Urine Analysis

Objectives :

At the end of the practical, the student should be able to,

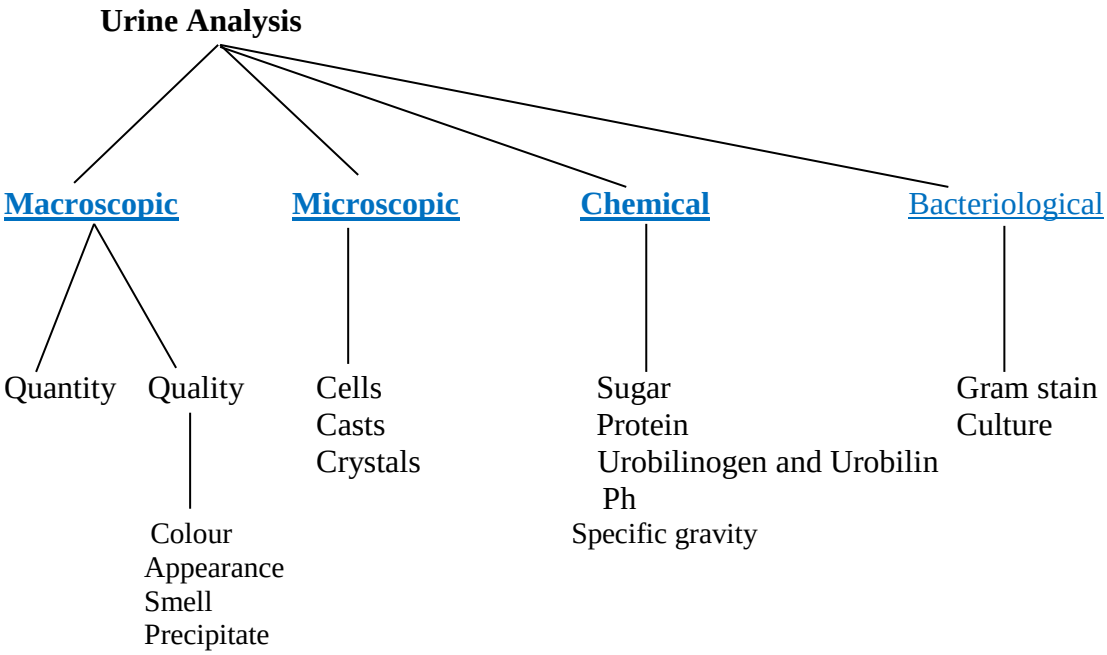
1. Describe how to collect a sample of urine for analysis
2. Interpret the results of macroscopic examination of a sample of urine
3. Identify the following in a sample of urine, under the microscope
    a. RBC
    b. WBC
    c. Epithelial cells
    d. Casts (Hyaline, cellular, granular)
    e. Crystals
4. Explain the physiological significance of the following,
    a. Observation of RBCs, WBCs, Casts or Crystals in a sample of urine
    b. Polyuria, Oliguria and Anuria.
5. Measure the specific gravity of a sample of urine.
6. Describe the technique of measuring urine abnormality.

Introduction:

A urinalysis is a laboratory test. It can help your doctor detect problems that may be shown by your urine. Many illnesses and disorders affect how your body removes waste and toxins. The organs involved in this are your lungs, kidneys, urinary tract, skin, and bladder. Problems with any of these can affect the appearance, concentration, and content of your urine. Your doctor may also order this test if they suspect that you have certain conditions, such as: diabetes, kidney disease, liver disease and urinary tract infection. Your doctor may also want to do a urinalysis if you experience certain symptoms, including: Abdominal pain , back pain ,blood in your urine and painful urination.**Urine** is a fluid obtained from the blood through the renal glomeruli with considerable changes before it is excreted as urine. The first step in urine formation is by ultrafiltration at the glomeruli which is about 170 – 200 L/24 hrs.. There is also active secretion at the renal tubules. Collection of urine specimen depends on the test required either random sample or 24- hours sample. Urine specimen tends to deteriorate unless the correct preservative is added (toluene, chloroform, thymol and formalin) or the specimen is refrigerated throughout the collection period.

