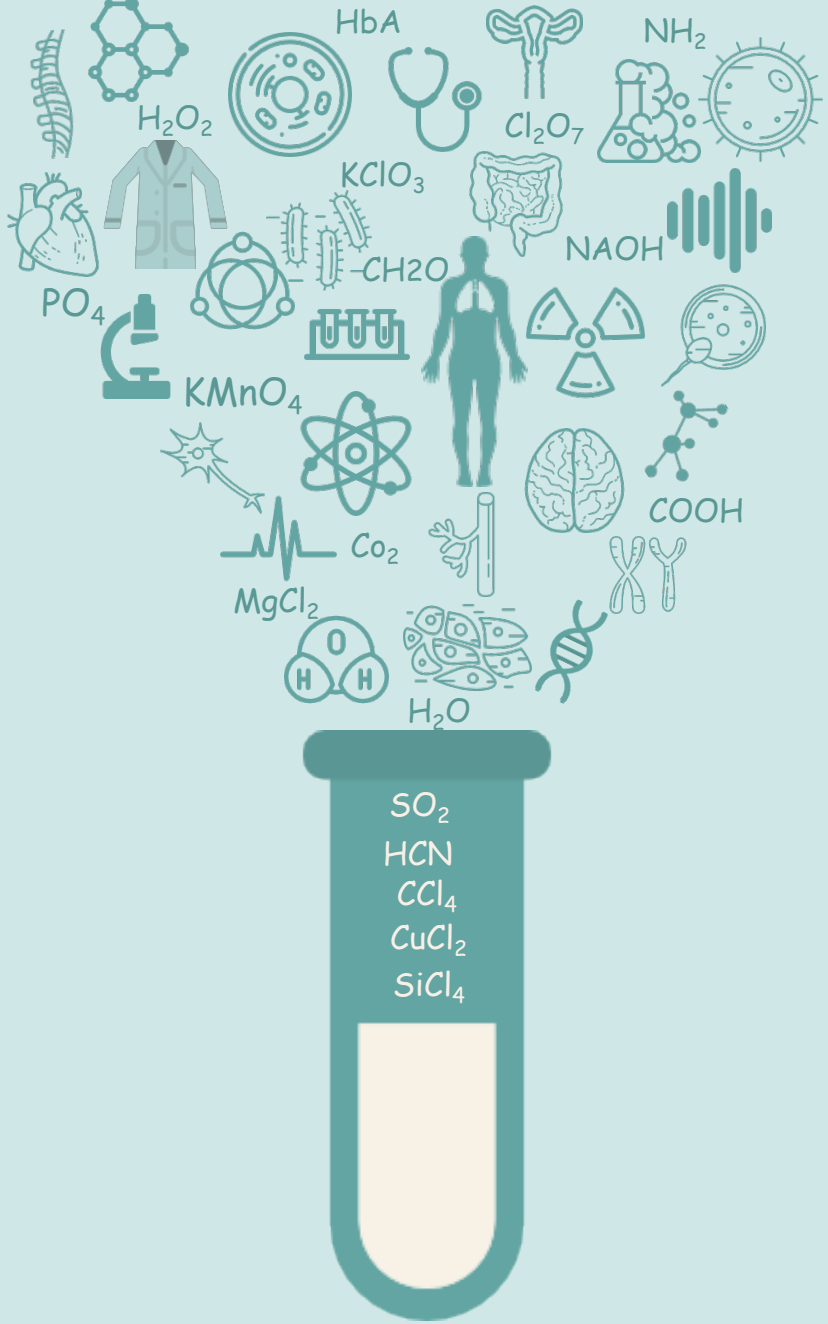


King Saud University

MEDICINE
KING SAUD UNIVERSITY

BIOCHEMISTRY TEAM 436

Important
Extra Information
Doctors slides
Doctors notes

Biochemistry

GNT BLOCK PRACTICAL SESSIONS

Editing file

Outline

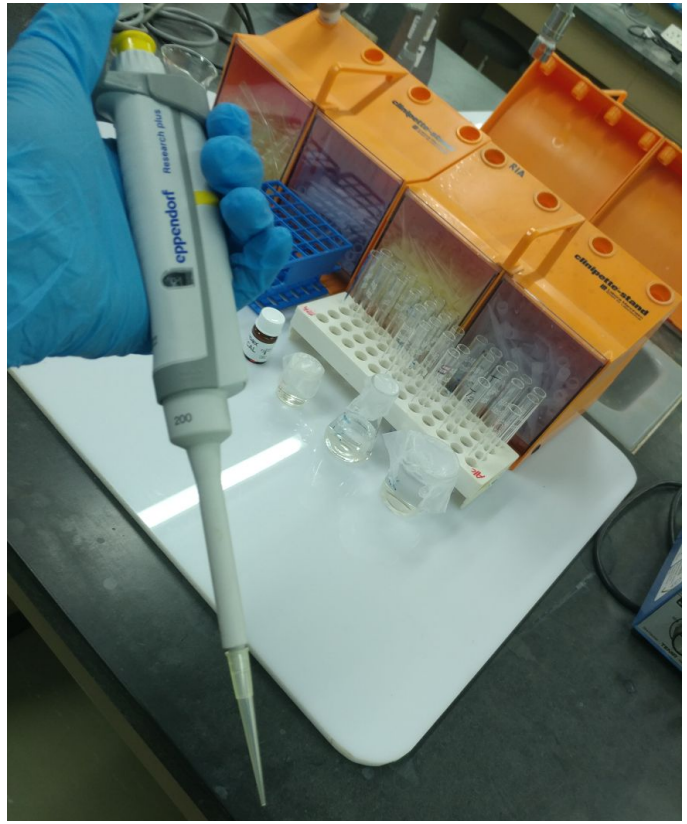
- This file contains the 2 lectures.
- This work is done by students, so if there are any mistakes please inform us .

1st Lecture :

Serum amylase & lipase
Accuracy & Precision

2nd Lecture :

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

