BIOCHEMISTRY PRACTICAL – GNT BLOCK

Color index:

- Important
- Extra explanation

Outline:

1st Part:

- Serum amylase & lipase
- Accuracy & Precision

2nd Part:

- LFTs & bilirubin cycle
- sensitivity & Specificity

AMYLASE:

Sources:

- Parotid Gland ⟶ Saliva
- Pancreas ⟶ Pancreatic Juice

Function:

hydrolyzes (breakdown) of **α (1,4) glycosidic bonds** in starch and glycogen

Products:

- short, branched and unbranched oligosaccharides (e.g. maltotriose).
- Disaccharides: Maltose and isomaltose

➢ **Amylase measurement is used in clinical practice as a diagnostic marker for Acute Pancreatitis. however, Amylase levels may also be significantly increased in patients having conditions other than pancreatic disease e.g.:**

- ✓ Gallbladder diseases
- ✓ Acute appendicitis
- ✓ Intestinal obstruction
- ✓ Perforated intestinal ulcer

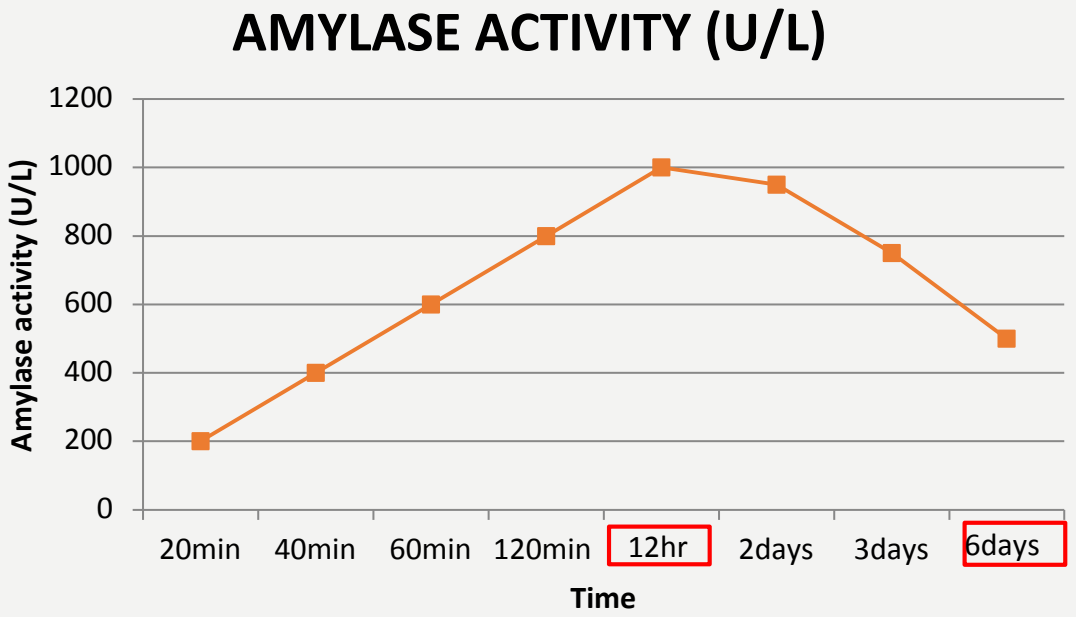
➢ Acute pancreatitis → damage of the **exocrine** part of the pancreas → release of the pancreatic enzymes "e.g. amylase + lipase" into the circulation.

Serum amylase vs. lipase for acute pancreatitis

	Condition	Serum amylase	Serum Lipase
Normal Ranges	Adults < 60	25-125 IU/L	10-140 IU/L
	Adults > 60	24-151 IU/L	18-181 IU/L
Ranges in Acute Pancreatitis	start rising at	2-12 hrs	4-8 hrs
	peak at (highest)	12-72 hrs	24 hrs
	return to normal	One week	8-14 days
	specificity and sensitivity	lower	**better**
	use for diagnosing pancreatitis	**widely used** because it is relatively **inexpensive.**	used

- Acute pancreatitis is positive if values are **three times more the normal** range **and** are associated with **clinical picture** e.g. upper abdominal pain radiating to the back, nausea…

*normal values will be given in the OSPE.



Amylase levels will be increasing over time, and will reach a peak within 12-72 hours. It will return to normal in few days (~ a week).

Why does it decrease?

1.The condition is self-limiting.

2. The circulating amylase will be excreted in urine.

3.The circulating amylase will be degraded (protein turnover).

* If the patient has a self limiting condition and is presented late, the diagnosis of acute pancreatitis based on the enzyme level could be missed.

➢ The rising trend of the levels of serum a-amylase as the acute inflammation is taking place is **more clinically significant than one single high reading**. This means that **multiple readings** should be taken (to see the rising trend) rather than one high reading.

