = 1 mL + 10% barium chloride = 2 mL	White precipitate of barium sulfate is seen	Urinary sulfate is precipitated as barium sulfate after reaction with barium chloride solution	• Sulphates are present in the urine • Dietary sulfur-containing amino acids are source of urinary sulfates • Normal excretion of sulfates per day is 0.8–1.0 g • *Increased urinary sulfates are seen in*: – Cystinuria – Homocystinuria – Melanuria – Obstructive jaundice – Hepatocellular jaundice – Cyanide poisoning – High protein diet intake • *Decreased urinary sulfates are seen in*: Renal function disorders

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Test for Urinary Phosphates

Experiment	Observation	Principle	Conclusion/Interpretation
Ammonium Molybdate test: Urine sample = 3 mL + Concentrated nitric acid = few drops + Ammonium Molybdate = pinch Heat	Canary yellow precipitate is seen	After heating with ammonium molybdate in presence of nitric acid, inorganic phosphates get precipitated as canary yellow colored ammonium-phosphomolybdate precipitate	• Phosphates are present in the urine • Normal excretion of phosphates per day is 1 g • *Increased urinary phosphates are seen in*: – Rickets – Osteomalacia – Hyperparathyroidism – Acidosis • *Decreased urinary phosphates are seen in*: – Diarrhea – Nephritis – Parathyroid hypofunction – Pregnancy – Hereditary fructose intolerance – Galactosemia

Test for Urinary Calcium

Experiment	Observation	Principle	Conclusion/Interpretation
Potassium oxalate test: Urine sample = 2 mL + 1% acetic acid = 5 drops + Potassium oxalate = 5 mL	White precipitate of calcium oxalate is seen	In acidic medium and in presence of potassium oxalate, calcium gets precipitated as calcium oxalate	• Calcium is resent in the urine • Normal excretion of calcium is 0.1–0.3 g per day • *Increased urinary calcium is seen in*: – Hyperparathyroidism – Hyperthyroidism – Hypervitaminosis D – Multiple myeloma – Renal stones – Renal tubular acidosis – Steroid therapy – Diuretic therapy • *Decreased urinary calcium is seen in*: – Hypoparathyroidism – Pseudohypoparathyroidism (decreased response of PTH and not its decreased secretion) – Steatorrhea – Nephrosis

Test for Urinary Ammonia

Experiment	Observation	Principle	Conclusion/Interpretation
Phenolphthalein test: Urine sample = 8 mL + Phenolphthalein = 1 drop + 0.1 N NaOH = few drops + Hold a glass rod dipped in phenolphthalein at the mouth of the test tube + Heat all the contents	Phenolphthalein of glass rod changes pink	• pH range of phenolphthalein is 8.3–10.0 (colorless to pink) • Ammonium salts of urine, in basic medium, release ammonia vapors • Ammonia emerging out from the test tube turns the indicator to pink color as ammonia is basic	• Ammonium salts are present in the urine • Excretion of ammonia as ammonium salts is important in maintaining acid base balance of the body • Urinary ammonia is derived from glutamine and other amino acids • Normal excretion of ammonia in urine per day is 0.6–0.8 g • *Increased urinary ammonia is seen in*: – Diabetic ketoacidosis – Intake of foods which can form acids – Excess water intake – Infections of urinary tract • *Decreased urinary ammonia is seen in*: – Alkalosis – Nephritis