Urine samples are usually examined for the main items:

- **Physical examination.**
- **Biochemical examination.**
- **Microscopic examination.**



Collection of a sample of urine

Over the course of a 24-hour period, the composition and concentration of urine changes continuously. For this reason, various types of specimens may be collected, including:

- First morning specimen
- Single random specimen
- Timed short-term specimens
- Timed long term specimens: 12 or 24 hours
- Catheterized specimen or specimen from an indwelling catheter
- Double voided specimens (test for sugar and acetone)
- Clean-catch (midstream) specimen for urine culture and cytological analyses

Urine specimens need to be examined within 2 hours. Urine that is left to standing too long becomes alkaline because bacteria begins to split the urea contained in urine into ammonia. Visualization of urine and other tests are inaccurate if the pH of the urine specimen has become highly alkaline. A urine specimen should be refrigerated if it cannot be sent to the laboratory within 2 hours.

Macroscopic appearance of urine

Quantity: **Volume, normally** 700 - 2500 ml/day

↑ Volume - ↑ fluid intake
Consumption of Alcohol
Eating salty foods

↓ Volume - ↓ fluid intake
Dehydration

Anuria is the absence of a urine output and is seen in,

- ✓ Acute renal failure
- ✓ Urinary tract obstruction

Oliguria is a urine output of < 400 ml/day in an adult. Is seen in,

- ✓ Severe dehydration
- ✓ Acute renal failure
- ✓ Acute glomerulonephritis

Polyuria is a urine output of > 2500 ml/day and is seen in,

- ✓ Diabetes Mellitus
- ✓ Diabetes Insipidus
- ✓ Chronic Renal Failure

Quality: Color

Characteristic colour of urine is due to the pigments, urochrome and uroerythrin

◊ **Normal color:** is pale yellow or amber yellow (due to urochrome and urobilin pigments).

◊ **Colorless urine:** very diluted urine due to:
-Physiological causes: excessive fluid intake in normal person.
-Pathological causes: uncontrolled diabetes mellitus, diabetes insipidus and chronic renal failure.

◊ **Orange urine: is due to:**

- Ingestion of large amount of carotenoids (vitamin A).
- Concentrated urine (hot weather, high fever, dehydration..etc).

◊ **Yellow - green urine:** is due to bilirubin or biliverdin (jaundice).

◊ **Red urine: is due to:**

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- Some drugs (rifampin for treatment of T.B., carmurit…etc).
- Blood or hemoglobin.

◊ **Dark brown or black urine: is due to:**

- Methemoglobin.
- Melanin (melanoma).
- Malignant malaria (black water fever).

◊ **Smoky urine:** is due to presence of RBCs in acute glomerulonephritis.

Smell (Odor)

a. **Urineferous odor**: normal odor of fresh voided urine (due to presence of aromatic acids).

b. **Fruity odor:** is due to acetone (diabetic ketoacidosis).

c. **Ammoniacal odor:** is due to release of ammonia as a result of the bacterial urease enzyme in the contaminated and long standing exposed urine sample.
d. **Mousy odor:** is due to PKU (Phenylketonuria).

e. **Burnt sugar odor:** is due to maple syrup urine disease.

Deposits:

a. **Normally**, the urine contains no deposits.
b. **Deposits** are mainly due to:

- Crystals, salts or cells.
- Blood clots, necrotic tissues and urinary stones.
- Whitish precipitate seen in heavy proteinuria

Chemical examination:

Reaction (pH):

a. Normal range: 4.6 - 7.0 (the average pH is about 6.0).

b. Acidic urine: is due to ketosis (diabetes mellitus & starvation), severe diarrhea, metabolic and respiratory acidosis, excessive ingestion of meat and certain fruits (cranberries).

c. Alkaline urine: is due to:
- Respiratory and metabolic alkalosis.
- Urinary tract infection.
- Vegetarians.

Normal composition of urine:

Urine is a fluid composed of water (95 %) and inorganic and organic solids (5%) that include:

A) **Chief inorganic solids include:** - Sodium - Potassium - Chlorides.
In addition, smaller amounts of calcium, magnesium, sulfate and phosphates, and traces of iron, copper, zinc and iodine.

