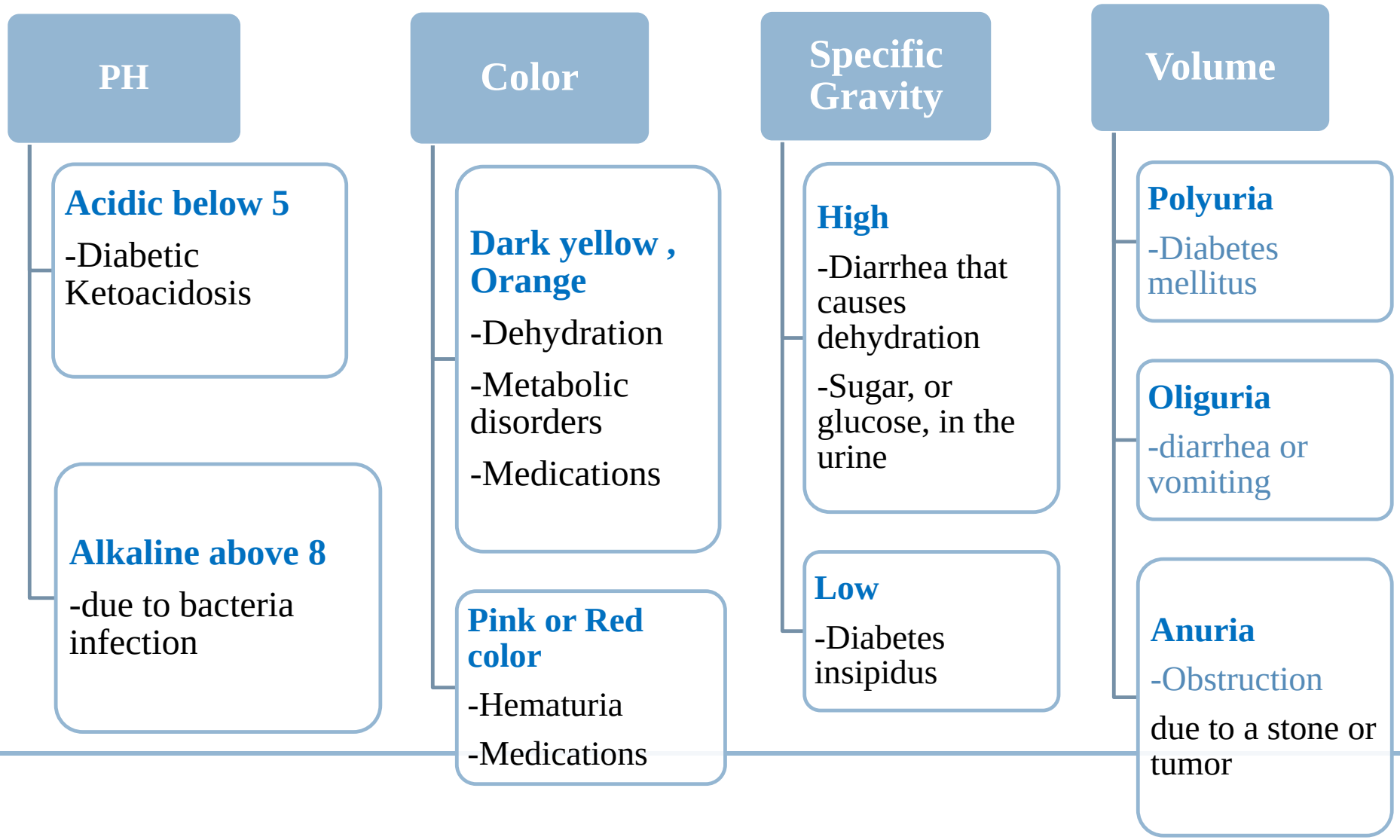
Examination of Urine: Detection and Estimation of Some Abnormal Constituents.