1. short, branched and unbranched oligosaccharides (e.g. maltotriose
2. 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 hours	4-8 hours
	peak at (highest)	12-72 hours	24 hours
	return to normal	One week	8-14 days
Specificity and sensitivity	Most associated conditions	Lower	Higher
Using for diagnosis	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

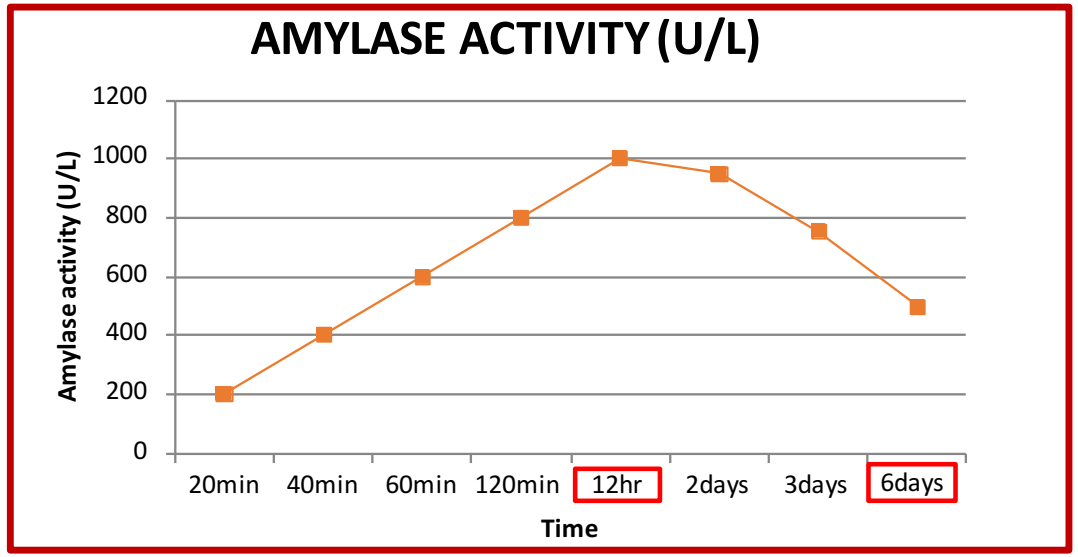
Amylase levels will be increasing over time, and will reach a peak within 12-72 hours. It will return to normal in few days (~ a week).