B) **Chief organic solids are:**
1. Non-protein nitrogen (NPN) compounds.
2. Organic acids
3. Sugars.

Abnormal constituents of urine:

I. Proteinuria:

- Normally less than 200 mg protein is excreted in the urine daily; more than this level leads to a condition called " proteinuria".
- Proteinuria is either glomerular or tubular. **Glomerular** proteinuria is due to increased glomerular permeability leading to filtration of high molecular weight proteins (e.g. glomerulonephritis).**Tubular** proteinuria occurs as a result of decreased reabsorption with normal glomerular permeability leading to excretion of low molecular weight proteins (e.g. chronic nephritis).

The urine should then be proceeded with :

1- **Sulpho-salicylic acid test:** This test for protein is very reliable and does not require heat. In a test tube place about 5 mls of urine and add drops of 20 % sulpho-salicylic acid. The formation of a cloud indicates the presence of protein. The cloud is seen best when looked for against a black background.

2- **Boiling test:** Fill a small test-tube two-thirds full of urine. Add 10 % acetic acid drop by drop and boil, if the cloud disappears it consist of phosphates, if it persist protein is present. Acid should be added drop by drop till no further precipitation of protein occurs.

Clinical finding of proteinuria is classified into three forms:

1. Pre-renal proteinuria:

- **Bence-Jones protein:**
  - This abnormal gamma globulin (light chains only) is synthesized by malignant plasma cells (multiple myeloma).

2. Renal proteinuria:

- After prolonged standing (orthostatic).
- Severe muscular exercise.
- Congestive heart failure, hypertension, fever, stress.
- Gestational (in the 3rd trimester of pregnancy).
- Glomerulonephritis.
- Diabetic nephropathy.

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3. Post-renal proteinuria:

- Lower urinary tract infection, tumors or stones.

II. Glycosuria

presence of detectable amount of any sugar in urine) includes the following:

1. **Glucosuria:** (presence of detectable amount of glucose in urine).
   - **Uncontrolled DM**: The concentration of glucose in the plasma exceeds the renal threshold.
   - **Renal glucosuria**: Normal plasma glucose concentration with proximal tubular malfunction leads to decreased renal threshold (gestational diabetes and Fanconi's syndrome).

2. **Fructosuria:** (Presence of fructose in urine)
   - **Alimentary:** due to increased fructose intake.
   - **Metabolic:** deficiency of fructokinase or aldolase B enzyme in the liver.

Biochemical tests for diagnosis the sugar in urine:

The presence of a reducing substance in the urine may be detected by means of **Benedict's test** or by the use of **Clinitest tablets**.

Benedict's test: To 5 ml of Benedict's reagent add 8 drops of the urine, boil for 2 minutes and allow to cool. If a reducing substance is present a precipitate will appear, varying from a light green turbidity to a red precipitate. If the reduction is due to glucose the test gives approximately quantitative results as following:

Alight **green** turbidity = 0.1 to 0.5 % of sugar
A **green** precipitate = 0.5 to 1.0 % of sugar
A **yellow** precipitate = 1.0 to 2.0 % of sugar
A **red** precipitate = 2.0 % of sugar or over

III. Ketonuria: (Presence of ketones "Acetone, acetoacetic acid and β- Hydroxybutyric acid" in urine)

- Diabetic ketoacidosis.
- Glycogen storage diseases.
- Starvation.
- Prolonged vomiting.
- Unbalanced diet: high fat and low carbohydrate diet.

IV. Nitrite:

- Positive nitrite test is significant of bacteruria in urine.

V. Choluria:

a) Bilirubin / bile salts: in cases of

- Hepatocellular damage.
- Obstruction of bile duct: extrahepatic (stone) or intrahepatic (tumors).

b) Urobilinogen:

- Normally, present in trace amounts in urine.
- Markedly increased in:
  - Hemolytic anemia.
  - Hepatocellular damage.