Urinalysis:

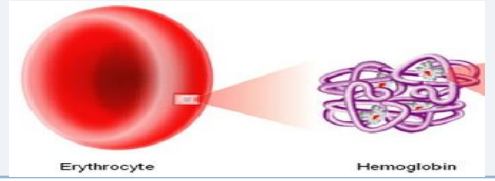
- The urinalysis is one of the most commonly ordered clinical tests in pediatrics.
- This frequency is partly due to the ease of urine collection and testing.
- Two types, **Physical** and **chemical** analysis.

Physical Analysis:

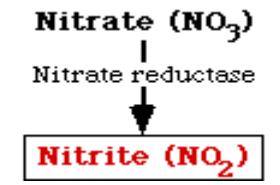


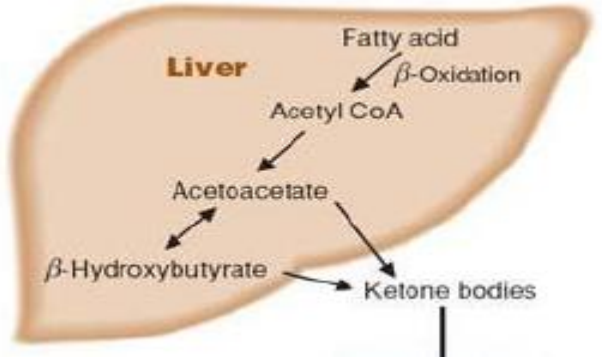
Some abnormal constituents in urine

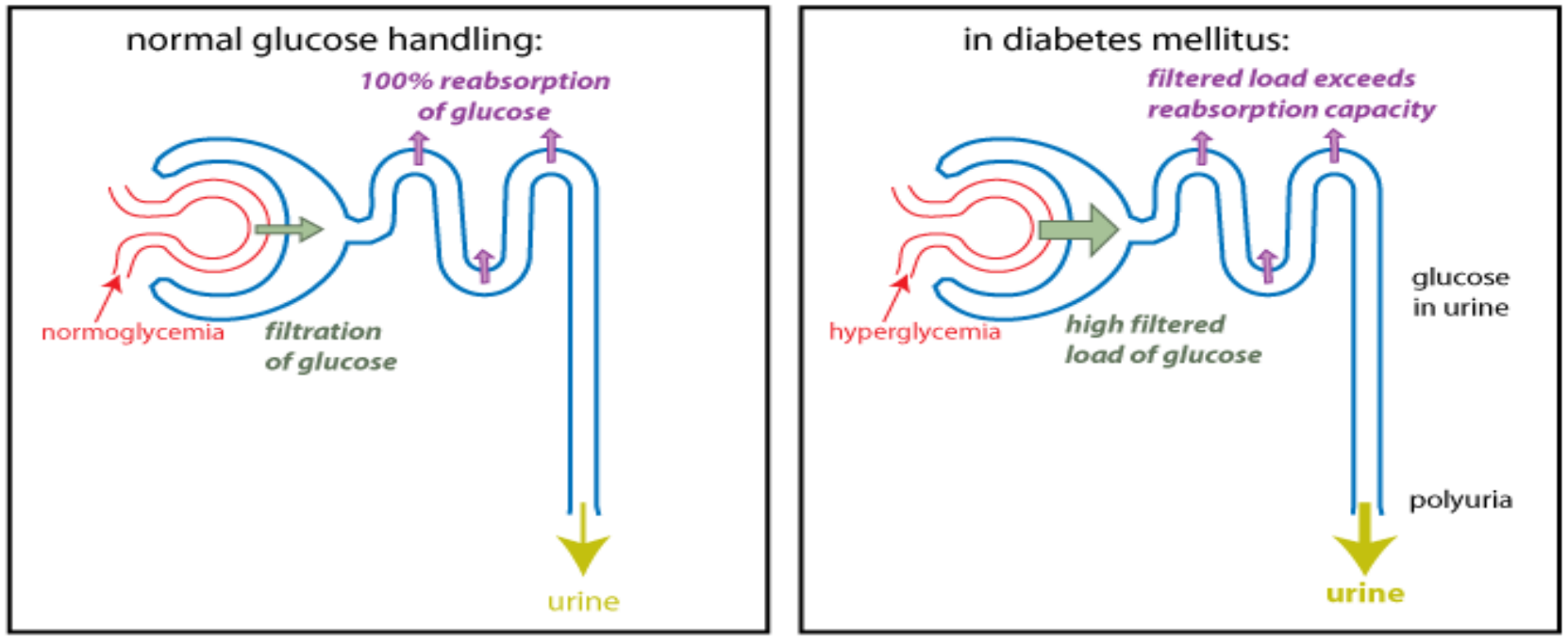
The following are some abnormal constituent that not normally found in detectable amount:

Positive in Urine	Cause	Notes
Blood (hematuria)	• Bleeding because of damage to kidney or genitourinary system,eg: Renal Calculi, Renal Tumor, Trauma to kidneys 	• Any pink, red or brown urine must be considered as bloody until proved otherwise. 
Haemoglobinuria	• Is due to intravascular haemolysis. 	
Leukocyte	• Urinary tract bacterial infection	
Ascorbic acid	• Large urinary concentrations arise from therapeutic doses of vitamin C	

Some abnormal constituents in urine

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 glomeroular filtrate and reabsorbed by the proximal tubules
Ketone bodies	• Occur whenever increased amounts of fat are metabolized eg, Diabetes mellitus, Starvation	• Urine may have a fruity (aceton) smell
Nitrite	• Urinary tract infection, Bacteria that can reduce the nitrate to nitrite	Bacteria that can reduce the nitrate to nitrite 



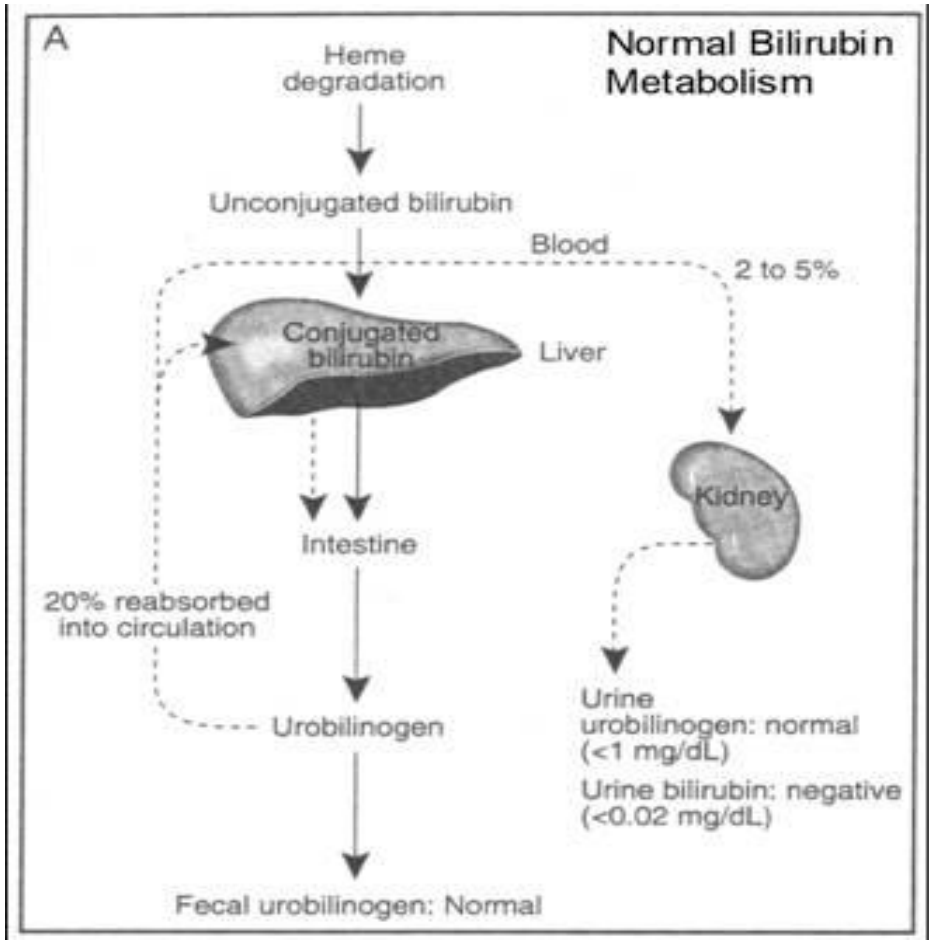


Glucose level exceeds the reabsorption capacity in diabetes.

