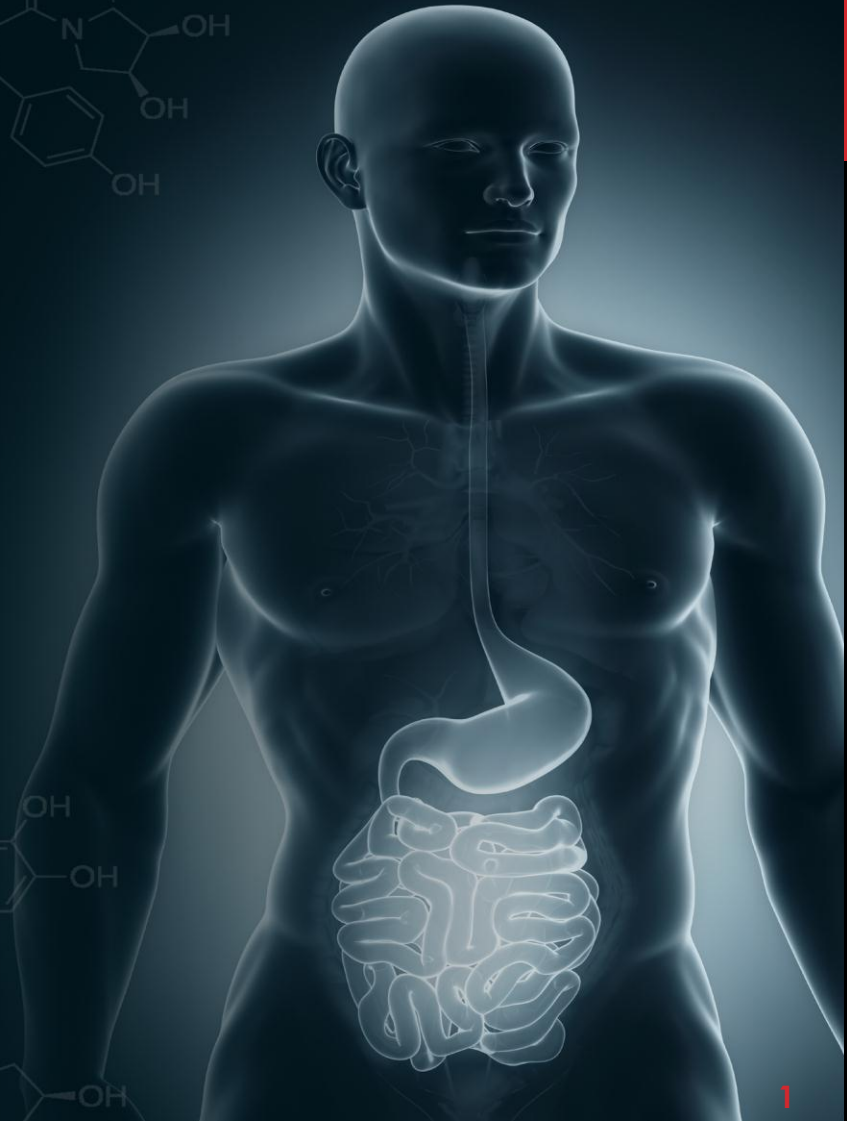**Bi**ochemistry
Bandits **434**

BIOCHEMISTRY PRACTICAL SESSIONS

❖ **1ˢᵗ Part**
➢ Serum amylase & lipase
➢ Accuracy & Precision

❖ **2ⁿᵈ Part**
➢ LFTs
➢ SENSETIVITY & SPECIFICITY

*IMPORTANT *EXTRA **CORRECTIONS LINK**

AMYLASE

Sources:	Function:	Products:
Parotid Gland →Saliva Pancreas →Pancreatic Juice	hydrolyzes (breakdown) α(1,4) glycosidic bonds in starch and glycogen	short oligosaccharides (both branched & unbranched) Disaccharides: Maltose and isomaltose

- **high levels of serum amylase & lipase are used to diagnose Acute Pancreatitis, but 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

- The specificity and sensitivity of **serum amylase** are lower than those of **serum lipase**, but serum amylase is used for diagnosing pancreatitis because it is **inexpensive.**

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

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 presented late, the diagnosis of acute pancreatitis based on the enzyme level could be missed.