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OSCE

1. **What are normal physical characteristics of urine?**
   Color: Pale yellow to amber (due to urochrome pigment)
   Clarity: Clear to slightly cloudy
   Odor: Mildly aromatic
   Volume: 800–2000 mL per day indicates normal hydration
   Specific gravity: 1.005–1.030 shows urine concentration
2. **Case scenario 1:**
   A healthy person gives a urine sample with these findings: pale yellow color, clear, specific gravity 1.015, no glucose, protein, or blood on dipstick. What is your interpretation?
   Findings: Pale yellow, clear, specific gravity 1.015, no abnormal dipstick results
   Interpretation: The sample is normal indicating adequate hydration without any pathological findings.
3. **Case scenario 2:**
   A patient drinks 3 liters of water daily. Urine is colorless, specific gravity is 1.005. Assess this?
   Findings: Colorless urine, specific gravity 1.005.
   Interpretation: Normal dilute urine because of high water intake.
   Explanation: High water intake results in increased urine output with less specific gravity, that is physiologically normal.
4. **Case scenario 3:**
   Urine sample exhibits a mild ammonical odor after standing for some hours. Is it normal?
   Findings: Mild ammonia odor after standing for few hours.
   Interpretation: Normal.
   Explanation: Urea of hydrolyzes down into ammonia as time passes due to bacterial action, leading to characteristic odor in stored urine.
5. **Case scenario 4:**
   A healthy athlete gives a urine sample after a marathon. Sample is dark yellow, specific gravity is 1.030. What does it indicate?
   Findings: Dark yellow urine, specific gravity 1.030.
   Interpretation: Urine is concentrated, normal after excessive physical activity/dehydration.
   Explanation: Exercise and dehydration results in reduced water excretion, leading to concentrated urine that is a normal physiological response.
6. **Case scenario 5:**
   A pregnant woman's urine sample exhibits pale yellow color, clear, pH of 6.5, no protein, and no glucose. What does it indicate?
   Findings: Pale yellow, pH 6.5, no protein or glucose.
   Interpretation: Normal urine sample.
   Explanation: Pregnancy results in high rate of glomerular filtration, but since there is absence of protein and/or glucose, this indicates towards normal renal function.
7. **Case scenario 6:**
   Urine sample from a person on a high-protein diet exhibits pH of 5.5 with no other pathological findings. Is this normal?
   Findings: pH 5.5, no other abnormalities.
   Interpretation: Normal acidic urine because of high-protein intake.
   Explanation: High-protein diets increase formation of acid and lowering the urine pH. This is normal physiological adaptation state.
8. **Case scenario 7:**
   Urine is pale yellow and gives a negative result for ketones, glucose, protein, and blood. Patient has no complaints. How would you interpret this?
   Findings: Pale yellow urine, no abnormal dipstick findings.
   Interpretation: Normal urine sample exhibiting healthy renal and metabolic functions.
9. **Case scenario 8:**
   A patient's urine has specific gravity: 1.020, pH: 7.0. No glucose, protein, or blood present. Give your interpretation.
   Findings: Specific gravity 1.020, pH 7.0, no abnormalities found.
   Interpretation: Normal urine sample with optimal hydration and no metabolic or renal issues in the patient.
10. **Case scenario 9:**
    A urine sample from a fasting person is pale yellow with no abnormalities on dipstick testing. What does it indicate?
    Findings: Pale yellow urine, no dipstick abnormalities.
    Interpretation: Normal urine despite fasting.
    Explanation: The body conserves glucose/ketone levels during fasting state, leading to normal urine composition unless prolonged fasting result in ketosis.

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11. Case scenario 10:
Urine pH: 6.8, specific gravity: 1.010. The patient takes balanced diet with no complaints. Interpret this.
Findings: pH 6.8, specific gravity 1.010, no complaints.
Interpretation: Normal urine reflecting balanced diet/healthy hydration.

12. What are the factors that influence normal urine composition?
Diet: High protein/acidic urine), vegetarian/alkaline urine.
Hydration: More fluids dilute the urine; dehydration concentrates the urine.
Physical activity: Strenuous exercises increase urine concentration.
Circadian rhythm: Urine output/composition vary in between day and night.

CLINICAL MCQS

1. What is the normal range for urine pH under physiological conditions?
a. 4.5–8.0
b. 3.2–5.0
c. 7.4–9.0
d. 6.2–10.0
Answer: a. 4.5–8.0

2. A patient's urine sample turns red after standing. Which condition is associated with this finding?
a. Hematuria
b. Porphyria
c. APKU
d. Glycosuria
Answer: b. Porphyria

3. Which test is done for detecting the presence of reducing sugars in urine?
a. Benedict's test
b. Biuret assay
c. Rothera's test
d. Sulkowitch method
Answer: a. Benedict's test

4. In urine analysis, the presence of ketone bodies is suggestive of which clinical condition?
a. UTI
b. Diabetic ketoacidosis
c. Nephrosclerosis
d. Glomerulonephritis
Answer: b. Diabetic ketoacidosis

5. Which parameter in a routine urine dipstick test is used to screen for UTI?
a. Plasma
b. Leukocyte esterase
c. Acetone
d. Ketones
Answer: b. Leukocyte esterase

6. A urine sample exhibits frothy appearance with positive proteins. Which condition is most likely?
a. Nephrotic syndrome
b. Diabetes insipidus
c. Pyelonephritis
d. Hematuria
Answer: a. Nephrotic syndrome

7. What does the presence of bilirubin in urine suggest?
a. Liver dysfunction or biliary obstruction
b. Renal damage
c. Hematuria
d. Normal state
Answer: a. Liver dysfunction or biliary obstruction

