Students Selective /S5/2022-2023
Basic of Biochemical Testing

General Urine Examination (Urinalysis)

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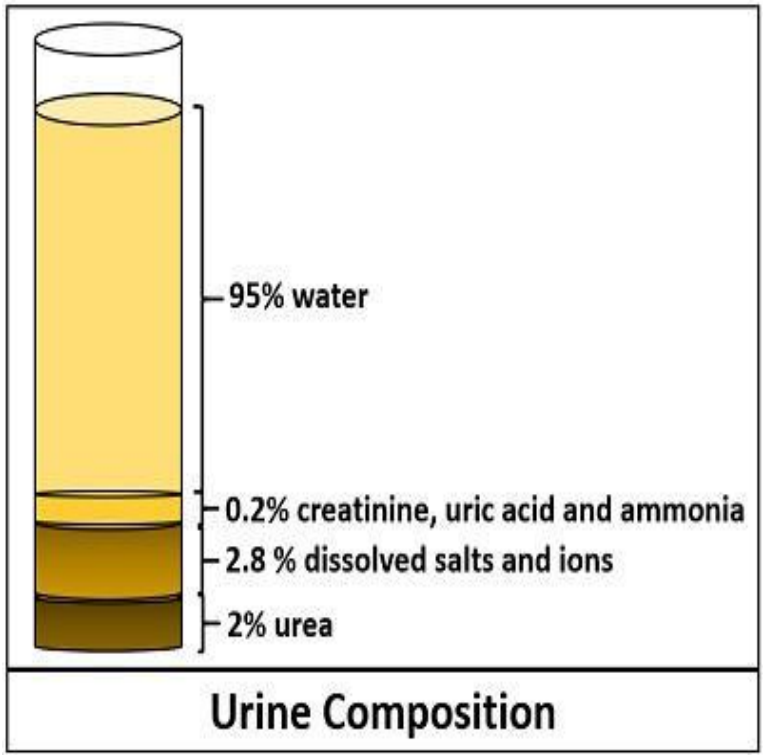
Urine Formation & Composition

- Urine is **an excretory** product (fluid) of the body .

- kidneys continuously form urine as an **ultra filtrate of plasma.** **Reabsorption** of water and essential filtered substances converts approximately 170,000 mL of this filtrate to the average daily **urine output of 1200 mL.**

Urine Composition

- Urine is normally 95% water and 5% solutes (urea and other organic and inorganic chemicals dissolved in water)



- **Urea**, a metabolic waste product, accounts for nearly half of the total dissolved solids in urine. Other organic substances include primarily **creatinine** and **uric acid**.

- The major ***inorganic solid*** dissolved in urine is **chloride**, followed by sodium and potassium.

- substances such as hormones, vitamins, and drugs also found in urine.

- Although not a part of the original plasma filtrate, the urine also may contain formed elements, such as cells, casts, crystals, mucus, and bacteria.

❖Variations in the concentrations of these solutes can occur owing to the influence of factors such as :

- Dietary intake
- Physical activity
- Body metabolism
- Endocrine functions.

- Analyzing urine was actually the beginning of laboratory medicine, references to the study of urine can be found in the drawings of cavemen and in Egyptian hieroglyphics,

- Two unique characteristics of a urine specimen account for this continued popularity:

1. Urine is a readily available and easily collected specimen.
2. Urine contains information, which can be obtained by inexpensive laboratory tests, about many of the body's major metabolic functions.

Specimen Collection

- Specimens must be collected in **clean**, **dry**, **leak-proof , disposable** containers.

- Containers are available in a variety of sizes and shapes, including bags with adhesive for the collection of pediatric specimens and large containers for 24-hour samples.

Adhesive bag

24-hour urine collection container

❖Specimen Integrity

Following collection, specimens should be delivered to the laboratory promptly and tested **within 2 hours**, otherwise sample should be refrigerated or have an appropriate chemical preservative added.

Types of Specimens

1-Random Sample

- Most commonly used because of its ease of collection & convenience for the patient (can be collected at any time of the day).

- However, it may also show erroneous results resulting from dietary intake or physical activity just before collection. The patient will then be requested to collect an additional specimen under more controlled conditions.