Amylase activity (U/L)
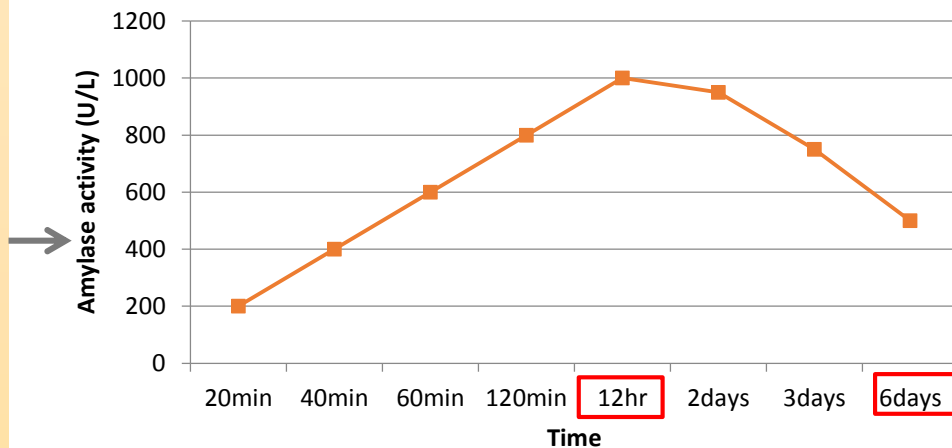
	Condition	Serum amylase	Serum Lipase
Normal Ranges	adults < 60	25-125 IU/L	10-140 IU/L
	Adults > 60	24-151 IU/L	18-181 IU/L
Ranges in Acute Pancreatitis	start rising at	2-12 hrs	4-8 hrs
	peak at (highest)	12-72 hrs	24 hrs
	return to normal	One week	8-14 days

In acute pancreatitis, values are three times more the normal range and associated with clinical picture e.g. upper abdominal pain radiating to the back, nausea..

3

SOURCES OF VARIATION IN AMYLASE TEST RESULTS

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

LAB RELEATED

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:** The reliability of the method in determining <u>the true value</u> of the analyte.

- The extent to which the mean measurement is <u>close to the true value</u>.

- It is useful for comparison of original, gold stndard method with other methods.

❖ **Precision:** The reproducibility of the method when it is run <u>repeatedly</u> under identical conditions**.**

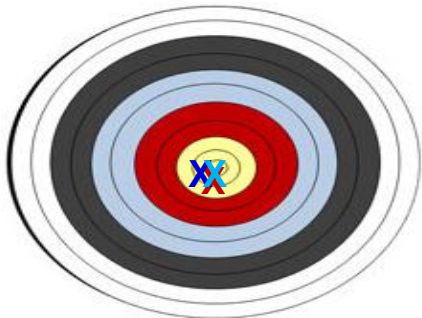✓ **They are independent, e.g., an inaccurate result can be extremely precise**
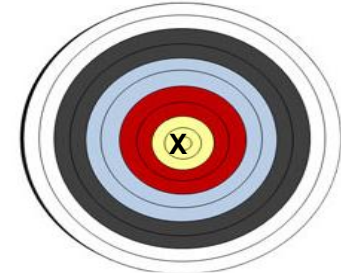
Close to the true value this is accurate

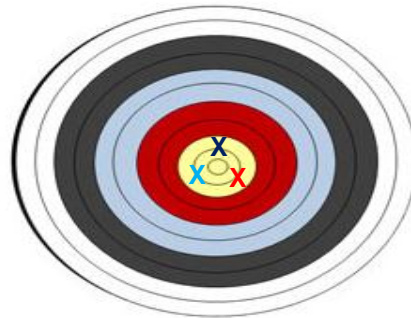**EX:**The Results of repeated tests were: 3 + 3,2 + 3,1

The results are close to each other; This is Precise
***Regardless their approximation to the true value**