SOURCES OF VARIATION IN AMYLASE TEST RESULTS

Analytical factors: e.g., accuracy (reliability) and precision (reproducibility)
LAB RELATED

Biological factors:
e.g., sex, age, diet, drugs..
KEEP IN MIND

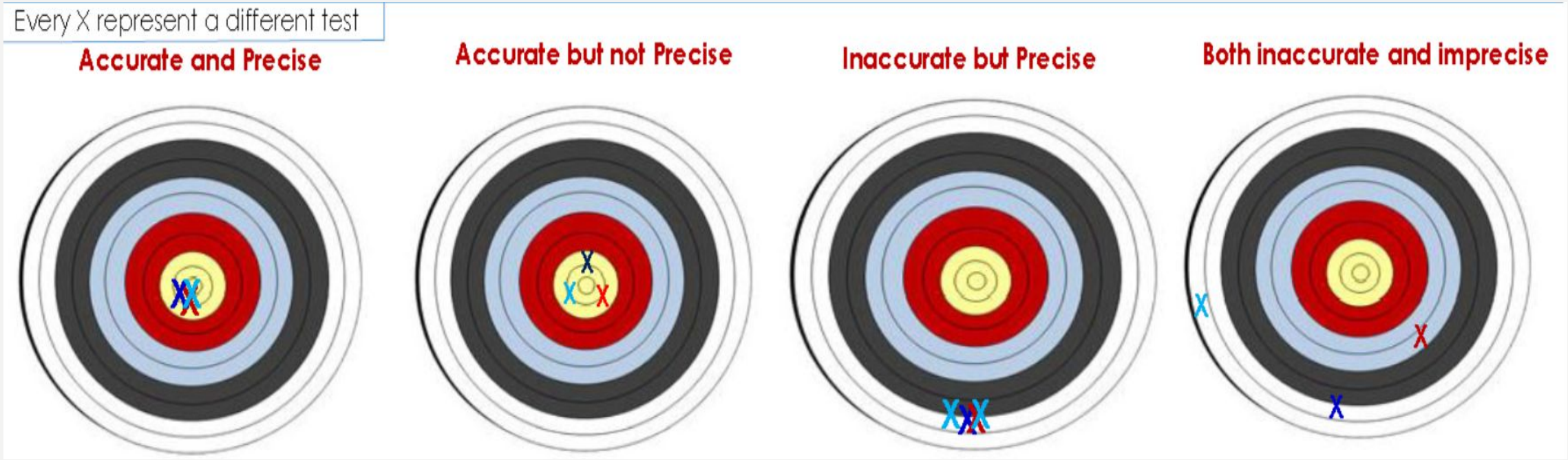
Pathological factors:
e.g., progression of the disease, complications
YOUR AIM

ACCURACY VS PRECISION

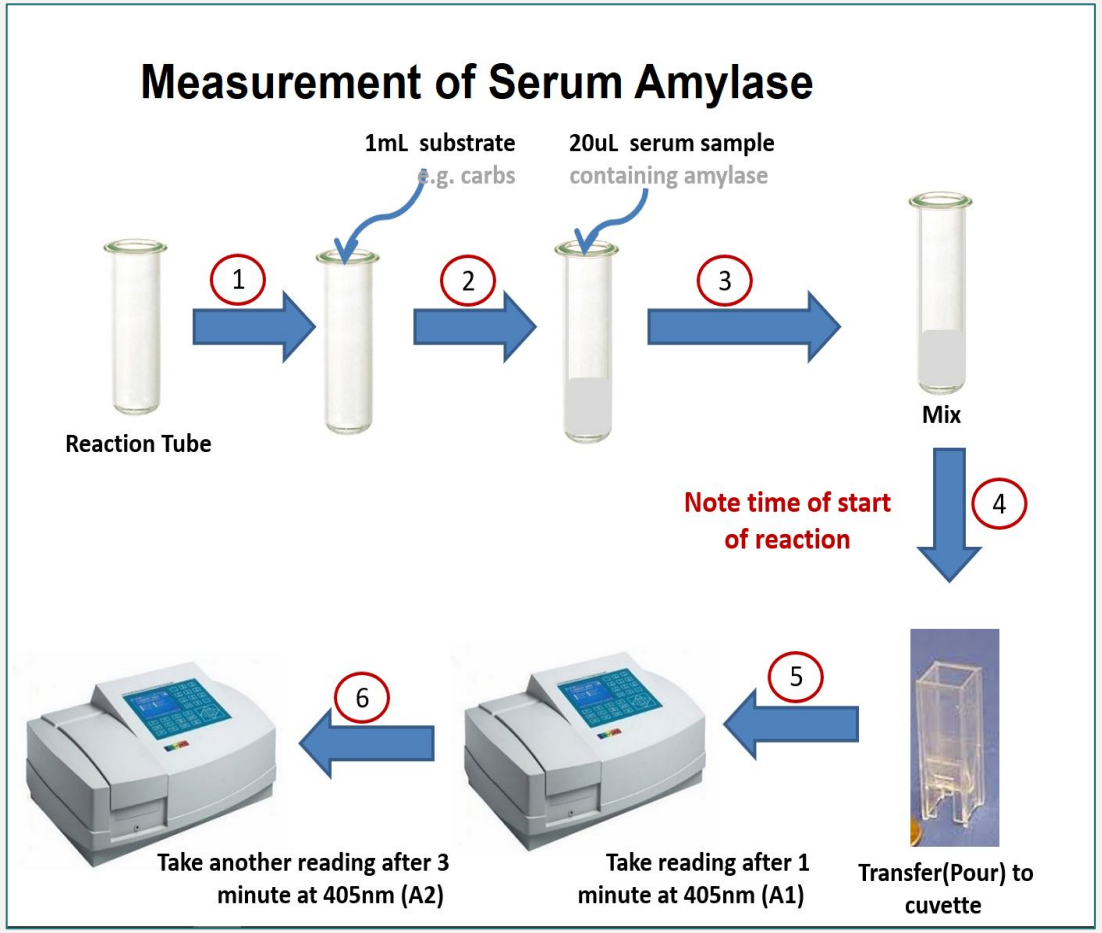
Accuracy	Precision
• The **reliability** of the method in determining the true value of the analyte. • The extent to which the mean measurement is close to the true value. It is useful for comparison of original/gold standard method with other methods. e.g. the true value obtained from the standard method is 5 , and the mean measurement obtained from another method is 5 or close to 5. 	The **reproducibility** of the method when it is run repeatedly under identical conditions. i.e. The results of the same experiment done by the same method are close to each other, Regardless of their approximation to the true value. e.g.The Results of repeated tests were: 3, 3, 2.9, 3, 2.8 

