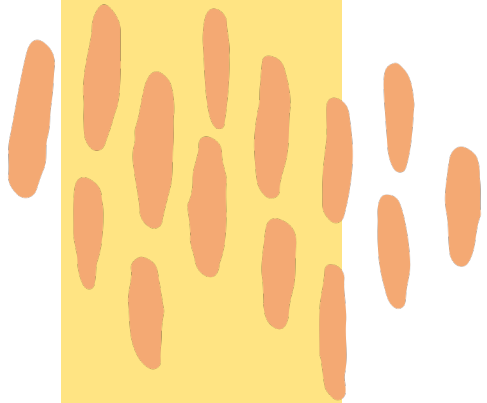
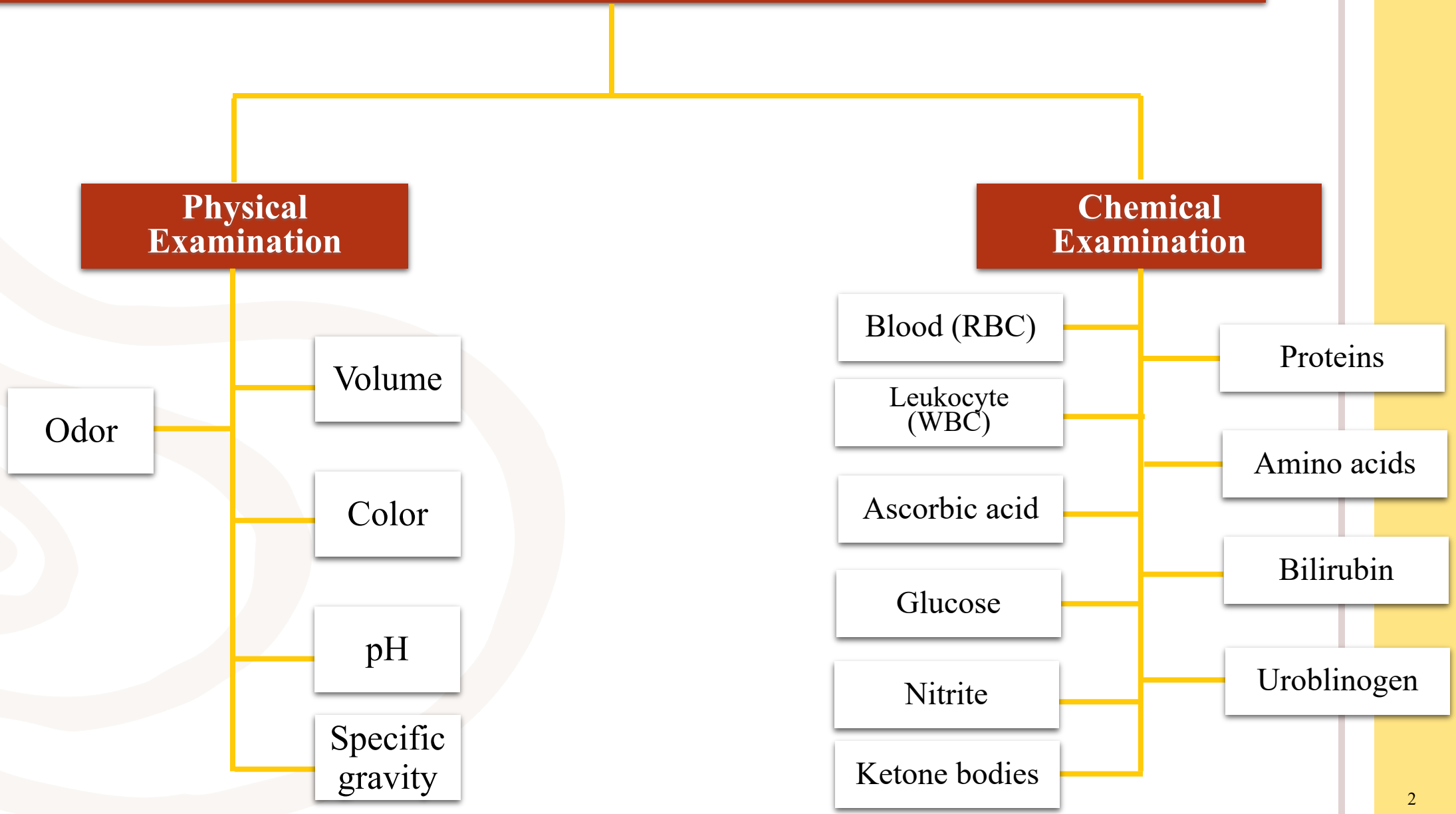
DETECTION AND ESTIMATION OF SOME ABNORMAL CONSTITUENTS IN URINE



Abnormal urine



1- Physical Examination (abnormal):

pH

Acidic:
-Diabetic Ketoacidosis.
-Starvation.
-UTIs (E. coli).