Every X represent a different test

Accurate and Precise

Accurate but not Precise

Inaccurate but Precise
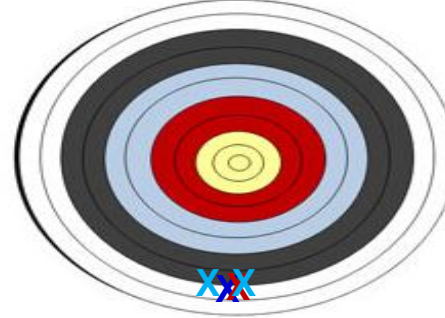
Both inaccurate and imprecise
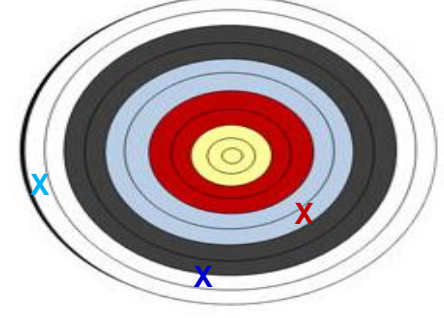
Measurement of Serum Amylase

1mL substrate

20uL serum sample

Reaction Tube → 1 → → 2 → → 3 → **Mix**

Note time of start of reaction

4 ↓

Transfer(Pour) to cuvette

← 5 ←

Take 1 minute reading (A1) at 405nm

← 6 ←

Take 3 minute reading (A2) at 405nm
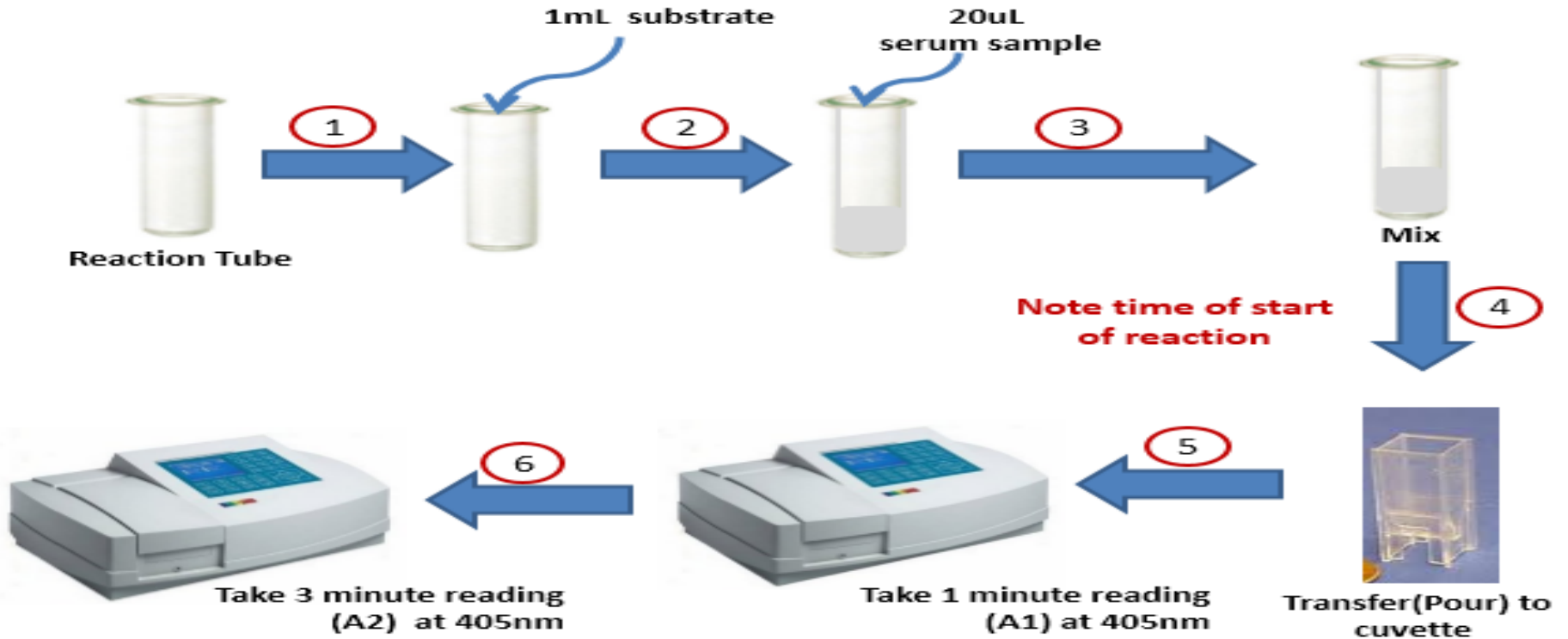
Calculation & Interpretation