ACCURACY VS PRECISION (cont.)

Accuracy	Precision
• They depends on the quality of instruments, expertise and other experimental factors. • The relation between accuracy and precision can be easily illustrated by its analogy to shooting at a target or 'dartboard'. • They are independent, e.g., an inaccurate result can be extremely precise.	



Note: you won't be asked about the steps of the experiment in the exam. absorbance (A) will be given and you will have to do the calculations only.



The experiment measures the amount of carbohydrates broken down by amylase per unit time (by measuring the absorbance of the substrate via spectrophotometer), and use that as reference to measure serum amylase.

Calculation & Interpretation:

1. $\Delta A = \frac{A2 - A1}{3 \text{ min.}}$

2. **Serum Amylase (U/L) = 5544 × Δ A**

   5544 is a constant number, set by the kit manufacturer.

3. **compare to normal range, which is** 25-125 U/L

TEST YOURSELF!

1st Part

- Talk about amylase regarding its sources & physiological action, briefly?

Amylase is an enzyme found in saliva, pancreatic juice and parts of plants; they help to convert starch into oligo- and di-saccharides by hydrolyzing (breakdown) a(1,4) glycosidic bonds in starch and glycogen.

- What are the uses of amylase in clinical practice, and what other markers that could be used in such condition?

Acute Pancreatitis, Serum lipase

- Name two conditions with elevated serum amylase other than acute Pancreatitis?

-Acute appendicitis -Intestinal obstruction

- Suppose a lab refrigerator holds a constant temperature of 38.0 F. A temperature sensor is tested 5 times in the refrigerator. The temperatures from the test yield the temperatures of: 29.4, 38.1, 39.3, 42.5, 36.3 what is your comment?

The Results show no tendency toward a particular value (no precision) and does not match the actual temperature (no accuracy)

- Suppose a lab refrigerator holds a constant temperature of 38.0 F. A temperature sensor is tested 5 times in the refrigerator. The temperatures from the test yield the temperatures of : 49.2, 49.3, 49.1, 49.0, 49.1. what is your comment?

The Results show a tendency toward a particular value (precision) but every measurement is not close from the actual temperature (no accuracy).

Hands out - summary

1st Part

1. What is amylase, and what are its sources in the human body?

Amylase are group of proteins found in saliva, pancreatic juice and parts of plants; they help to convert starch into sugar

2. What is the physiological action of amylase?

Amylase hydrolyzes (breakdown) the dietary starch and glycogen into:

- Short, branched oligosaccharides (e.g. maltotriose)
- Disaccharides (maltose and iso-maltose).

3. Would you expect a high level of amylase in blood under normal conditions?

No.

4. What are the uses of amylase measurement in clinical practice?

Acute Pancreatitis

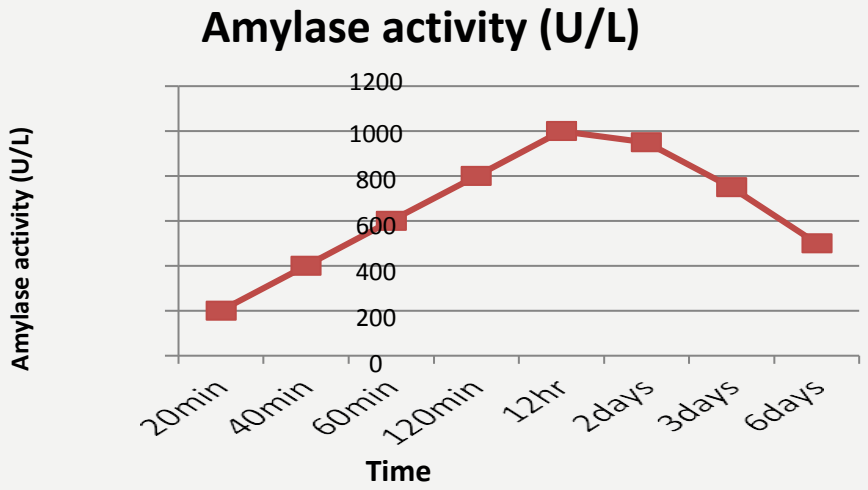
What other diagnostic marker that can be measured in this clinical condition?

