

Detection and Estimation of Some Abnormal Constituents in Urine

Abnormal urine

Physical Examination

Odor

Volume

Color

pH

Specific gravity

Chemical Examination

Blood (RBC)

Leukocyte

Ascorbic acid

Glucose

Nitrite

Ketone bodies

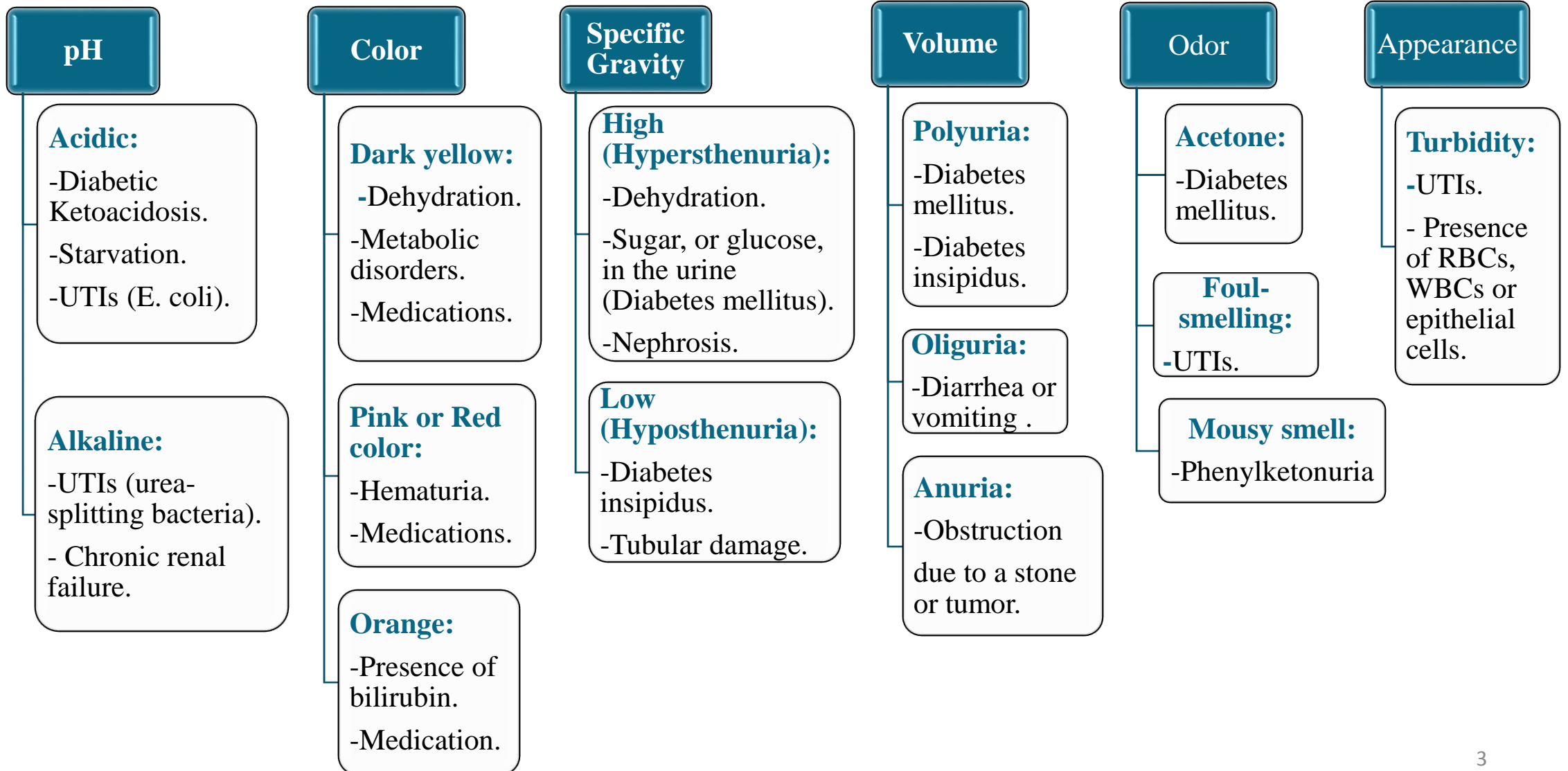
Proteins

Amino acids

Bilirubin


Uroblinogen

1- Physical Examination (abnormal):