$$\Delta A = \frac{A2 - A1}{3}$$

Serum Amylase (U/L) = 5544 × Δ A

Normal reference values:

Serum: up to 125 U/L (at room temperature)

Normal **range** 25-125 U/L

TEST YOURSELF!

➢ 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).

LIVER FUNCTION TESTS

They're used to:

1. Detect Hepatic Injury   2.Asses hepatic function.

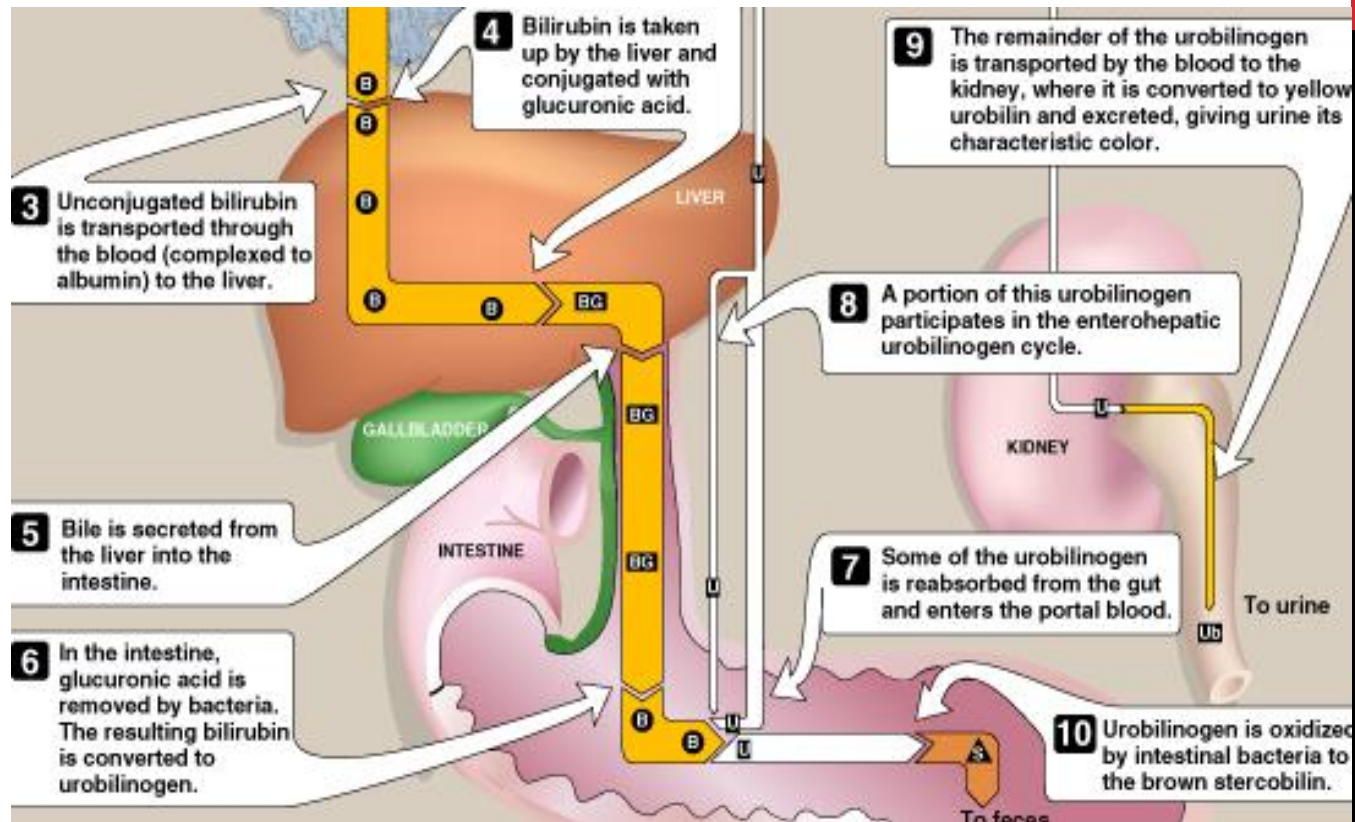| Common serum liver chemistry tests	
ALT + AST + lactate dehydrogenase[1]	For hepatocellular damage
Bilirubin	For cholestasis, impaired conjugation & biliary obstruction
Prothrombin time & Albumin	For synthetic function
ALP + GGT + Bile acids + 5-nucleotidase	For cholestasis or biliary obstruction