2-First Morning Specimen

- This is a concentrated specimen, thereby assuring detection of chemicals and formed elements that may not be present in a dilute random specimen.

- The patient should be instructed to collect the specimen immediately on awaking and to deliver it to the laboratory within 2 hours or keep it refrigerated.

3- 24-Hour (or Timed) Specimen

- Measuring the exact amount of a urine chemical is often necessary instead of just reporting its presence or absence. A carefully timed specimen must be used to produce accurate **quantitative results**

- All specimens should be refrigerated or kept on ice during the collection period and may also require addition of a chemical preservative.

Sample 24-Hour (Timed) Specimen Collection Procedure

Provide the patient with written instructions, and explain the collection procedure.

Provide the patient with the proper collection container and preservative.

Day 1: 7 a.m.: patient voids and discards specimen; collects all urine for the next 24 hours.

Day 2: 7 a.m.: patient voids and adds this urine to previously collected urine.

On arrival at laboratory, the entire 24-hour specimen is thoroughly mixed, and the volume is measured and recorded.

The preferred urine sample **mid-stream** : at first patients required to cleanse the urethral area, then void the first portion of the urine stream into the toilet, the urine midstream is then collected into a clean container (any excess urine should be voided into the toilet).

General Urine examination consist of 3 parts :

1. Physical examination
2. Chemical examination
3. Microscopic examination

Physical Examination

1. Urine color
2. Appearance
3. Volume
4. Oder

Urine color & Appearance

- Common descriptions include **pale yellow, yellow, and dark yellow**.

- This is attributed to the presence of pigments, such as **urochrome** (a product of endogenous metabolism),and **Urobilin** (an oxidation product of the normal urinary constituent urobilinogen) the later imparts an orange-brown color to urine that is not fresh.

- Freshly voided normal urine is usually **clear**, turbidity in a fresh specimen caused by RBCs, WBCs, bacteria, epithelial cells and mucus.

Laboratory Correlation of Urine Color

color	Cause
Pale yellow	polyuria : diabetes insipidus ,diabetes mellitus dilute random specimen (recent fluid consumption)
Dark yellow	concentrated specimen , increase Bilirubin, increase urobilinogen
Pink -Red	RBCs, Hemoglobin, Myoglobin beets , rifampin ,menstrual contamination
Brown -Black	Homogentisic acid (alkaptonuria)

Urine Volume

- normal daily urine output **600 to 2000 mL**.

❖Factors that influence urine volume include :

1. fluid intake
2. fluid loss from non-renal sources
3. variations in the secretion of antidiuretic hormone
4. Increased amounts of dissolved solids, such as glucose in urine.

❖Oliguria : a decrease in urine output (less than 400 mL/day), occur during dehydration as a result of excessive water loss from vomiting, diarrhea, or severe burns.

- Oliguria may result from acute kidneys injury (AKI) .

❖Polyuria: an increase in daily urine volume (greater than 2.5 L/day), is often associated with diabetes mellitus , diabetes insipidus and diuretics use.

Oder

- Freshly voided urine has a faint aromatic odor. As the specimen stands, the odor of ammonia becomes more prominent (the breakdown of urea is responsible for the characteristic ammonia odor).

Causes of unusual odors include

- ❖Bacterial infections, which cause a strong, unpleasant odor.
- ❖Diabetic ketoacidosis , which produce a sweet or fruity odor.
- ❖Maple syrup urine disease: metabolic defect results in urine with a strong odor of maple syrup