2- Chemical Examination:

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

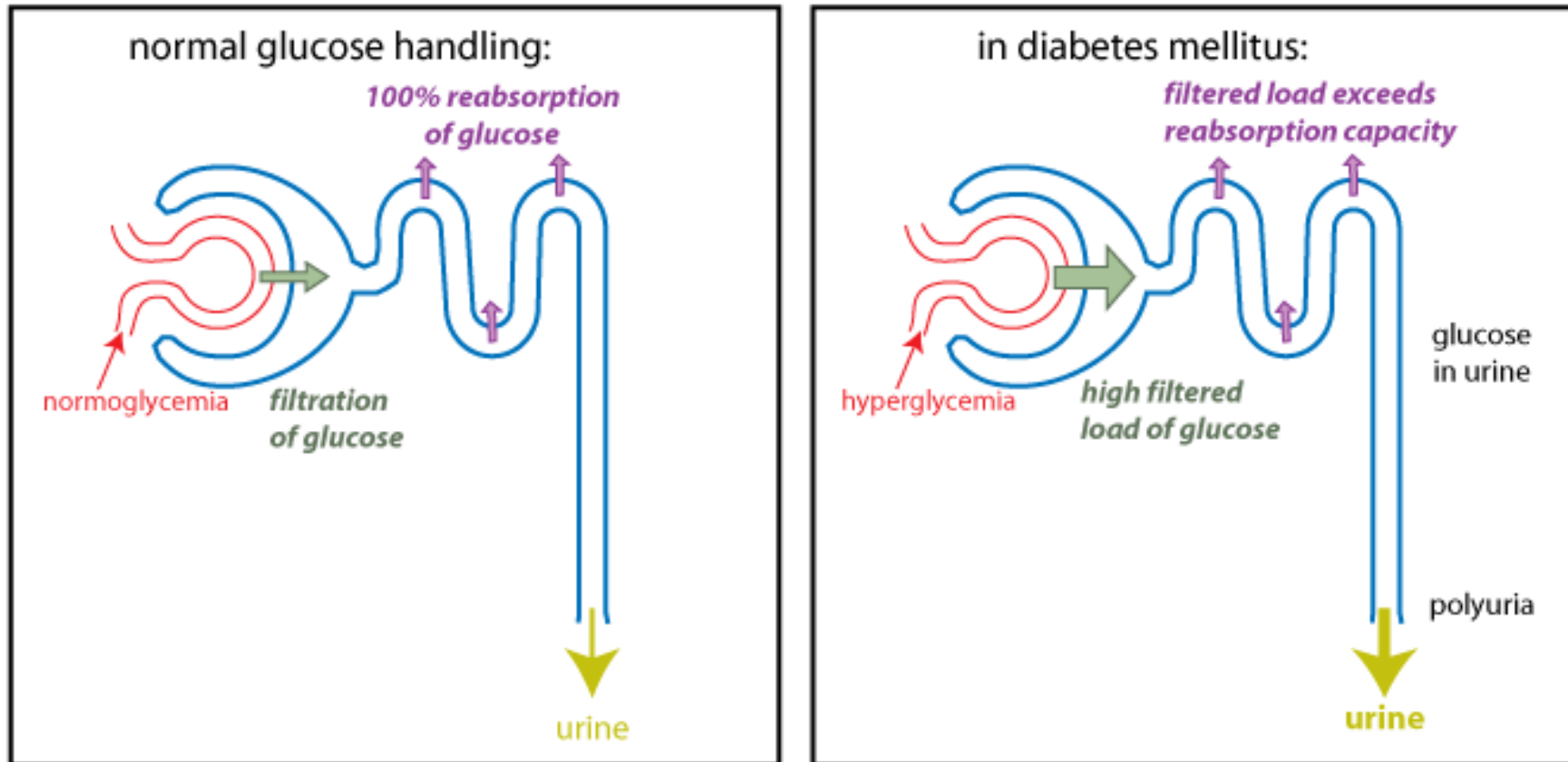
Positive in Urine	Cause	Notes
Blood (RBC) (hematuria)	<ul style="list-style-type: none"> Bleeding because of damage to kidney or genitourinary system, eg: Renal Calculi, Renal Tumor, Truma to kidneys. Urinary tract infection. Malignant hypertension. 	<ul style="list-style-type: none"> Any pink, red or brown urine must be considered as bloody until proved otherwise.
Hemoglobinuria	<ul style="list-style-type: none"> Intravascular hemolysis due to hemolytic anemia. 	
Leukocyte (WBC)	<ul style="list-style-type: none"> Urinary tract infection bacteria. 	
Ascorbic acid	<ul style="list-style-type: none"> Large urinary concentrations arise from therapeutic doses of vitamin C. 	<ul style="list-style-type: none"> Urine with positive results from the dipstick should be examined microscopically for WBCs and bacteria.