1.Not specific for hepatic disease

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 SER[1]) by the enzyme "Glucoronyl transferase"

5. Conjugated-bilirubin is secreted into the bile ➔ SI.

6.In small intestine: Bacteria removes Glucuronic acid from conjugated bilirubin➔ Bilirubin becomes unconjugated(free) again & Bacteria convert it to **Urobilinogen.**

4 Bilirubin is taken up by the liver and conjugated with glucuronic acid.

3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.

5 Bile is secreted from the liver into the intestine.

6 In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.

9 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.

8 A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.

7 Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.

10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.

LIVER
GALLBLADDER
INTESTINE
KIDNEY
To urine
To feces

1: Smooth Endoplasmic Reticulum

Conjugated vs Unconjugated

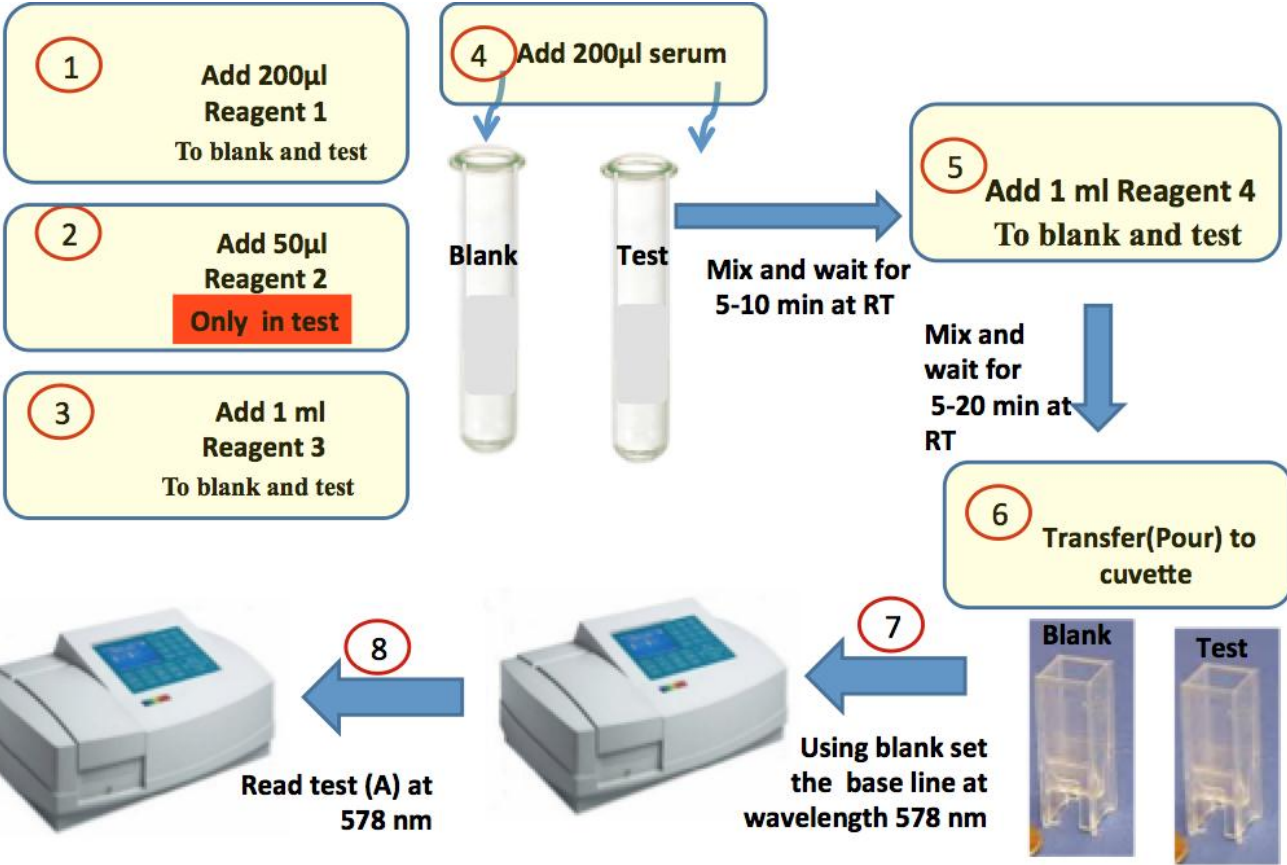| Conjugated	Unconjugated
✧ Water soluble Loosely bound to albumin	✧ Insoluble in water
	✧ Tightly complex to albumin
✧ Filtered through renal glomeruli and excreted in urine	✧ Not filtered through renal glomeruli, is not excreted in urine
✧ Non-toxic	✧ Toxic substance
✧ low conc. in the blood	✧ The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