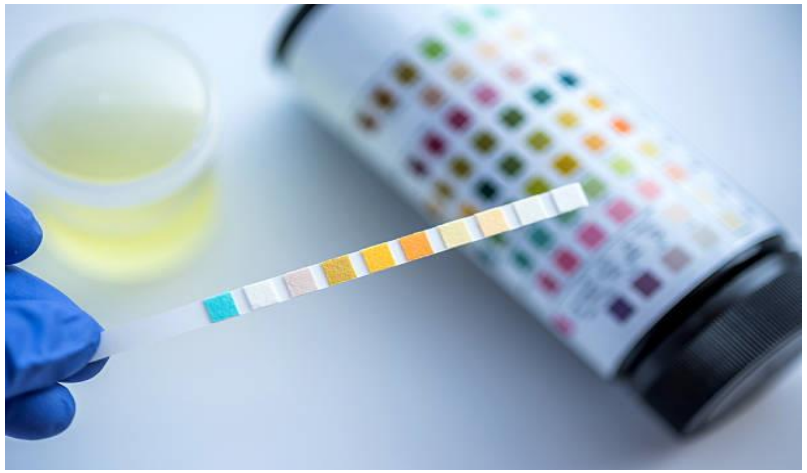
Chemical Examination

- Routine examination of urine has changed dramatically by the development of the **reagent strip method**, providing a simple, rapid means for performing medically significant chemical analysis of urine, including:

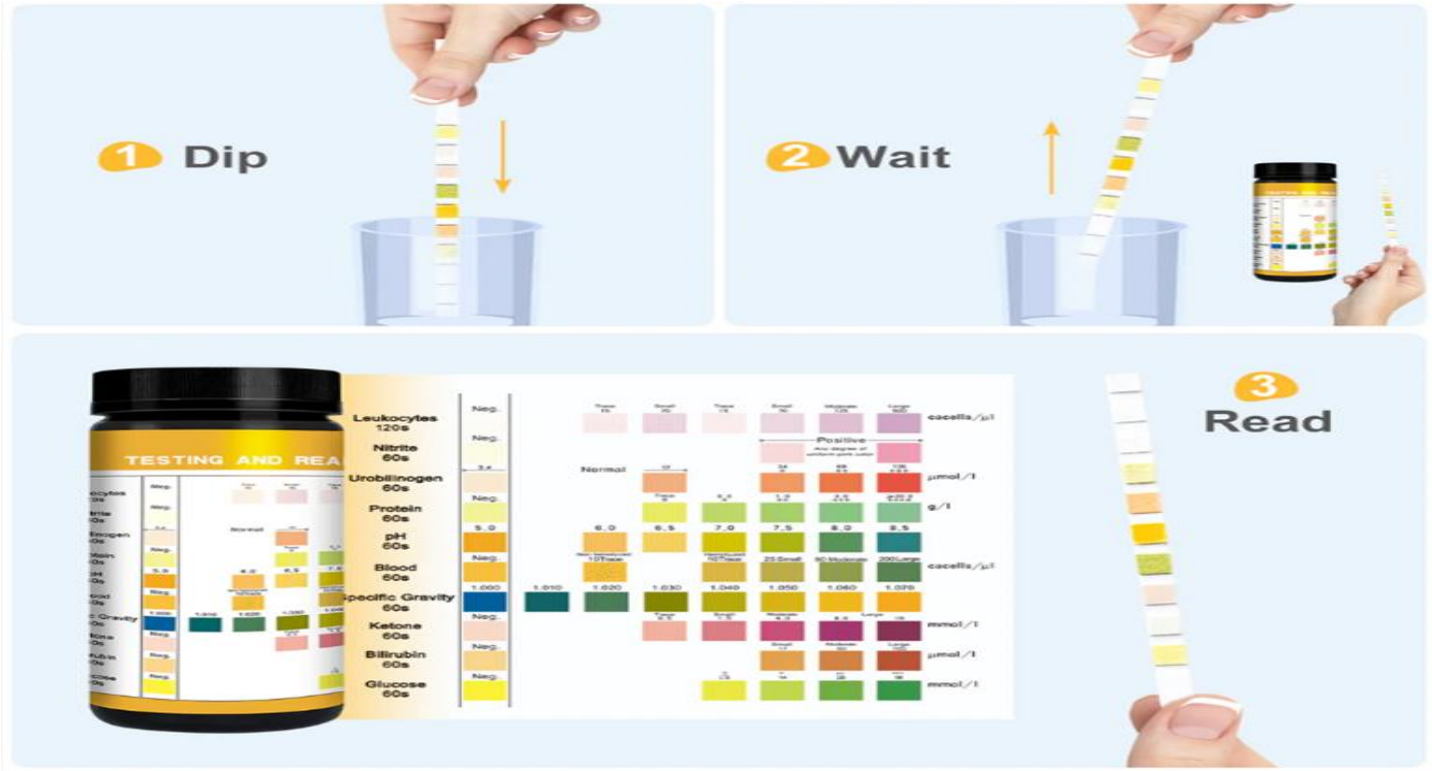
1. Protein
2. Glucose
3. Ketones
4. Blood
5. Bilirubin
6. Urobilinogen
7. Nitrite
8. Leukocytes esterase
9. PH
10. Specific gravity.