Serum lipase

Hands out - summary

1st Part

5. Changes in serum amylase activity during course of an injury (time course)



1. levels increase
2. peak: within 12-72 hours.
3. levels decrease
4. return to normal: in few days (~ a week).

a. What are the possible factors responsible for these changes in the curve?

Why does amylase level increase?	Why does it decrease?
Acute pancreatitis → damage of the exocrine part of the pancreas → release of the pancreatic enzymes into the circulation (α-Amylase is one of the pancreatic enzymes released).	1. The condition is self-limited 2. The circulating amylase will be excreted in urine 3. The circulating amylase will be degraded (protein turnover)

Hands out - summary

b- With knowledge about amylase activity overtime, what is the clinical application?

Three points can be derived from such a curve:

1. Measurement of α-amylase in the serum is limited by the time elapsed since the initiation of acute inflammation of the pancreas. If the patient presented late, and the condition was self limited, the diagnosis of acute pancreatitis based on the enzyme level at time of presentation **could be missed.**
2. The measurement of α-amylase in serum should not be interpreted on its own; it has to be evaluated in association with the clinical picture (e.g. the nature of abdominal pain).
3. The rising trend of the levels of serum α-amylase as the acute inflammation is taking place is more clinically significant than one single high reading. i.e. multiple readings should be taken rather than one high reading.

SENSITIVITY	SPECIFICITY
Sensitivity answers the following question: If a person has a disease, how often will the test be positive **(true positive rate)**?	Specificity answers the following question: If a person does not have the disease how often will the test be negative **(true negative rate)**?
i.e.: if the test is highly sensitive and the test result is negative you can be nearly certain that the **individuals don't have disease.**	i.e., if the test result for a highly specific test is positive you can be nearly certain that the individuals actually **have the disease.**
A Sensitive test helps rule out[1] or or "Snout" disease, when the result is negative.	**A very specific test rules in[2] or "Spin" disease with a high degree of confidence, when the result is positive**
★ $\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$	★ $\text{Specificity} = \frac{TN}{TN + FP} \times 100$

2 X 2 Contingency Table:

Test	Disease	
	+	-
+	True Positive (TP)	False Positive (FP)
-	False Negative (FN)	True Negative (TN)

Please pay attention to the colors, as they may help you remember.

In general = $\frac{\text{the true value}}{\text{the total value}} \times 100$

in case of Sen: true positive "rule out/snout"
in case of Sp: true negative "rule in/spin"

1: exclude the presence of the disease(i.e usually used for screening of large groups to exclude the disease)
2: ensure the presence of the disease(i.e usually after we finish screening with a sensitive test we use a specific test to diagnose)

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★ EXAMPLE:

2nd Part

Q: A Lab test to measure serum bilirubin was performed on 1000 individuals. The test gave the following results:

- ✧ Number of positive results in patients with acute hepatitis: 440
- ✧ Number of positive results in normal subjects: 50
- ✧ Number of negative results in normal subjects: 450
- ✧ Number of negative results in patients with acute hepatitis: 60

For this Serum bilirubin test, calculate the sensitivity and the specificity.

Answer Steps:

1. draw a 2 X 2 Contingency Table
2. Fill in the blanks with their values. (Values will be given)
3. Write the equations
4. Calculate. (DON'T forget your calculator)

Test	Disease +	Disease -
+	TP	FP
-	FN	TN

Test	Disease +	Disease -
+	440	50
-	60	450

Remember:

- ✓ Positive or negative >> regarding the test results.
- ✓ True or false >> regarding the matching of the patient's disease state with the test (whether both are positive or negative).

ANSWERS:

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100 = \frac{440}{440 + 60} \times 100 = 0.88 \times 100$$

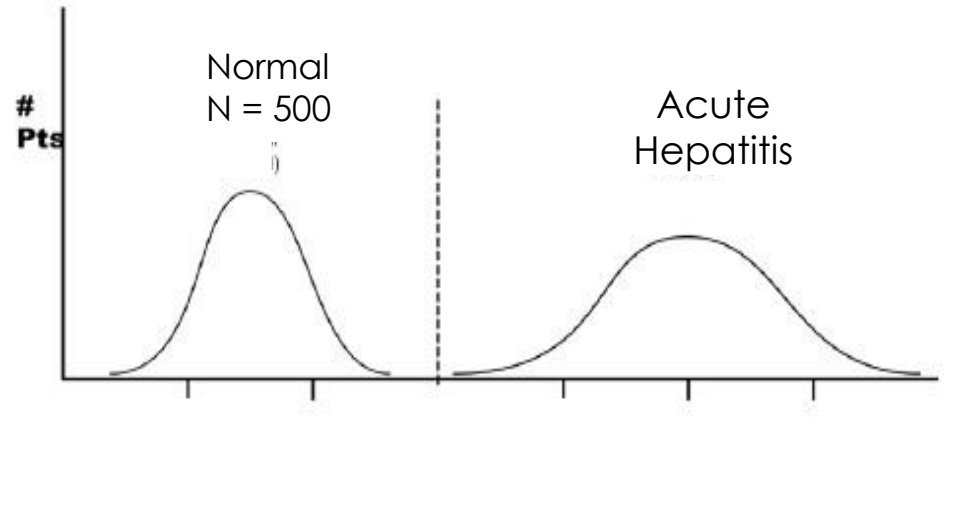
Sensitivity= 88%

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100 = \frac{450}{450 + 50} \times 100 = 0.90 \times 100$$