Abnormal constituents in urine

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 yellow foam if much is present
Uroblinogen	• Increased production eg, hemolytic anemia.	• Its presence does not give a colored foam
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.	

Bilirubin and Urobilinogen



Test strip (dipstick)

- **Normally,** substances such as nitrate, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are present in very **small quantities** that is not detectable 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	Pigmented urine	Ascorbic acid,nitrite
Uroblinogen	Alkaline Urine	Nitrite,formaline
Nitrite	Pigmented urine	Ascorbic acid
Leukocytes	Oxidizing detergent	protein

Collection of urine sample:

Types of urine specimens depend on the tests to be performed

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

Practical Part:

Experiments

1-Detection of some abnormal constituent of urine using test strip

2-Detection of amino acid using ninhydrin

3-The effect of the type of urine specimen on the detection of a constituents

Objectives:

- The semi-quantitative detection of some abnormal constituents using test-strips.
- The detection of amino-acids in urine sample using ninhydrine.
- The effect of the type of urine collection in the detection of urine constituents.

Test strips (dipsticks) principle:

The test strips consist of absorbent microfiber cellulose pads attached to it.

Each pad contains the dried reagents needed for a specific test that react with the compounds present in urine producing a characteristic **color**.

There are strips which serve different purposes, such as **qualitative** strips that only determine if the sample is positive or negative, or there are **semi-quantitative.**

semi-quantitative strips provide an estimation of a quantitative result, the color reactions are approximately proportional to the concentration of the substance being tested for in the sample.

The reading of the results is carried out by comparing the pad colors with a color scale provided by the manufacturer.

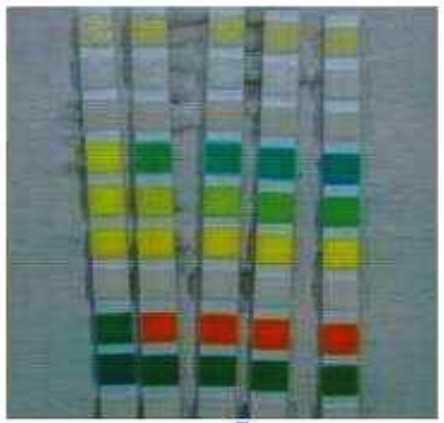
How to test your urine(visual read)?

A

Prepare some fresh urine sample.

B

Dip the dry strip into the urine.



C

Absorb the excess urine with absorbent paper.

D

Contrast color chart, close to which color?