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8. The presence of nitrites in a urine dipstick test is suggestive of which of the following?
a. Albuminuria
b. Bacterial infection, particularly by gram-negative bacteria
c. Dehydration
d. Heart failure

Answer: b. Bacterial infection, particularly by gram-negative bacteria

9. A patient's urine sample is positive for glucose and negative for ketone bodies. What is the most likely cause?
a. Diabetes insipidus
b. Renal glycosuria
c. Fasting
d. Alcohol

Answer: b. Renal glycosuria

10. Rothera's test is done to detect which substance in urine?
a. Protein
b. Ketone bodies
c. Biliverdin
d. Urea

Answer: b. Ketone bodies

REFLECTION

Analysis of Physiological Urine Sample

Test	Observation	Inference

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Result and Clinical Interpretation

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Teacher's Signature with Date

REFLECTION

Analysis of Physiological Urine Sample

Test	Observation	Inference

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Result and Clinical Interpretation

Teacher's Signature with Date

VIVA VOCE

Q1. Discuss normal constituents of urine/what is the significance of every constituent for maintaining body homeostasis?
Next question/Follow-up: How the factors like hydration status/diet influence the composition of normal urine?

Q2. Discuss about normal volume of urine produced in a 24-hour period/what factors can result in variations in urine output?
Next question/Follow-up: How do the renal system regulate urine concentration/volume in response to hydration levels?

SPOTTERS

Q1. Show a sample of normal urine having pale yellow color/no visible abnormal constituents. Interpret/what does this suggest about the patient's hydration status?

Hints: Pale yellow color suggests normal hydration. A clear sample with no visible abnormalities indicates the absence of infections/disease/other pathological conditions.

Q2. A urine sample shows specific gravity of 1.015, pH of 6.0/no protein/no glucose/no blood. Interpret/what does it suggest about the patient's renal function/health?

Hints: The results are typical for a healthy individual with normal renal functions. Specific gravity and pH are normal and indicative of proper kidney function in regulating water/electrolyte balance.

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Practical Biochemistry

With Clinical Correlation

This book represents a culmination of her years of dedication to both science and education, aimed at providing students and professionals with a comprehensive and up-to-date resource on the fascinating world of biochemistry.

Nita Sahi is a renowned expert in the field of Biochemistry with over 25 years of experience in both research and teaching. She holds PhD degree in (Medicine) Biochemistry from RNT Medical College, Udaipur (RUHS), Rajasthan. Throughout her career, Dr Sahi has contributed significantly to advancing the understanding of specific areas of biochemistry, e.g., metabolic pathways, enzyme kinetics, molecular biology, etc. She is currently serving as Dean-Academics (UG and PG), Professor and Head of Department of Biochemistry at Pacific Medical College and Hospital, Udaipur, and MEU Member.

She has published numerous papers in leading scientific journals, focusing on specific topics or areas of expertise. As a Professor and Head at PMCH Udaipur, she has inspired and mentored countless students pursuing careers in biochemistry. Her teaching approach combines rigorous theoretical knowledge with practical insights, making complex biochemical concepts accessible and engaging for learners at all levels.

In addition to her academic and research work, she is a frequent speaker at conferences, sharing her latest findings and innovative ideas. She is passionate about promoting scientific education and fostering a deeper understanding of biochemistry's role in medicine, biotechnology, and environmental science.

Disha Sahi MBBS is a medical graduate from Pacific Medical College and Hospital, a constituent college of Pacific Medical University, Udaipur, Rajasthan. With a strong academic foundation and a keen interest in medical education, she has developed a thoughtful and student-centric approach to learning.

Having earned a distinction in Biochemistry, she brings both personal experience and academic insight to this work. Her contribution reflects a genuine effort to make biochemistry more accessible, clinically relevant, and easier to understand for both undergraduate and postgraduate students.

Passionate about bridging the gap between theory and practice, she believes that effective learning happens when concepts are made simple, relatable, and rooted in real-life application. This book is a step in that direction. She has displayed a remarkable blend of clinical insight and academic passion early in her career.

With a deep interest in medical education, she brings a fresh perspective to traditional biochemistry teaching, making it more student-friendly, clinically relevant, and examination-oriented. Her collaboration on this book reflects a commitment to helping medical and postgraduate students overcome common learning hurdles through simplified explanations, real-life correlations, and practical insights. This is her first co-authored work—and just the beginning of what promises to be a prolific academic journey.

Shelving Recommendation
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ISBN 978-93-5696-074-9

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