Specificity= 90%

NOTE: since the specificity of this test is higher than the sensitivity; this test is better for a diagnosing purposes (to rule in)

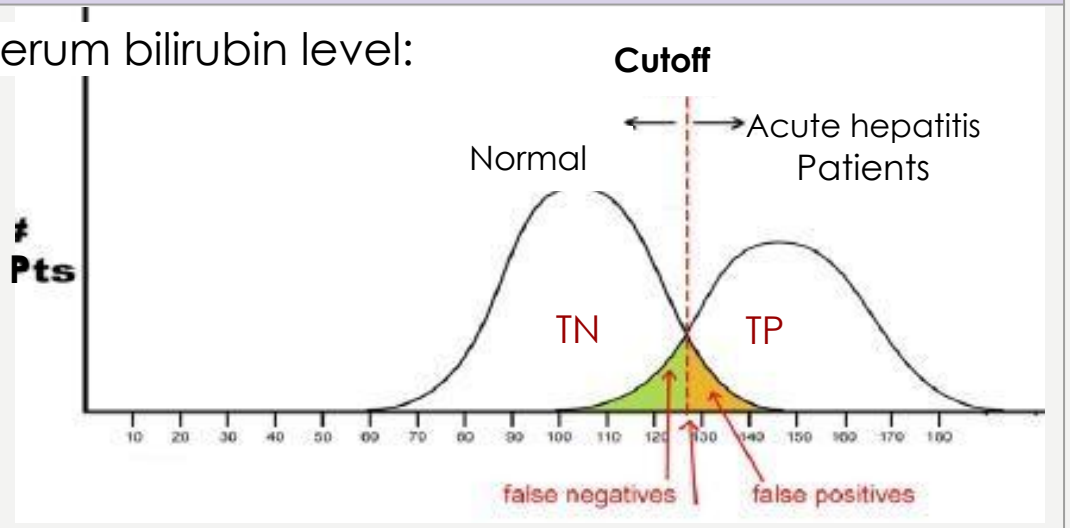
Ideal test



- * Shown above is an example of an **Ideal diagnostic lab test results for many subjects (normal and patients).** it is a perfect Test where there is no overlap between the results of normal and diseased.

- **It would be a perfect test to diagnose acute hepatitis. as the test identifies all patients with disease, and all subjects without disease 100% of the time.**
- In other words, this test has a sensitivity of 100% and a specificity of 100%.

Lab test



- ***shown above is what actually happens in the lab. The lab test results in normal and disease conditions overlap.**
- **To increase the overall accuracy of the test, the centermost point of overlapping is chosen as the cutoff value.**

- about 10% of the people without the disease are incorrectly classified as abnormal (false-positive) **in orange**.
- about 10% of the people with the disease are incorrectly classified as normal (false-negative) **In green.**
- In other words, this test has a sensitivity of 90% and a specificity of 90%.

LIVER FUNCTION TESTS

Q.1 What are the liver function tests (LFTs)?

They're used to:

1. Detect Hepatic Injury
2. Assess hepatic function.

Common serum liver chemistry tests

Liver chemistry test:	Clinical implication of abnormality:
Alanine aminotransferase (ALT)	**Hepatocellular damage.** e.g. viral hepatitis
Aspartate aminotransferase (AST)	
Bilirubin	Cholestasis, impaired conjugation, or biliary obstruction.
Alkaline phosphatase (ALP)	Cholestasis, infiltrative diseases, or biliary obstruction.
Prothrombin time	Synthetic function.
Albumin	
γ-glutamyltransferase (GGT)	Cholestasis or biliary obstruction.
Bile acids	

Bilirubin

Q.2 What is bilirubin and how is it produced in the body?

- Bilirubin is a yellow bile pigment.
- It is produced from the degradation of heme; which is one of the breakdown products of red blood cells.
- Hb (in senescent RBC) is taken up by macrophages in RES. It is then broken down into globin + heme "which release bilirubin"