SER
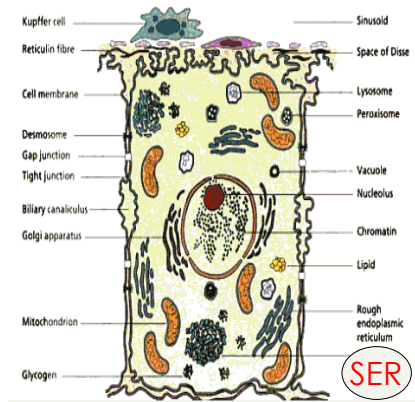
*EXAMPLES OF CLINICAL CONDITIONS DUE TO CONGENITAL DEFICIENCY OF THE CONJUGATING ENZYME (BILIRUBIN GLUCURONYL TRANSFERASE)?

1. Crigler-Najjar syndrome
2. Gilbert syndrome

FATE 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**

    ✓ **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.**

10

MEASUREMENT OF TOTAL BILIRUBIN

1. Add 200μl Reagent 1 To blank and test

2. Add 50μl Reagent 2 Only in test

3. Add 1 ml Reagent 3 To blank and test

4. Add 200μl serum

Blank   Test

Mix and wait for 5-10 min at RT

5. Add 1 ml Reagent 4 To blank and test

Mix and wait for 5-20 min at RT

6. Transfer(Pour) to cuvette

Blank   Test

7. Using blank set the base line at wavelength 578 nm

8. Read test (A) at 578 nm

Calculation of total bilirubin concentration

$A^1 \times 185 =$ ...... μmol/L

Note:(Normal range: 2 – 17 μmol/L)

1: (test result)

SENSETIVITY

A Sensitive test helps <u>rule out[1] or or "Snout"</u>

Disease, when the result is negative

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.**

SPECIFICITY

A very specific test <u>rules in[2] or "Spin"</u> disease with a high degree of confidence, when the result is positive

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.**

2 X 2 Contingency Table

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

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

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

1: exclude the presence of the disease(i.e usually used for <u>screening</u> 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 <u>diagnose</u>)

ROC CURVE

*SHOWN BELOW IS AN EXAMPLE OF A PERFECT TEST WHERE THERE IS NO OVERLAP BETWEEN THE RESULTS OF NORMAL AND DISEASE.