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 α-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)

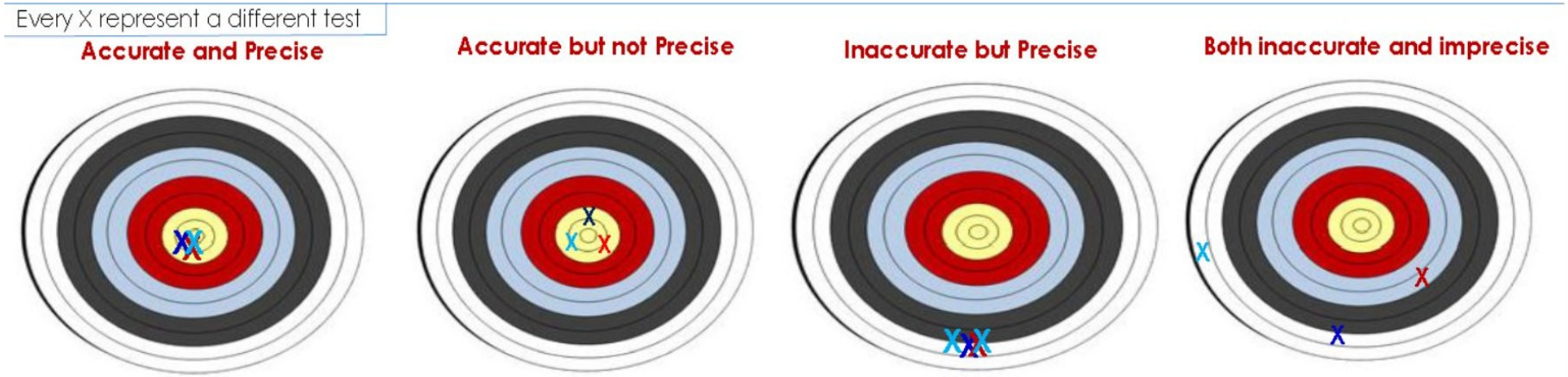
Biological factors: e.g., sex, age, diet, drugs..