VI. Blood:

a) Hematuria (Presence of detectable amount of blood in urine):

- Acute and chronic glomerulonephritis.
- Local disorders of kidney and genito-urinary tract (trauma, cystitis, renal calculi, tumors …..etc).
- Bleeding disorders (hemophilia).

b) Hemoglobinuria (Presence of hemolysed blood in urine):

- Hemoglobinopathies (sickle cell anemia and thalassemia).
- Transfusion reaction (blood incompatibility).
- Malaria (plasmodium falciparum)

Urine analysis (using dipstick):

Principle:

Dipsticks are plastic strips impregnated with chemical reagents which react with specific substances in the urine to produce **color-coded visual results**. They provide quick determination of pH, protein, glucose, ketones, bilirubin, hemoglobin, nitrite, leukocytes and specific gravity. The depth of color produced relates to the concentration of the substance in urine**.** Color controls are provided against which the actual color produced by the urine sample can be compared.The reaction times of the impregnated chemicals are standardized.

Procedure:

1- Dip the dipstick in the urine sample provided then remove it immediately.

2- Remove the excess urine.

3- Read the color produced within 60 seconds.

4- Match the color changes to the control charts.

5- Give a full report about:

- Physical examination.
- Chemical examination.

Microscopic examination of urine :

1 -A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 r.p.m) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube.

2. The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube.

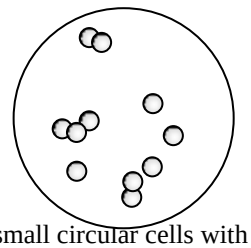
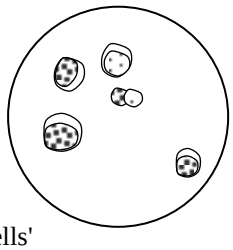
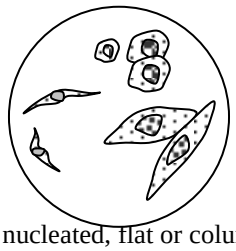
3. The sediment is resuspended in the remaining supernatant by flicking the bottom of the tube several times.

4. A drop of resuspended sediment is poured onto a glass slide and cover-slipped.

5. The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. Next, examination is carried out at high power to identify crystals, cells, and bacteria