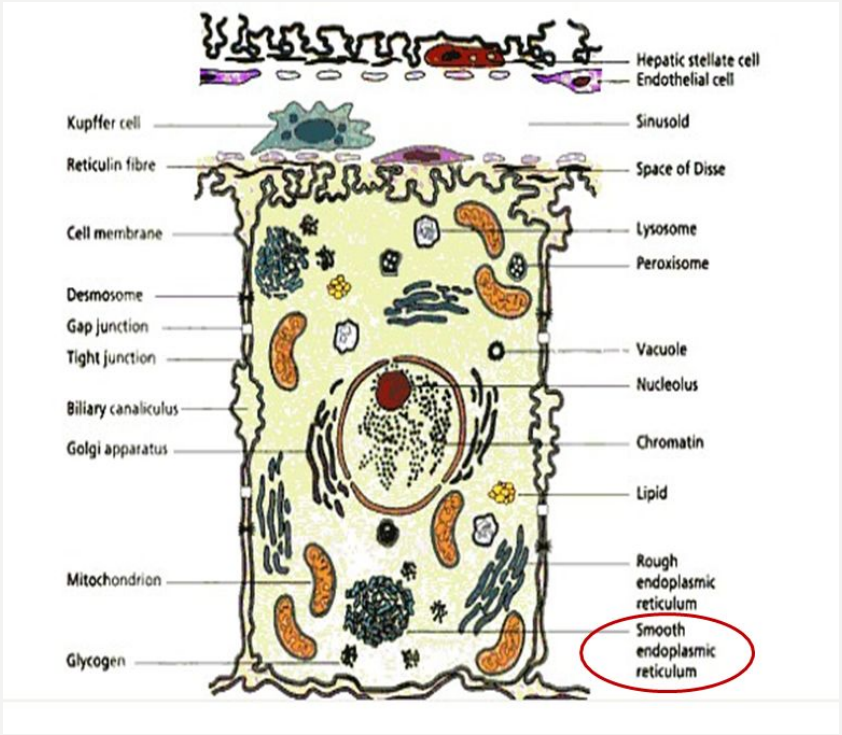
Q3. Which form of bilirubin is carried to the liver and how?

- Unconjugated (free) Bilirubin forms a complex with Albumin and transported to the Liver

Q.4

4a- How and why is bilirubin conjugated?

- **How?** Bilirubin is conjugated by addition of 2 Glucuronic acid in Hepatocytes -specifically in smooth endoplasmic reticulum (SER)- by the enzyme "**Glucoronyl transferase**"
- **Why?** The conjugated-bilirubin is water soluble and can be excreted in the urine and feces. This prevents precipitation and deposition in tissues.



4b- On the picture, mark the intracellular location for the process of conjugation.

smooth endoplasmic reticulum (SER)

2nd Part

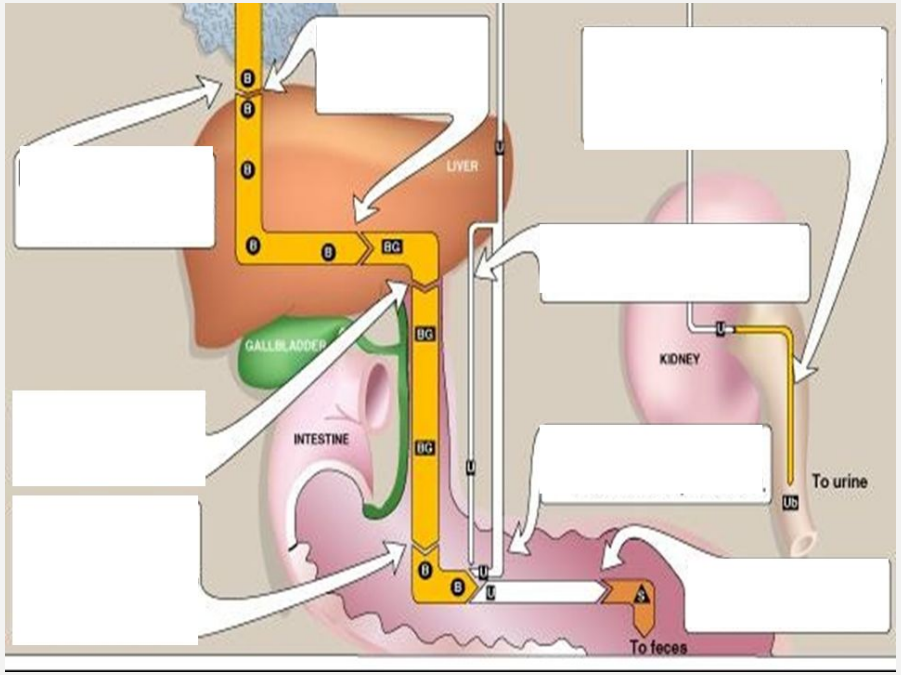
Conjugated vs Unconjugated bilirubin:

Conjugated	Unconjugated
✧ Water soluble	✧ Insoluble in water
✧ Loosely bound to albumin	✧ Tightly complexed to albumin
✧ Filtered through renal glomeruli and excreted in urine	✧ Not filtered through renal glomeruli, is not excreted in urine
✧ Non-toxic	✧ Toxic substance
✧ low conc. in the blood	✧ The chief form of bilirubin in the blood

4c- Mention 2 syndromes due to congenital deficiency of the conjugating enzyme (bilirubin glucuronyl-transferase).

clinical conditions (imp.):

1. Crigler-Najjar syndrome
2. Gilbert syndrome



Q. 5A How is bilirubin eliminated from the body?

Q.5 B What are the fates of bilirubin in the intestine?

see next slide

Note: In hepatic and post-hepatic jaundice, The conjugated bilirubin formed can not pass into small intestine (due to obstruction) and it returns back into blood. **Conjugated bilirubin** is then filtered through the kidney and appears in **urine** giving it **dark brown color.**
Extrahepatic causes of brown urine may include UT bleeding or infections, hemolytic anemia, some food and drugs.