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

Accuracy vs. precision

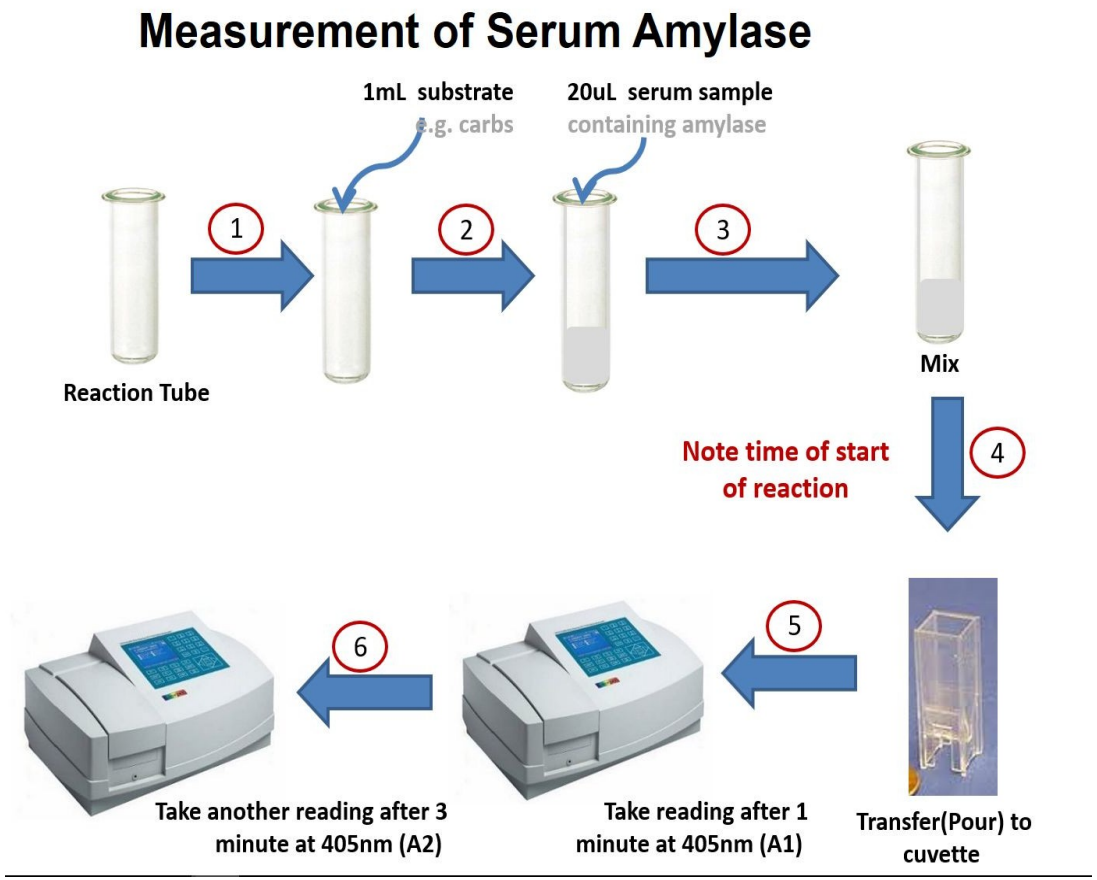
Accuracy	Precision
• The reliability of the method in determining the true value of the analyte. • The extent to which the mean measurement is close to the true value. **It is useful for comparison of original/gold standard method with other methods.** • e.g. the true value obtained from the standard 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 

- 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.