- A reagent strip, also called a **dipstick**, is a narrow strip of plastic with small pads attached to it. Each pad contains reagents for a different chemical reaction, thus allowing for the simultaneous determination of several tests.

- The color intensity generated on each reagent pad vary according to the concentration of the analyte present.



- The reactions are then interpreted by comparing the color produced on the pad within the required time frame with a chart supplied by the manufacturer.
- By careful comparison of the colors on the chart and the strip, a semi quantitative value of trace, 1+, 2+, 3+, or 4+ can be reported



Protein

- Of the routine chemical tests performed on urine, the most indicative of renal disease is the protein determination. Proteinuria is often developed in the early stage renal disease.

- The loss of plasma proteins through the glomeruli is restricted by the **size** of the pores and by a **negative charge** on, the basement membrane that repel negatively charged protein molecules.

- **less than 200 mg** of protein is normally excreted in the urine each day (half of which is Tamm–Horsfall protein, secreted by tubular cells).

Causes of proteinuria:

a. **Nephrotic Syndrome** is a clinical condition caused by increased glomerular permeability, resulting in a daily urinary protein loss of more than 3 g with hypoalbuminemia, edema, hyperlipidemia.

b. **Microalbuminuria**: urinary albumin loss at a range of 30-300 mg per 24 hours, which usually **undetected by reagent strip** and required quantitative determination using more sensitive immunoassays. Microalbuminuria is shown to precede the overt renal disease and is an indicative of increased risk for development of diabetic nephropathy.

❖Blood and pus in the urine also give positive tests for protein.

Glucose

- Under normal circumstances, **almost all the filtered glucose** is actively reabsorbed in the proximal convoluted tubule.

- The quantity of glucose that appears in the urine is dependent upon the ***blood glucose level***, the ***rate of glomerular filtration***, and the degree of ***tubular reabsorption***.

❖The blood level at which tubular reabsorption stops **(renal threshold)** for glucose is approximately 160 to 180 mg/dL, when the blood glucose exceeds the renal threshold, the tubules cannot reabsorb all of the filtered glucose, and **glycosuria** occurs.

Glycosuria may be due to:

- Diabetes mellitus.
- Renal glycosuria: in which the renal threshold for glucose decreases so that glucose is present in urine despite of normal blood sugar level.
- Pregnancy

Blood

- blood may be present in the urine either in the form of intact red blood cells (**hematuria**) or as the product of red blood cell destruction, hemoglobin **(hemoglobinuria**).

- hematuria produces a cloudy red urine, and hemoglobinuria appears as a clear red specimen

❖Major causes of **hematuria** include

1. renal calculi
2. glomerular diseases
3. tumors
4. trauma
5. pyelonephritis
6. exposure to toxic chemicals and anticoagulant therapy

- **Haemoglobinuria**: (Presence of free Hb in urine), seen in intravascular hemolysis when the binding capacity of Haptoglobin is exceeded.
- **Myoglobinuria**: presence of myoglobin in urine(seen in crush injuries and muscular disorders) also give positive test for blood .

How to differentiate between hematuria and hemoglobinuria?

Microscopic Examination of Urine

Microscopic examination of the sediment obtained from the centrifugation of a fresh urine sample shows the presence of :

- **Few cells** (erythrocytes, leukocytes, and cells derived from the kidney and urinary tract)
- **Casts** (composed predominantly of Tamm-Horsfall glycoprotein, other proteins present in the urinary filtrate, such as albumin and immunoglobulins, are also incorporated into the cast matrix)

❖Casts are the only elements found in the urinary sediment that are unique to the kidney.