Bilirubin

Fates of bilirubin in the intestine:

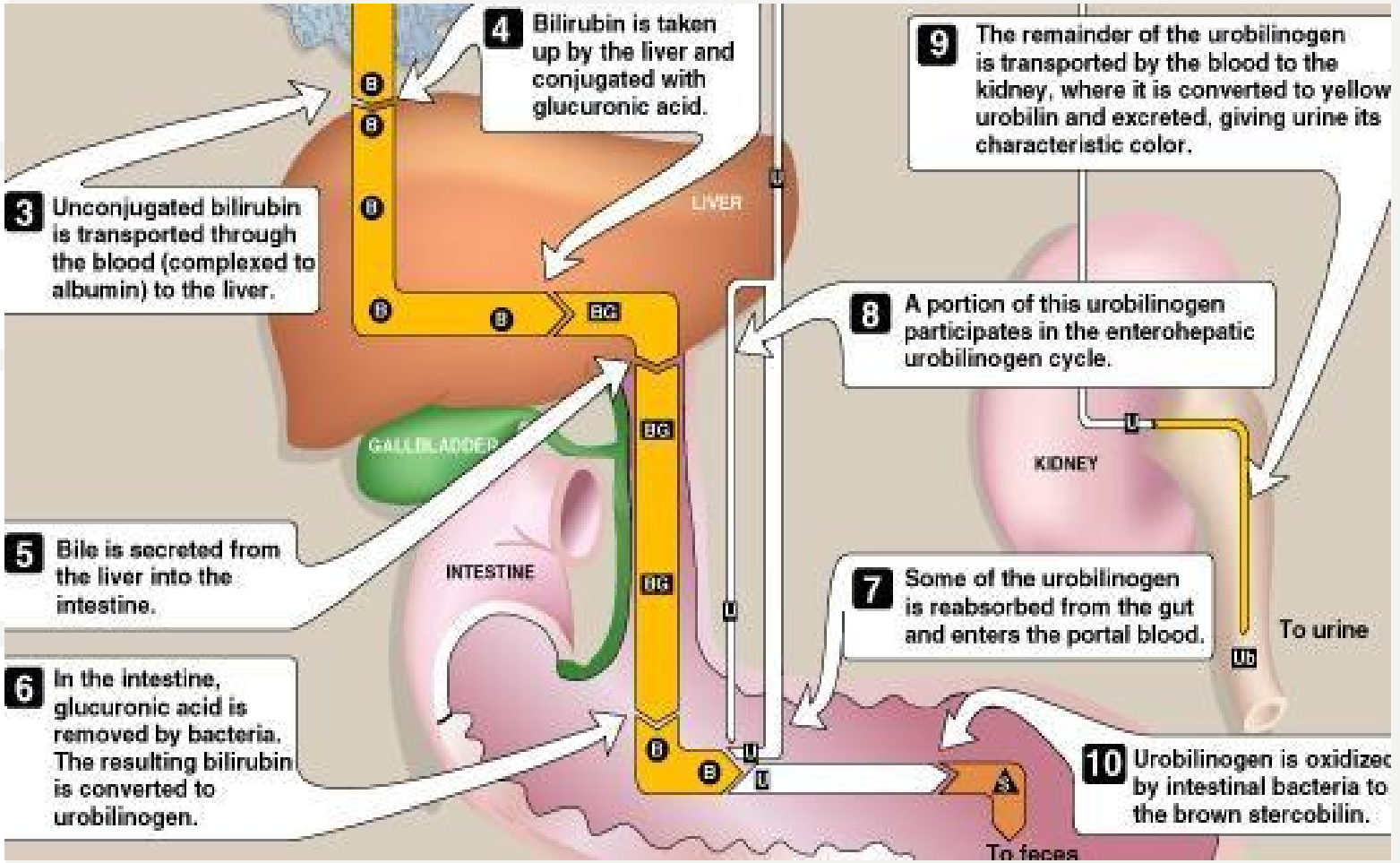
Conjugated bilirubin is hydrolyzed and reduced by bacteria in the gut to yield **urobilinogen** "colorless".

- **Feces: Most of the urobilinogen is** oxidized by intestinal bacteria to **stercobilin**, which gives feces the characteristic **brown color**.