Measurement of serum amylase

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

Calculations

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

2. Serum Amylase (U/L) = $5544 \times \Delta A$
   - 5544 is a constant number, set by the kit manufacturer.

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

* (A) stands for absorbance

Liver function tests

- They are used to :
1. Detect hepatic injury
2. Assess liver functions

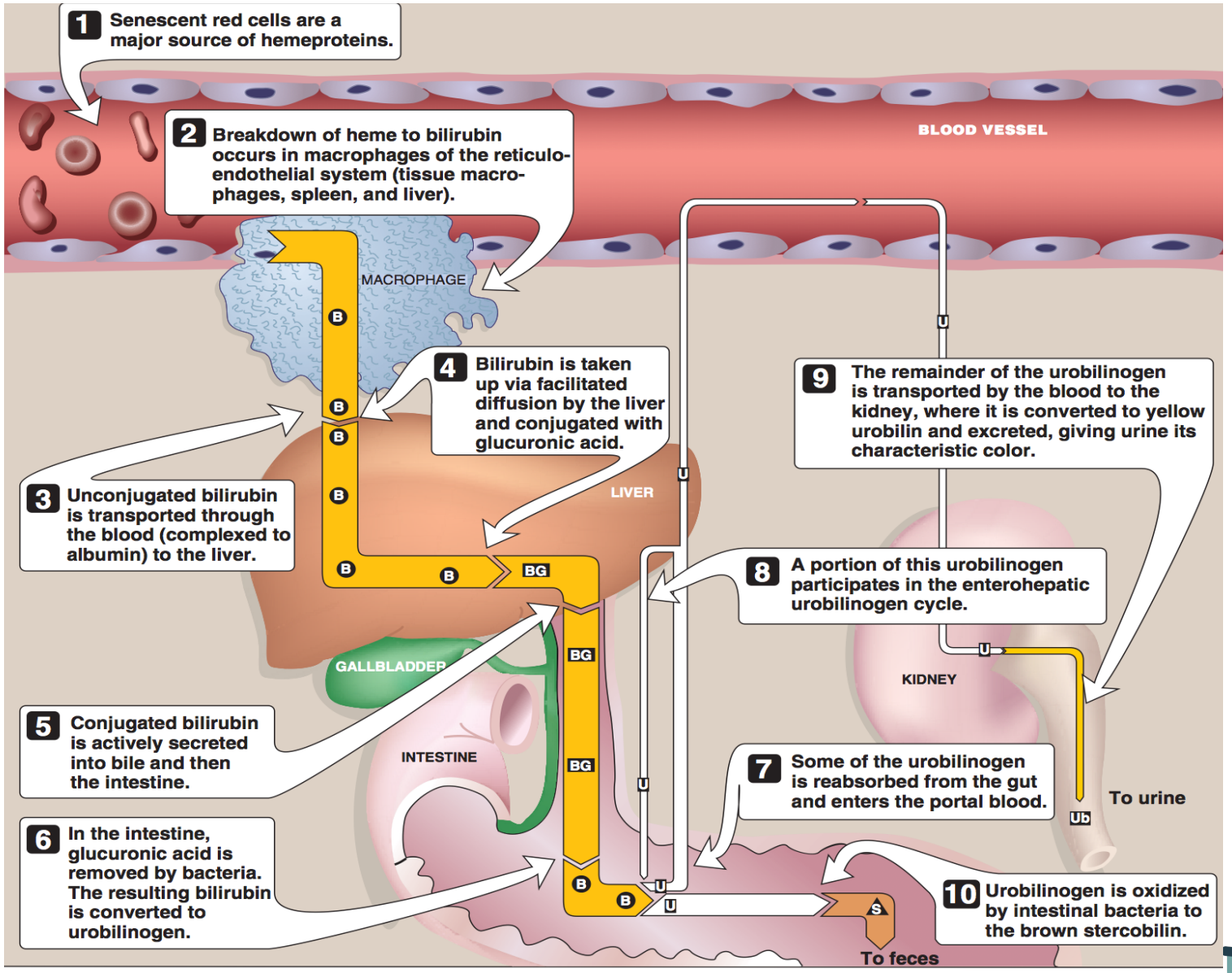
Chemistry test	Clinical importance of abnormality
ALT + AST + lactate dehydrogenase[1]	For hepatocellular damage
Bilirubin	For cholestasis, impaired conjugation & biliary obstruction
Prothrombin time & Albumin	For synthetic function
ALP + GGT + Bile acids + 5-nucleotidase	For cholestasis or biliary obstruction

1: Not specific for hepatocellular damage

Bilirubin

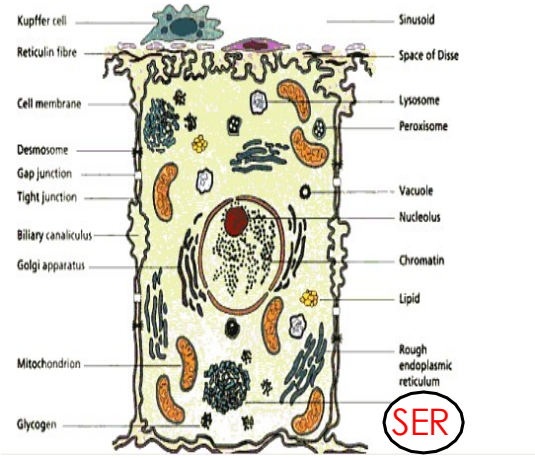
- Bilirubin is a yellow bile pigment.
- It is produced from degradation of heme (One of the breakdown products of RBCs).

1. Hb (in senescent RBC) > (RES)
2. In RES : (Heme) BILIRUBIN (FREE)
3. Unconjugated (free) Bilirubin forms a complex with Albumin and transported to the Liver
4. Bilirubin is conjugated to Glucuronic Acid in Hepatocytes (in smooth endoplasmic reticulum) by the enzyme "Glucoronyl transferase"
5. Conjugated-bilirubin is secreted into the bile toward small intestine .
6. In small intestine: Bacteria removes Glucuronic acid from conjugated bilirubin then Bilirubin becomes unconjugated (free) again & Bacteria convert it to Urobilinogen .