Alkaline:
-UTIs (urea-splitting bacteria)

Color

Dark yellow:
-Dehydration.
-Metabolic disorders.
-Medications.

Pink or Red color:
-Hematuria.
-Medications.

Orange:
-Presence of bilirubin.
-Medication.

Specific Gravity

High (Hypersthenuria):
-Dehydration.
-Sugar, or glucose, in the urine (Diabetes mellitus).

Low (Hyposthenuria):
-Diabetes insipidus.
-Tubular damage.

Volume

Polyuria:
-Diabetes mellitus.
-Diabetes insipidus.

Oliguria:
-Diarrhea or vomiting.

Anuria:
-Obstruction due to a stone or tumor.

Odour

Acetone:
-Diabetes Ketoacidosis.

Foul-smelling:
-UTIs.

Mousy smell:
-Phenylketonuria

Appearance

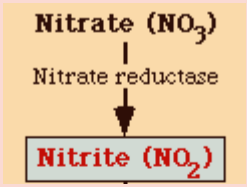
Turbidity:
-UTIs.
- Presence of RBCs, WBCs or epithelial cells.

2- Chemical Examination

- The following are some abnormal constituents that **not normally** found in detectable amount:

Positive in Urine	Cause	Notes
Blood (RBC) (hematuria)	• Bleeding because of damage to kidney or genitourinary system, **eg: Renal Calculi, Renal Tumor, Trauma to kidneys.** • Urinary tract infection. • Malignant hypertension.	• Any pink, red or brown urine must be considered as bloody until proved otherwise. 
Hemoglobinuria	• Intravascular hemolysis due to hemolytic anemia.	
Leukocyte (WBC)	• Urinary tract infection bacteria.	• Urine with positive results from the dipstick should be examined microscopically for WBCs and bacteria.
Ascorbic acid	• Large urinary concentrations arise from therapeutic doses of vitamin C.	

2- Chemical Examination

Positive in Urine	Cause	Notes
Glucose (Glycosuria)	• Blood glucose level exceeds the reabsorption capacity of the tubules, eg, **Diabetes mellitus.** • Defect in the tubular reabsorption eg. **fanconi syndrome.**	Normally, glucose is present in the glomerular filtrate and reabsorbed by the proximal tubules. (*see next slide*)
Ketone bodies (ketonuria)	• Occurs whenever increased amounts of fat are metabolized eg, **Diabetes mellitus, starvation** and **altered carbohydrate metabolism.**	Urine may have a fruity (acetone) smell.
Nitrite	• Urinary tract infection.	Bacteria that can reduce the nitr**ate** to nitr**ite**. 

Note:

Glucose level **exceeds** the reabsorption capacity in diabetes patients:

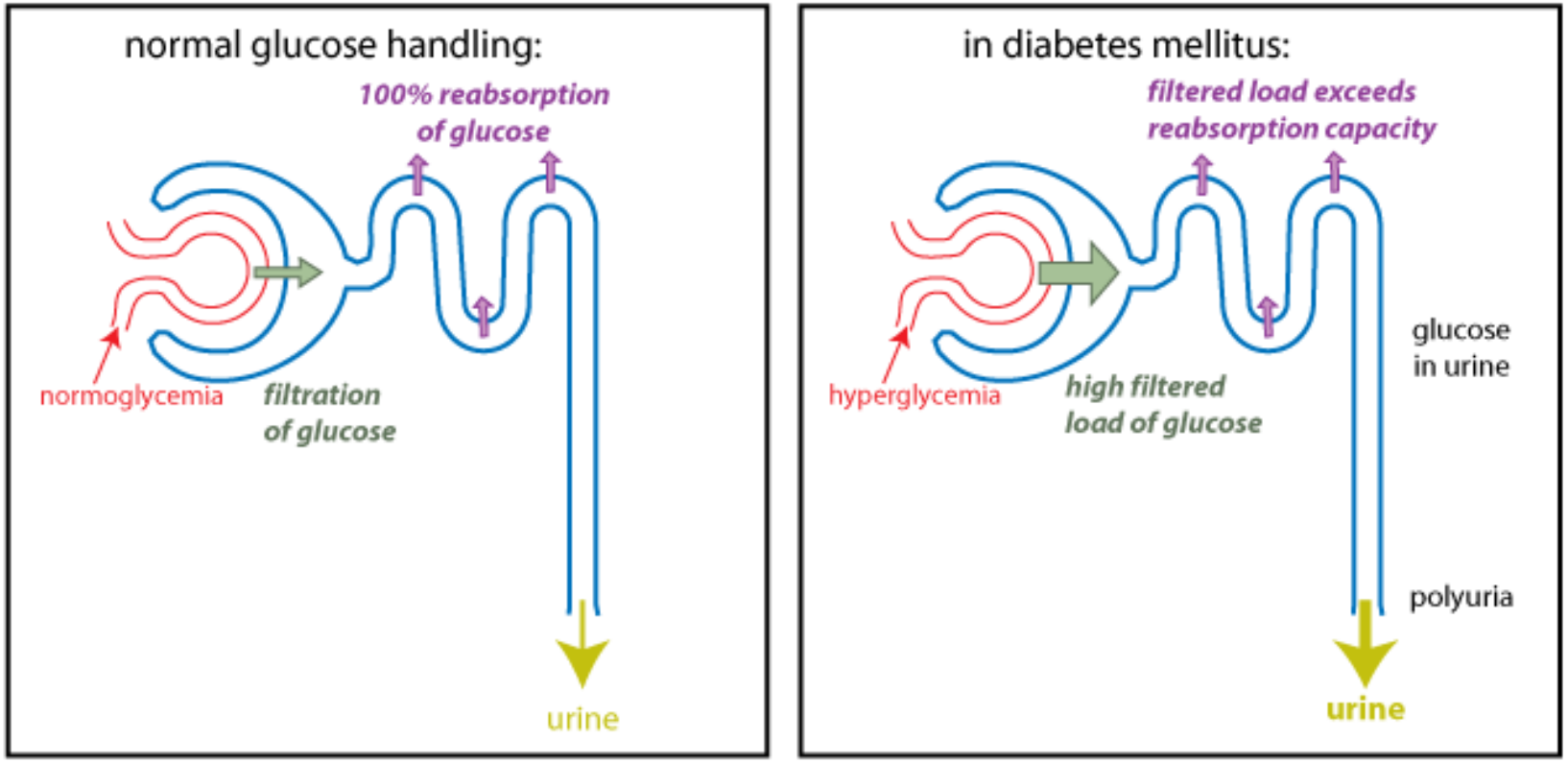


Figure 1. Glucose reabsorption by the kidney

How are high glucose levels related to polyuria?

2- Chemical Examination

Positive in Urine	Cause	Notes
Bilirubin	▪ Elevated amount of bilirubin in the blood stream, eg, **Bile duct obstruction.**	▪ The urine may be dark with a yellowish foam if much is present.
Uroblinogen	▪ Increased production eg, **hemolytic anemia.**	▪ Its presence does not give a colored foam (urobilinogen is colorless).
Amino acid (aminoaciduria)	▪ Blood amino acid level exceeds the reabsorption capacity of the tubules eg, **Phenylketonuria, Alkaptouria.** ▪ Defect in the tubular reabsorption eg, **fanconi syndrome, cystinuria.**	
Protein	▪ Acute infection. ▪ Primary kidney disease. ▪ Secondary kidney disease.	

Common Correlations in Urinalysis:

Microscopic Elements	Physical Examination	Dipstick Measurement
Red blood cells	Turbidity, red to brown color	Blood
White blood cells	Turbidity	Protein Nitrite Leukocytes
Epithelial cast cells	Turbidity	Protein
Bacteria	Turbidity, odor	pH Nitrite Leukocytes
Crystals	Turbidity, odor	pH

Test strip (dipstick):

- Normally, substances such as nitrite, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are present in **very small quantities** that is **not** capable of detection by this method.

➔ But present in detectable amount are **not normal.**

(False positive and false negative are common when using dipstick)

	False-positive	False-negative
Protein	Alkaline Urine Ammonia	Dilute Urine
Glucose	Strong oxidizing agent	Ascorbic acid
Blood	Oxidizing contaminants	High ascorbic acid
Bilirubin	Certain drugs	Ascorbic acid, nitrate
Uroblinogen	Alkaline Urine	Nitrite, formaline
Nitrite	Pigmented urine	Ascorbic acid

Notes in using test strip:

- Reagent strips should be stored in their original container.
- The lid should be kept tightly closed.
- Strips should **not** be used if expired or discolored.
- Strips should **not** be exposed to sunlight, moisture, heat, or cold.
- Specific reagents should be read at the appropriate time after dipping in urine, as recommended by the manufacturer.
- The strip should **not** be dipped for more than a second in the urine, and excess urine should be blotted off on the edge of absorbent paper to prevent mixing of reagents.

Types of urine specimens:

- Type of specimen and collection procedure are determined by physician and depend on the tests to be performed.
- There are basically four types of urine specimens:

Sample type	Sampling	Purpose
Random specimen	No specific time most common, taken anytime of day	Routine screening, chemical
Morning sample	First urine in the morning, most concentrated	Pregnancy test, microscopic test
Clean catch midstream	Discard first few ml, collect the rest	Culture
24 hours	All the urine passed during the day and night and next day Ist sample is collected.	used for quantitative and qualitative analysis of substances

- **Note:** 24h sample is necessary for accurate quantitative results.