- **Portal blood: some of the urobilinogen is** reabsorbed from the gut and enters the portal blood:

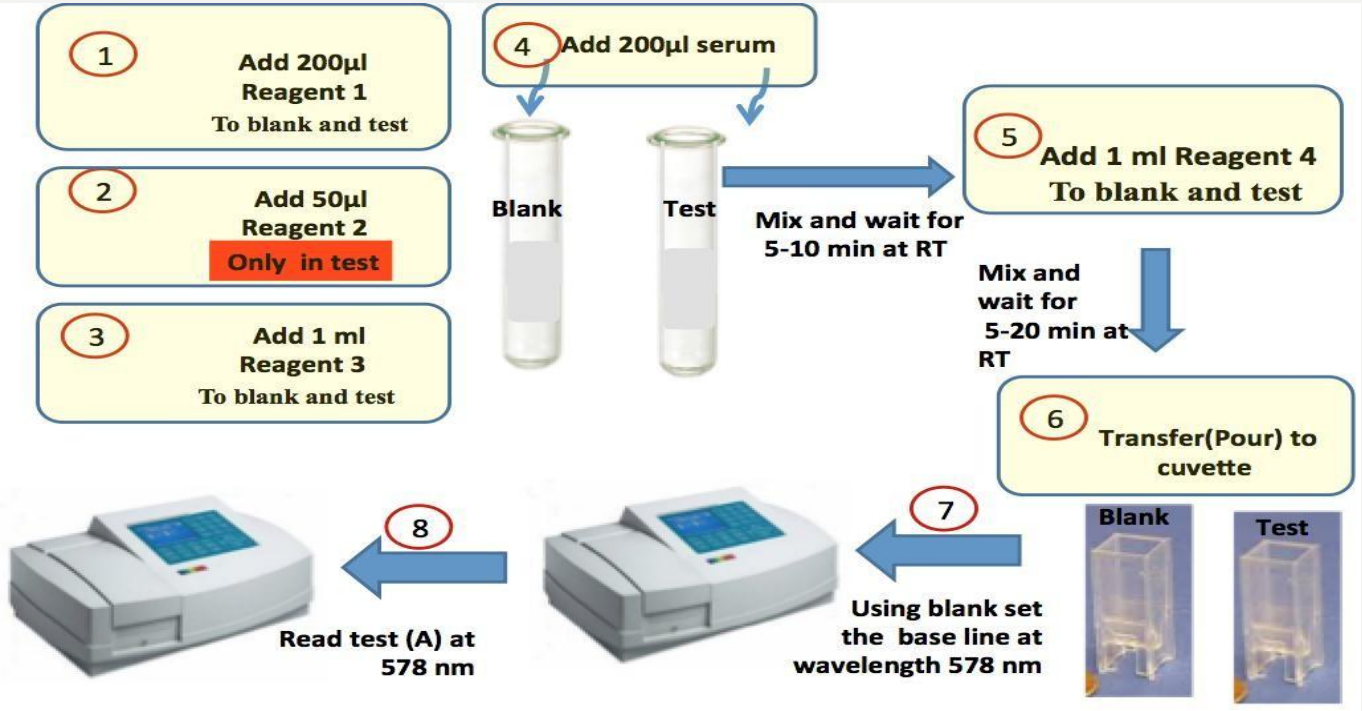
  - **Liver:** A portion of this urobilinogen participates in the **enterohepatic** urobilinogen cycle in which it is taken up by the **liver**, and then **re-secreted into the bile**.

  - **Kidneys:** The remainder of the urobilinogen is transported by the blood to the **kidney**, where it is converted to **yellow urobilin** and excreted, giving urine its characteristic color.



MEASUREMENT OF TOTAL BILIRUBIN

Note: you won't be asked about the steps of the experiment in the exam. absorbance (A) will be given and you will have to do the calculations only.



Calculation of total serum bilirubin concentration:

$A^1 \times 185 =$ …… µmol/L

(Normal range: 2 – 17 µmol/L)

1: The test result. It is the absorbance of bilirubin by spectrophotometer.

2: 185 is a constant set by kit manufacturer

Biochemistry Team 435

TEST YOURSELF!

2nd Part

A 43-year-old engineer who works with Saudi construction in Jazan is brought to KKUH by ambulance because of loss of consciousness and bloody vomiting. He looks pale and sclera of his eyes are yellow in color. Evidence of liver cirrhosis is shown by ultrasound.

LFT	Patient's result	Normal range
Bilirubin	83	0-19 µmol/L
AST	72	0-40 IU/L
ALT	59	0-50 IU/L

- What is the most likely type of elevated form of bilirubin in this case?
  Conjugated & unconjugated bilirubin
- In this case, why the levels of conjugated and unconjugated bilirubin are elevated?

Unconjugated>>the decreasing ability of the hepatocytes to conjugate.

Conjugated>>The remaining intact Hepatocytes will conjugate some, this escape to the circulation by ductal cells destruction or intrahepatic biliary duct obstruction (swelling of cells and edema due to inflammation)

- What do you expect to see in this patient urine and stool sample?

Stool ➔ pale grayish in color due to deficiency of stercobilin.

Urine ➔ dark brown due to filtration of excess conjugated bilirubin through the kidney

- Why does bilirubin get conjugated in the liver in normal person?
  To be converted to a water-soluble molecule which is non toxic and easily excreted from the body.
- Give one example for congenital deficiency of the conjugating enzyme (bilirubin glucuronyl transferase)?

Crigler-Najjar syndrome & Gilbert syndrome

- Describe the role of the intestine in excretion of Bilirubin?

Bilirubin get deconjugated by the bacteria in SI which results in transforming bilirubin to Urobilinogen.

Recourses:

- 435 slides and handout
- 434 & 433 teams
- Biochemistry team 435 (theory)

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