Bilirubin

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



❖ Examples of clinical conditions due to congenital deficiency of the conjugating enzyme (Glucuronyl transferase) .

1. Crigler-Najjar syndrome
2. Gilbert syndrome

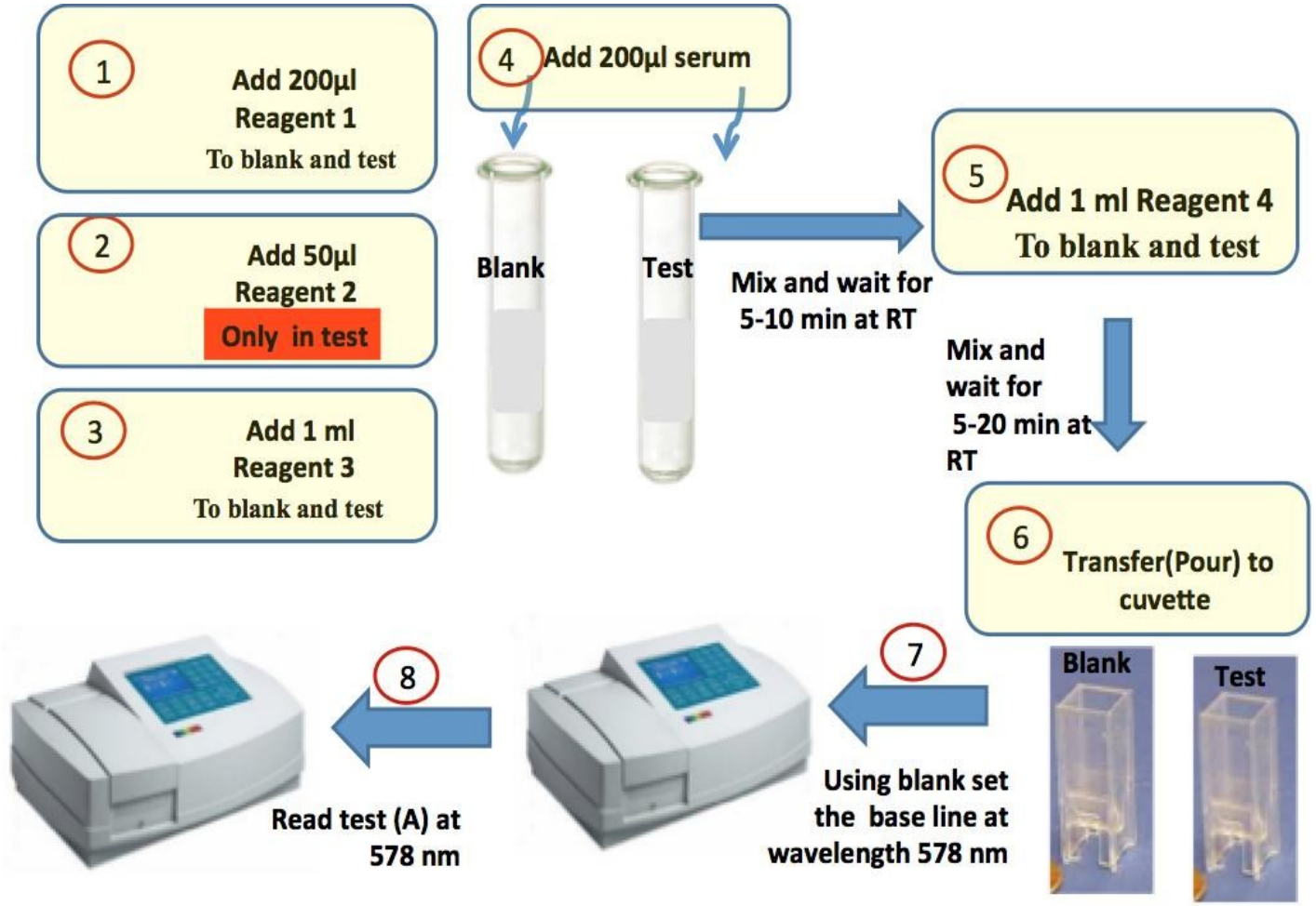
❖ Fates of bilirubin

✓ 10% of the conjugated bilirubin returns to the plasma either **directly** into the liver sinusoids or **indirectly** by absorption from the bile ducts or lymphatics.