Microscopic appearance of urine

Cells

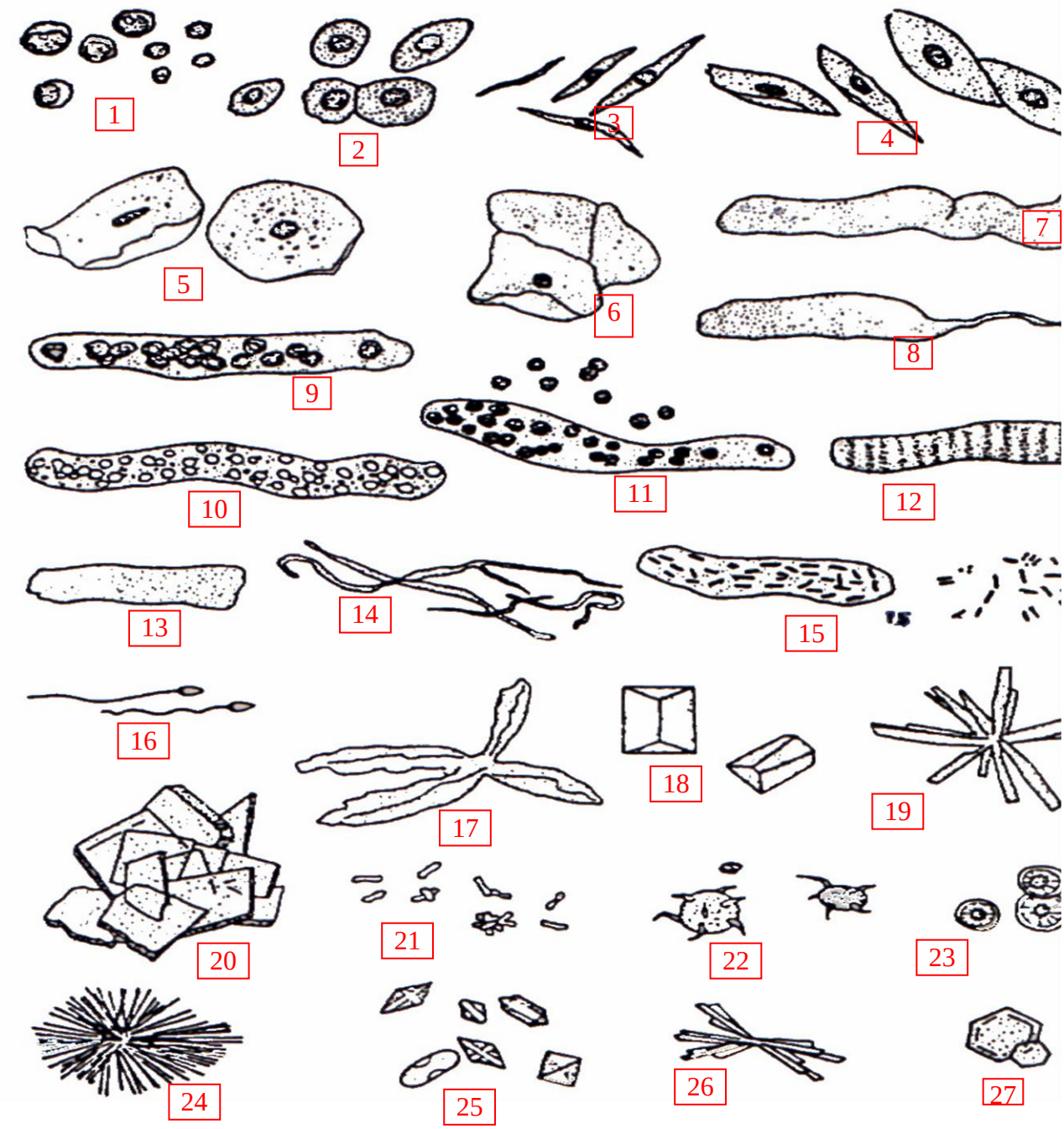
Red Blood Cells	White Blood Cells	Epithelial Cells
 - Are small circular cells with a yellowish center - 2 -3 cells/mm^3 in females - normally no cells in males - Increased amounts seen in, Glomerulonephritis UTI Urinary calculi	 - 'Pus cells' - Larger than RBC - Round shaped with lobed nuclei and a granular cytoplasm - Normally <10cells/mm^3 - Increased amounts in, Cystitis Urethritis Nephritis Prostatitis	 - Are nucleated, flat or columnar cells - Normally, only little amounts seen. - If large amounts, there may be some tubular damage

Casts

- Are cylindrical structures formed by precipitation of muco-proteins (Tamm - Horsefal proteins) in the tubular lumen.
- Hyaline Casts - Pale, transparent and homogenous apperance
- Cellular Casts - Cells are embedded on the hyaline casts, to form RBC, WBC, Epithelial cell casts
- Granular Casts - Disintegrated cells on the cellular casts give the granular Appearance

Crystals

- Are formed by precipitation of chemicals in urine.
- Ammonium, Magnesium, Calcium phosphate crystals (formed in alkaline urine) also known as Triple Phosphate crystals have a "coffin lid" shape.
- Calcium Oxalate crystals are formed in acid urine and have an envelope shape.
- Uric acid crystals maybe normal, but are significantly increased in hyperuricaemia (gout).



1 Leucocyte and pus cell
2,3,4 Polygonal, epithelial cells
5,6 Squamous epithelial cells
7 Hyaline casts
8 Pseudohyaline
9 Epithelial cell cast
10 Erythrocyte cast
11 Leucocyte cast and leucocytes
12 Granular casts (coarse granules)
13 Waxy cast (fine granules)
14 Fibres
15 Bacterial casts and bacteria
16 Spermatozoa
17 ,18 Triple phosphate crystals
19 Calcium hydrogen phosphate
20 Uric acid crystals
21 Calcium carbonate crystals
22 Ammonium urate crystals
23 Leucine crystals
24 Tyrosine crystals
25 Calcium crystals
26 Calcium urate crystals
27 Cysteine crystals