2- Chemical Examination cont':

Positive in Urine	Cause	Notes
Glucose (Glycosuria)	<ul style="list-style-type: none"> 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)	<ul style="list-style-type: none"> Occur whenever increased amounts of fat are metabolized eg, Diabetes mellitus, Starvation and altered carbohydrate metabolism. 	<ul style="list-style-type: none"> Urine may have a fruity (acetone) smell .
Nitrite	<ul style="list-style-type: none"> Urinary tract infection. 	<p>Bacteria that can reduce the nitrate to nitrite.</p> <div style="text-align: center;"> <pre> graph TD A[Nitrate (NO3)] -- Nitrate reductase --> B[Nitrite (NO2)] </pre> </div>

Note:

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

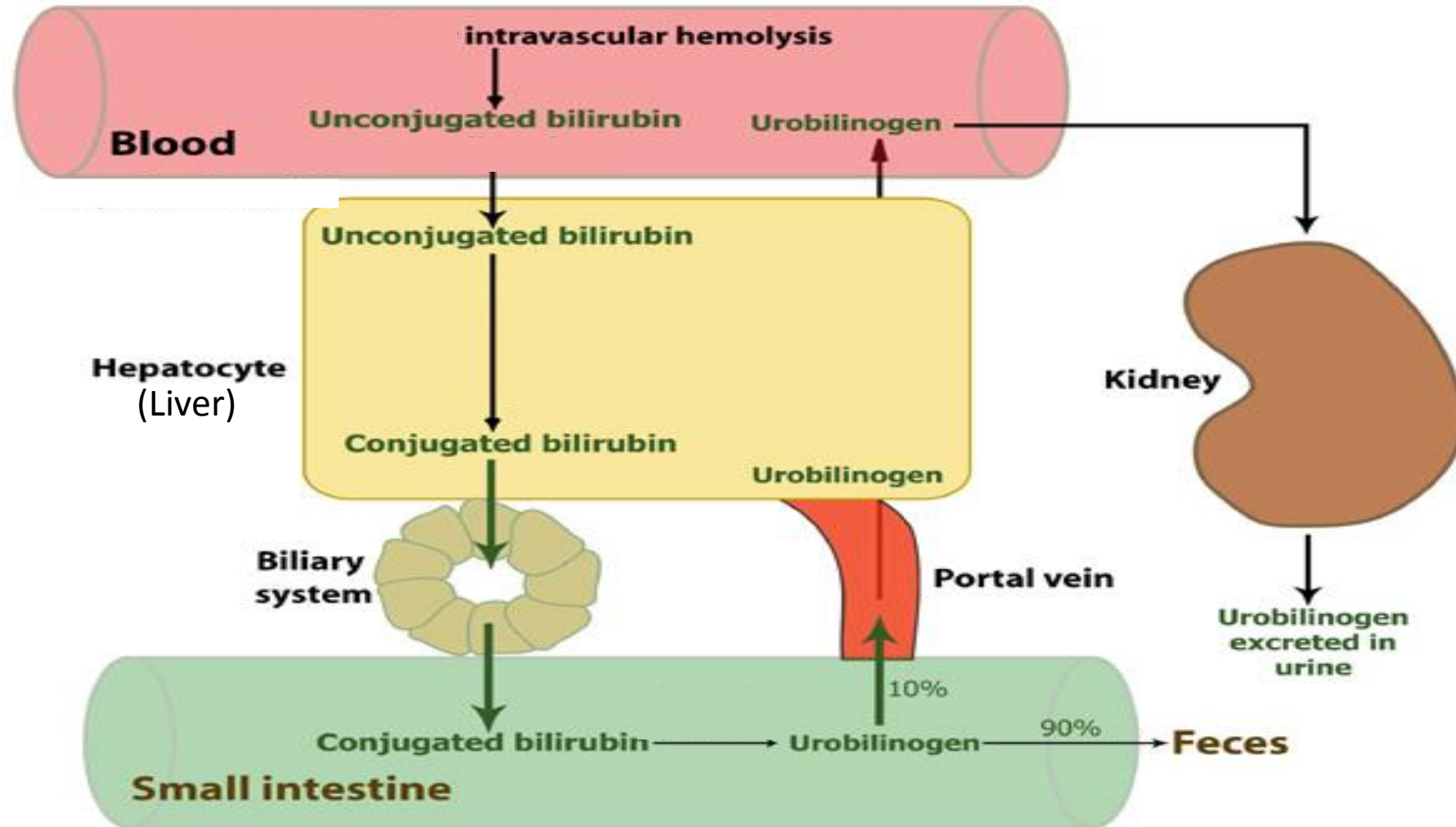


2- Chemical Examination cont':

Positive in Urine	Cause	Notes