✧ **AN IDEAL DIAGNOSTIC** LAB TEST RESULTS FOR MANY SUBJECTS (NORMAL AND PATIENTS)

✧ THE TEST IDENTIFIES ALL PATIENTS WITH DISEASE AND ALL SUBJECTS WITHOUT DISEASE 100% OF THE TIME.

✧ IT WOULD BE A PERFECT TEST TO DIAGNOSE ACUTE HEPATITIS.

Normal
N = 500

Hepatitis
N= 500
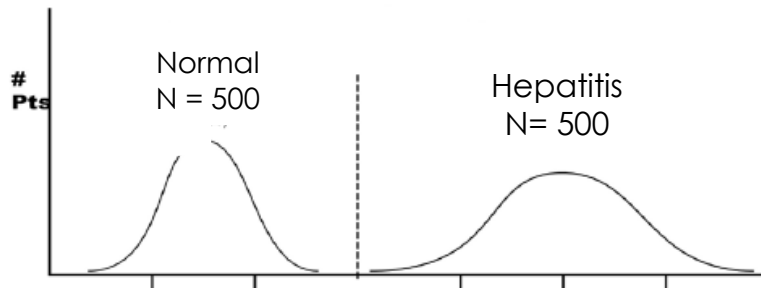
*shown below is what actually happen in the lap.
✧ **The lab test results in normal and disease conditions overlap.**
✧ **To increase the overall accuracy of the test, the centermost point of overlapping is chosen as the cutoff value.**

✧ 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%.

Cutoff
Acute hepatitis Patients
Normal

TN   TP

Serum bilirubin level

false negatives   false positives
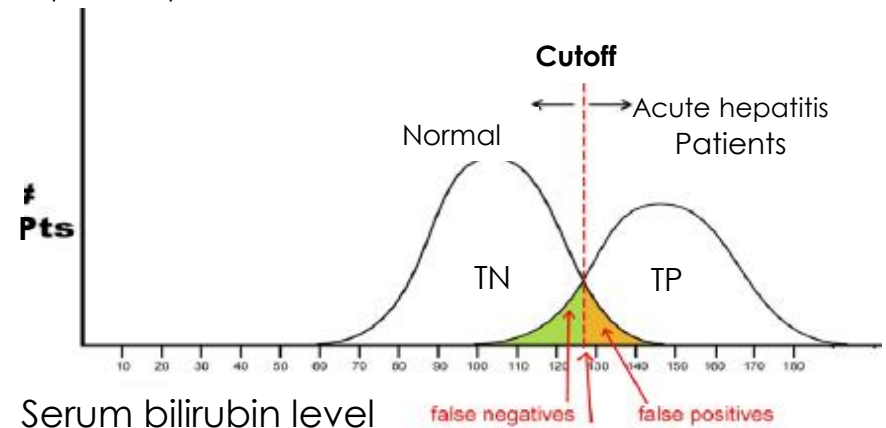
EXAMPLE:

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

✧ Number of <u>positive</u> results in <u>patients</u> with acute hepatitis: 440

✧ Number of <u>positive</u> results in <u>normal</u> subjects: 50

✧ Number of <u>negative</u> results in <u>normal</u> subjects: 450

✧ Number of <u>negative</u> results in <u>patients</u> 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 their right values. (Values will be given)
3. Write the equations
4. Calculate.(DON'T forget your calculator)

Remember:
✓ True or false>>regarding the person
✓ Positive or negative>>regarding the test.

Test	Disease	
	+	-
+	TP	FP
-	FN	TN

Test	Disease	
	+	-
+	440	50
-	60	450

1. **The sensitivity**: $\dfrac{TP}{TP+FN}$ X 100 = $\dfrac{440}{440+60}$ X 100 = 0.88 X 100 = 88%

2. **The specificity**: $\dfrac{TN}{TN+FP}$ X 100 = $\dfrac{450}{450+50}$ X 100 = X 0.90 X 100 = 90%

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

TEST YOURSELF!

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.

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والله وَلي التَوفِيق

Done by:

Mohammad Alotaibi & Najla Aldraiweesh