❖ They are formed within the lumens of the distal convoluted tubules and collecting ducts, providing a microscopic view of conditions within the nephron. Their shape is representative of the tubular lumen.

❖The most frequently seen cast is the **hyaline type**, which consists almost entirely of Tamm-Horsfall glycoprotein .

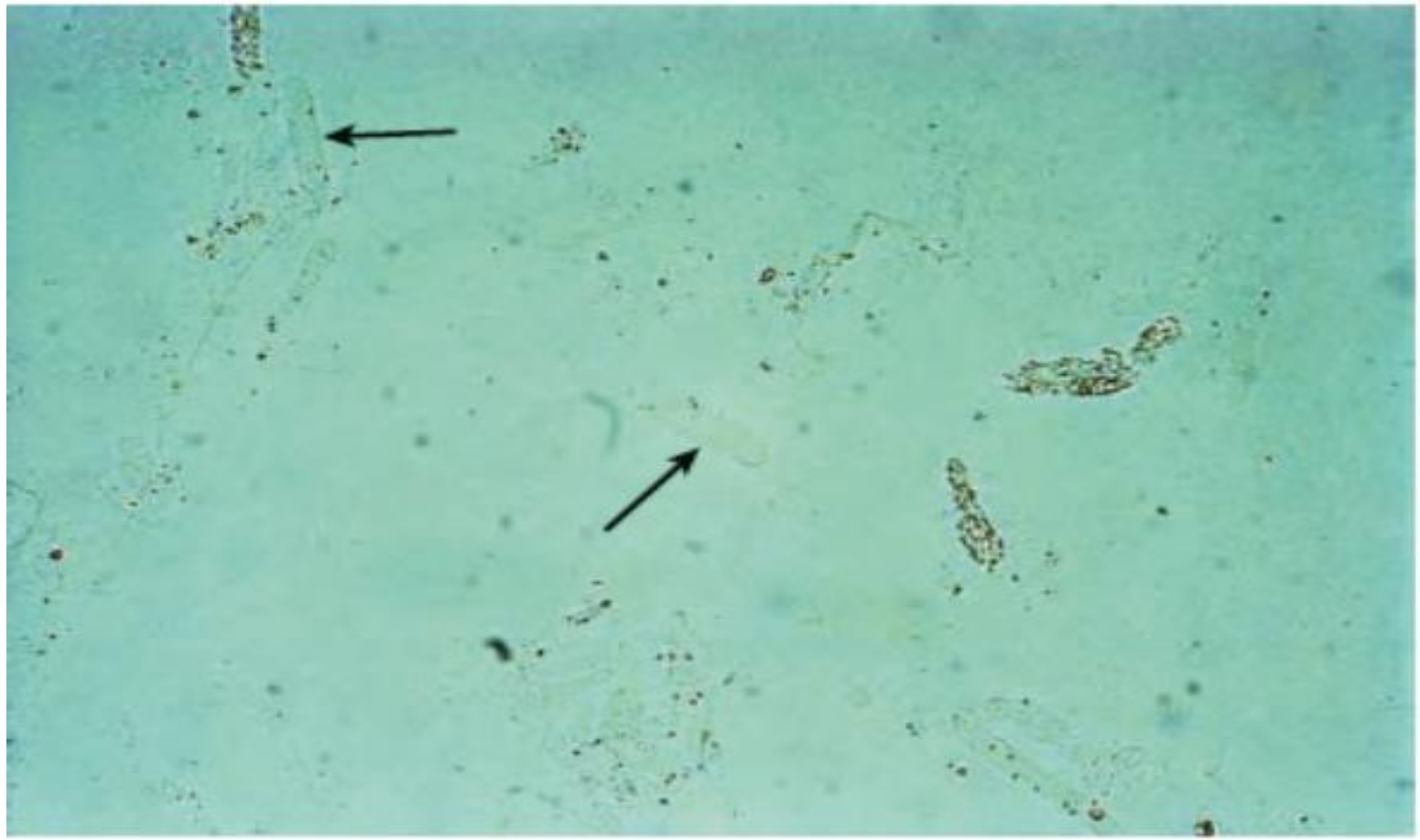


Figure 6–43 Hyaline casts under low power (×100).