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

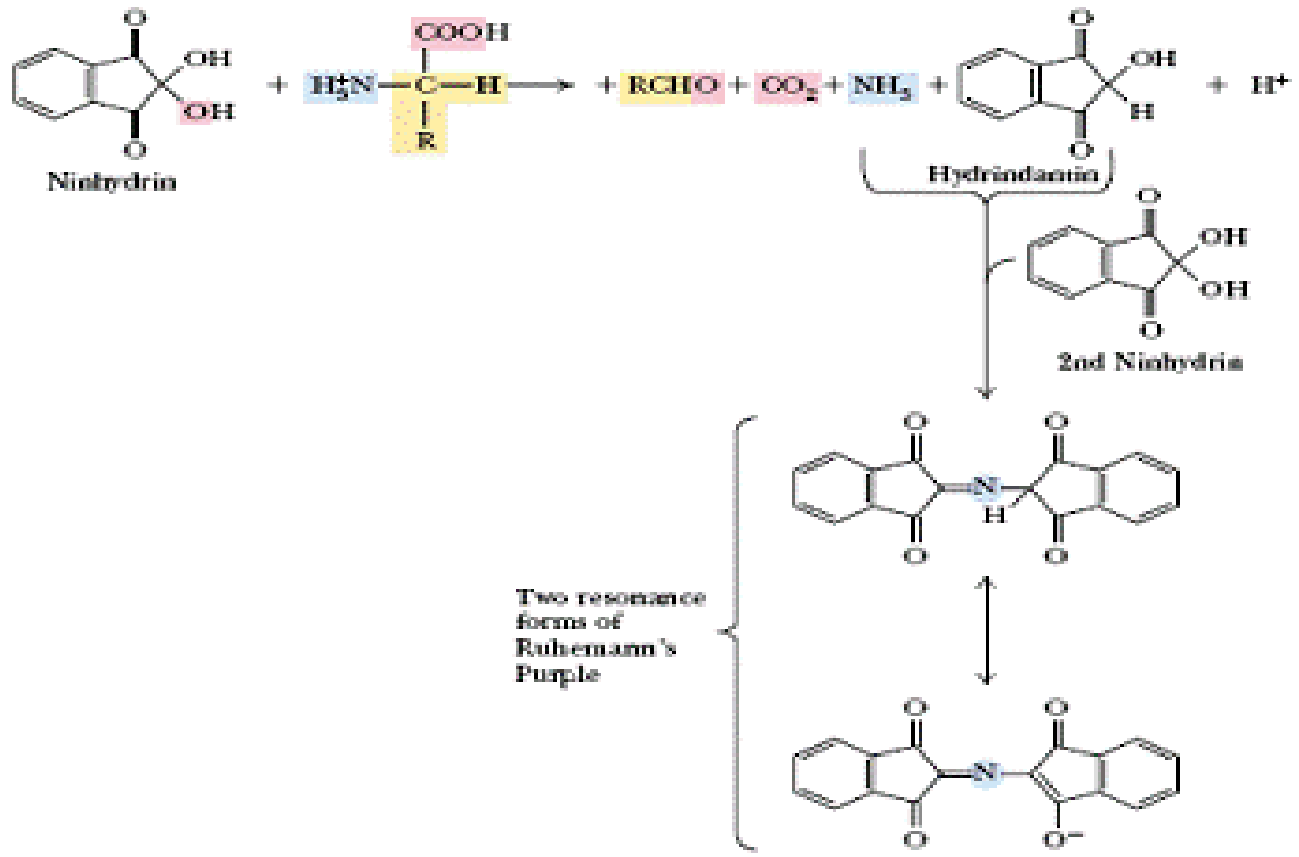
- You will have 3 different urine sample:
- You should fill the following information and then the probable diagnosis:

Test	Sample 1	Sample 2	Sample 3
Volume	3000 ml	900 ml	1000 ml
Color			
Odor			
pH			
Specific gravity			
Protein			
Blood			
Bilirubin			
Uroblinogen			
Glucose			
Ketone			
Nitrite			
Clinical Diagnosis:			

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.

- Initially, the amino acid is oxidized to an aldehyde containing one carbon atom less together with the release of ammonia and carbon dioxide. Then the ammonia, ninhydrin and the reaction product hydrindantin react to form the purple product.


Ninhydrin
Hydrindantin
2nd Ninhydrin
Two resonance forms of Ruhemann's Purple

Method:

- As standard, use proline and glycine :

Glysine	Add 1 ml of Glysine
Proline	Add 1 ml of proline
Urine Sample A	Add 1 ml of sample A

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

Solution	**Observation**
Glycine	
Proline	
Urine sample A	

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

- You have two samples, one is random urine sample, the other is 24-hour urine sample from the same patient,
- Compare between the two samples using the test strip

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

Questions:

Why random urine sample doesn’t used for qualitative and quantitative analysis?

Why ketone bodies are present in the urine of patients with diabetes ?