Bilirubin	<ul style="list-style-type: none"> Elevated amount of bilirubin in the blood stream, eg, Bile duct obstruction. 	<ul style="list-style-type: none"> The urine may be dark with a yellowish foam if much is present.
Urobinogen	<ul style="list-style-type: none"> Increased production eg, hemolytic anemia. 	<ul style="list-style-type: none"> Its presence does <u>not</u> give a colored foam (urobilinogen is colorless).
Amino acid (aminoaciduria)	<ul style="list-style-type: none"> Blood amino acid level exceeds the reabsorption capacity of the tubules eg, Phenylketonuria, Alkaptonuria Defect in the tubular reabsorption eg, fanconi syndrome, cystinuria. 	
Protein	<ul style="list-style-type: none"> Acute infection. Primary kidney disease. Secondary kidney disease. 	

Note:

-Bilirubin and Urobilinogen :



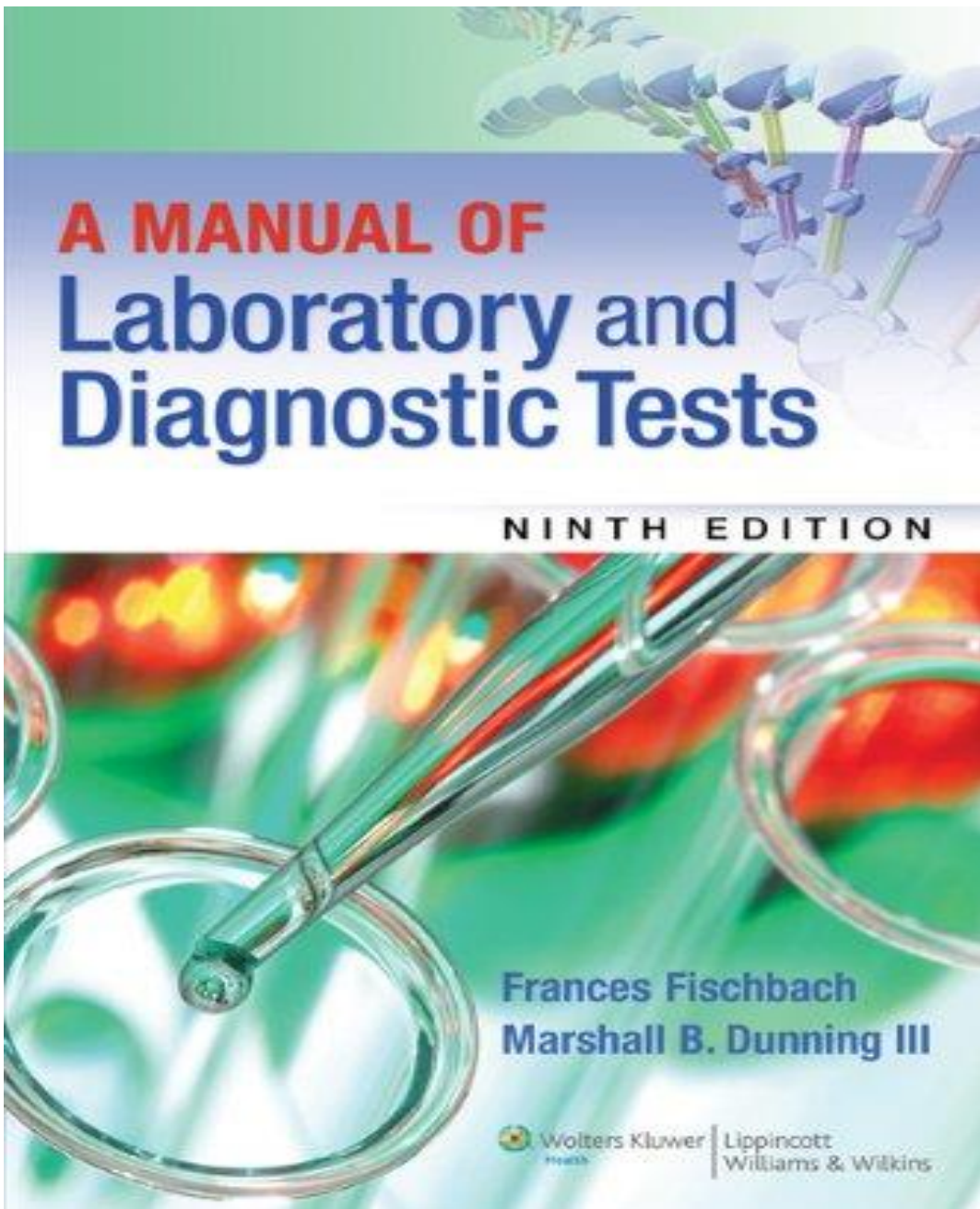
-Bilirubin and Urobilinogen:

Test	In Health	In Hemolytic Disease	In Hepatic Disease	In Biliary Obstruction
Urine urobilinogen	Normal	Increased	Increased	Low or absent
Urine bilirubin	Negative	Negative	Positive or negative	Positive

NOTE: Biliary obstruction refers to the blockage of any duct that carries bile from the liver to the gallbladder or from the gallbladder to the small intestine.

-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



For more information...

-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** 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
Leukocytes	Oxidizing detergent	Protein

-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 1 st 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 normal constituents using test-strips.
2. The detection of amino-acids in a urine sample using ninhydrine.
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:

- You will have 3 different urine samples.
- You have to fill the following table 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			
Leukocyte			
Clinical Diagnosis for sample 1:			
Clinical Diagnosis for sample 2:			
Clinical Diagnosis for sample 3:			

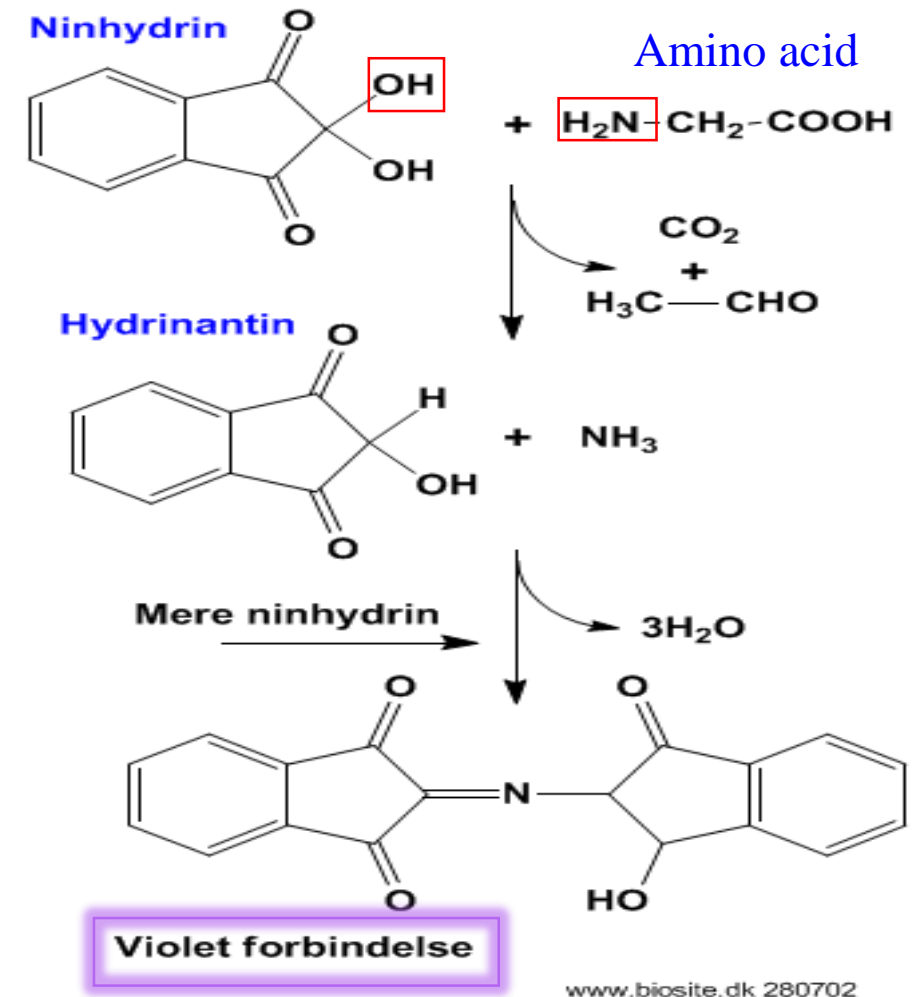
2- Detection of amino acid using ninhydrin:

- Principle :

- Ninhydrin reacts with **all amino acids** except proline and hydroxyproline 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)
Glycine	1
Proline	1
Urine Sample	1

- Add a 1ml 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 3	

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

-Method:

- 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 in the presence of the proteins using the test strip.

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

Homework:

-Read the two urine analysis reports, then write a short comments:

URINE ROUTINE EXAMINATION			
Voucher No. :	[REDACTED]	Date : 31-MAR-2012	Del.Date : 01-APR-2012
Patient Name :	[REDACTED]	Age : 50 Y	Sex : [REDACTED]
Referred By :	[REDACTED]	MBBS FCPS(MEDICINE) MD(NEPHRO)	
Sample : URINE	Lab. No. : 11203171139	()	
Tests : Urine R/M/E			
PHYSICAL EXAMINATION		MICROSCOPIC EXAMINATION	
Quantity	19 ml	CELLS /HPF	
Color	Straw	R B C	Nil
Appearance	Clear	Pus Cells	0 - 2
Sediment	Nil	Epithelial Cells	0 - 3
CHEMICAL EXAMINATION		CASTS/LPF	
Reaction	Acidic	R B C	Nil
Albumin	Trace	W B C	Nil
		Epithelial	Nil
		Granular	Nil
		Hyaline	Nil
		CRYSTALS & OTHERS	
		Urates	Nil
		Uric Acid	Nil
		Cal. Oxalate	Nil
		Amor. Phos	Nil
		Tripple Phos	Nil

MACROSCOPIC / CHEMICAL EXAMINATIONS			
→ COLOR	straw	→ pH (REACTION)	acidic (6.5)
→ TURBIDITY (CLARITY)	cloudy	PROTEIN	negative
GLUCOSE (SEMI-QUANTITATIVE)	negative	UROBILINOGEN	0.2 EU/dl
BILE	negative	NITRITES	negative
KETONE	negative	BLOOD	large
SPECIFIC GRAVITY	<= 1.005	LEUCOCYTES	large

URINE SEDIMENT ANALYSIS BY FLOW CYTOMETRY			
			Reference Normal Range
→ RED BLOOD CELLS	7	/hpf	(M) 0-3.0 / (F) 0-4.3
→ WHITE CELLS	70	/hpf	(M) 0-3.5 / (F) 0-6.0
EPITHELIAL CELLS	2	/hpf	(M) 0-2.1 / (F) 0-4.0
CASTS	0	/lpf	(M) 0-1.5 / (F) 0-3.0
→ BACTERIA	278	/hpf	(M) 0-475 / (F) 0-773

REMARKS
*Results double-checked.

DR. MUNIRUL HUQ
MBBS, MCPS (Clinical Path), DCP
Consultant, Clinical Pathology
LABAID LTD.

793-8, 9670210-3, Fax : 88-02-9615497, E-mail : info@labaidgroup.com, Web : www.labaidgroup.com

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