Practical Part

Objectives:

1. The semi-quantitative detection of some abnormal constituents using **test-strips.**
2. The detection of amino acids in a urine sample using **ninhydrin**.
3. The effect of the type of urine collection in the detection of urine constituents.

1- Detection of some abnormal constituents of urine using test strip:

-Method:

1. You will have one urine samples.
2. You have to fill the following table and then the probable diagnosis.

Test	Sample 1
Volume	3000 ml
Color	
Odor	
pH	
Specific gravity	
Protein	
Blood	
Bilirubin	
Uroblinogen	
Glucose	
Ketone	
Nitrite	
Leukocyte	
Clinical Diagnosis for sample 1:	

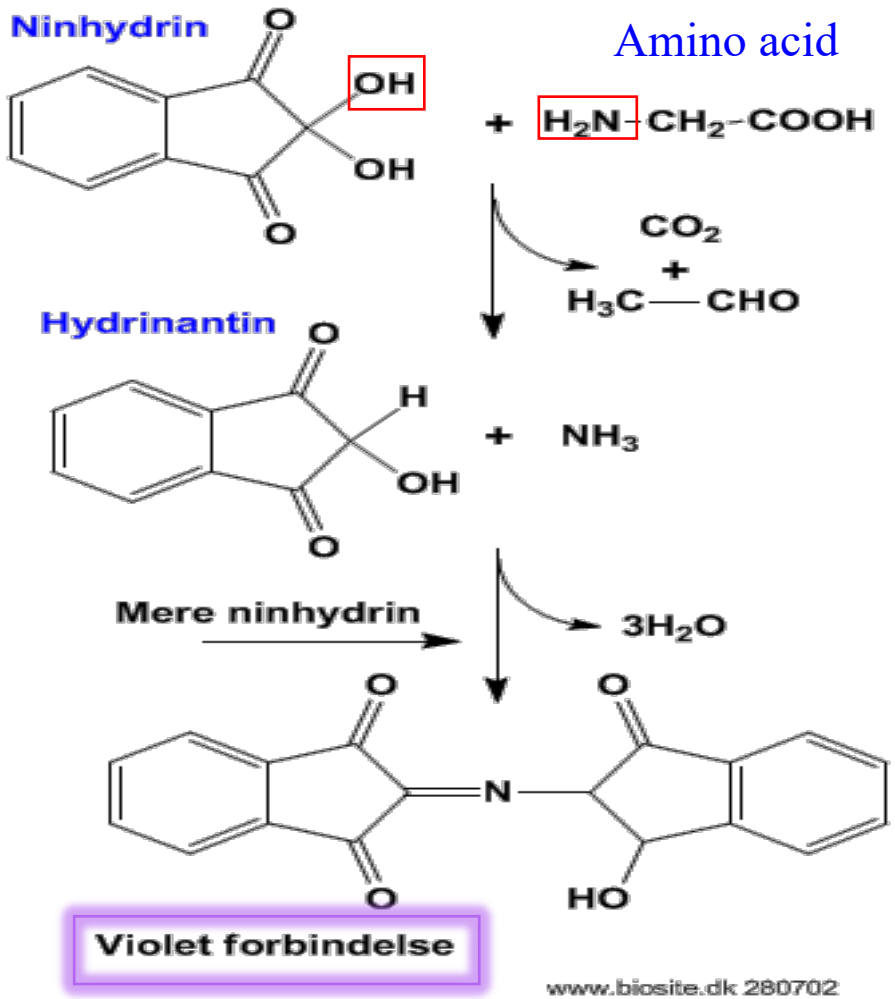
2- Detection of amino acid using ninhydrin:

Principle :

Ninhydrin reacts with **all amino acids** except proline and hydorxyproline at pH 3-4 to give a purple colored compound.
➔**Proline** will give a yellow color.

1. Initially, the amino acid is oxidized to an aldehyde containing one carbon atom less, together with the release of **ammonia** and **carbon dioxide**.

2. Then the **ammonia**, **ninhydrin** and the reaction product **hydrindantin** react to form the purple product.



Method:

- As standards, use proline and glycine as the following table:

Solution	Volume (ml)
Glysine	1
Proline	1
Urine Sample	1

- Add a 1 ml of ninhydrin solution to each test-urine.
- Boil the contents of each test tube for 2 minutes.
- Record your observations.

3- The effect of the type of urine collection on the detection of Urine constituents:

-Method:

1. You have two samples, one is random urine sample, the other is 24-hour urine sample from the **same patient.**
2. Compare between the two samples in the presence of the proteins using the test strip.

Test Parameter	24 hour Urine sample	Random urine Sample
Protein (+ or -)		

References:

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4. BCH 472 BCH practical note