✓ 90% of conjugated bilirubin is **deconjugated** in the small intestine and then converted to **Urobilinogen (UBG)**

- ✓ 70% of UBG is oxidized to Stercobilin (by intestinal bacteria) excreted in feces which gives it brown appearance.
- ✓ 20% of UBG Reabsorbed into portal blood and either **reexcreted by the hepatic cells** in the bile after re-conjugation (enterohepatic circulation) or Excreted in Urine in form of Urobilin which Gives the Urine its yellow color.

Measurement of bilirubin



Calculation of total bilirubin concentration

A (Test result) × 185 = …... µmol/L

Note : (Normal range: 2 – 17 µmol/L)

Sensitivity & Specificity of tests

Sensitivity

- Sensitivity answers the following question:
- If a person has a disease, how often will the test be positive (true positive 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.
  A Sensitive test helps rule out disease (when the result is **negative**).
- Sensitivity rule out* or "Snout"

*Exclude the presence of the disease(i.e usually used for screening of large groups to exclude the disease)

Specificity

- 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 result for a highly specific test is positive you can be nearly certain that the individuals actually have the disease.
  A very specific test rules in disease with a high degree of confidence (when the result is positive).
- Specificity rule in** or "Spin"

** Ensure the presence of the disease(i.e usually after we finish screening with a sensitive test we use a specific test to diagnose)

2 X 2 Contingency Table

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

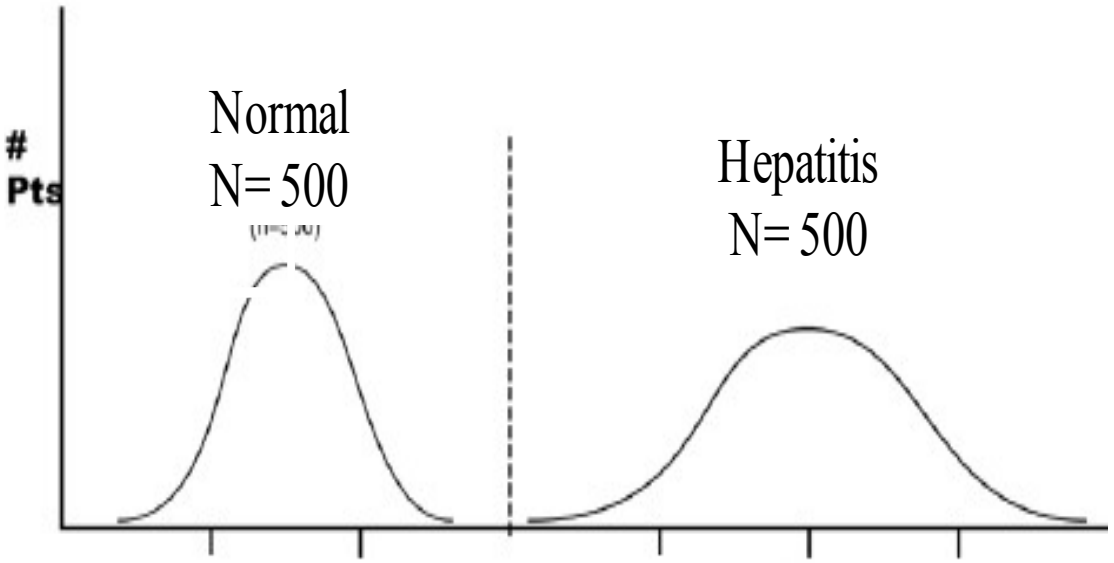
$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100$$

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

Ideal vs. Lab test

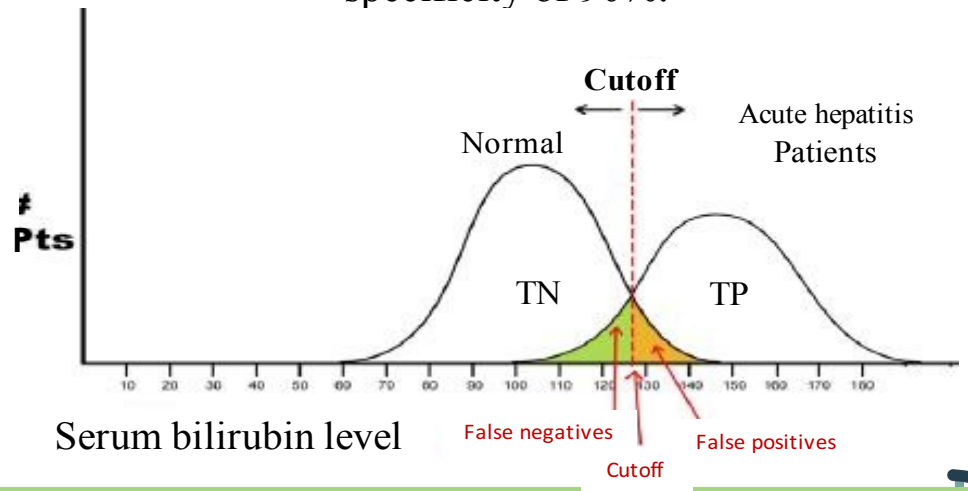
Ideal test

- The curve shown below ia an example of 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.



Lab test

- The curve shown below 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%.



Example

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 following quality measures:

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

Test	Disease	
	+	-
+	TP	FP
-	FN	TN

Test	Disease	
	+	-
+	440	50
-	60	450

1. The sensitivity : $\frac{TP}{TP+FN} \times 100 = \frac{440}{440+60} \times 100 = 0.88 \times 100 = 88\%$

2. The specificity : $\frac{TN}{TN+FP} \times 100 = \frac{450}{450+50} \times 100 = 0.90 \times 100 = 90\%$

NOTE: since the specificity of this test is higher than the sensitivity; this test is better for a diagnosing purposes.

Hands out - summary

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?

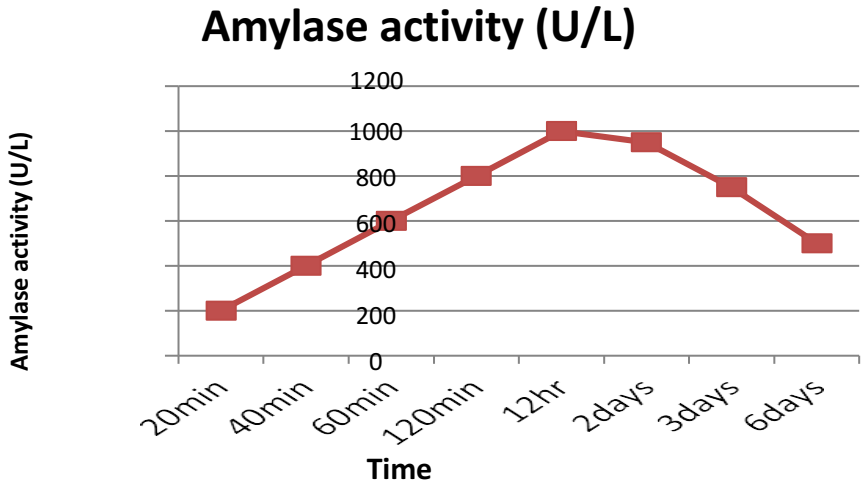
Acute Pancreatitis

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

Serum lipase

Hands out - summary

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.

QUIZ

- 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) α(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).

QUIZ

- 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. and 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 deunconjugated by the bacteria in SI which results in transforming bilirubin to Urobilinogen.

THANK YOU

FOR CHECKING OUR WORK

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

TEAM LEADERS

Mohammad Almutlaq

Atikah kadi