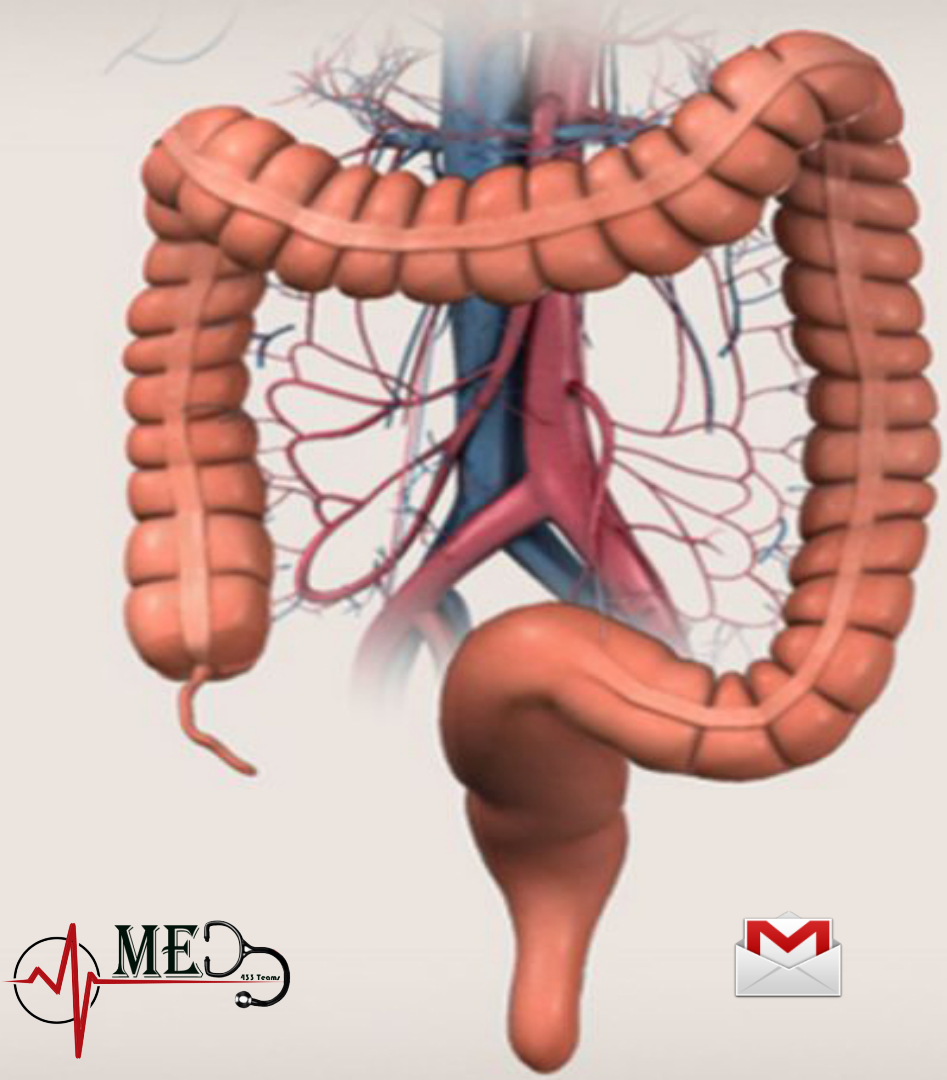
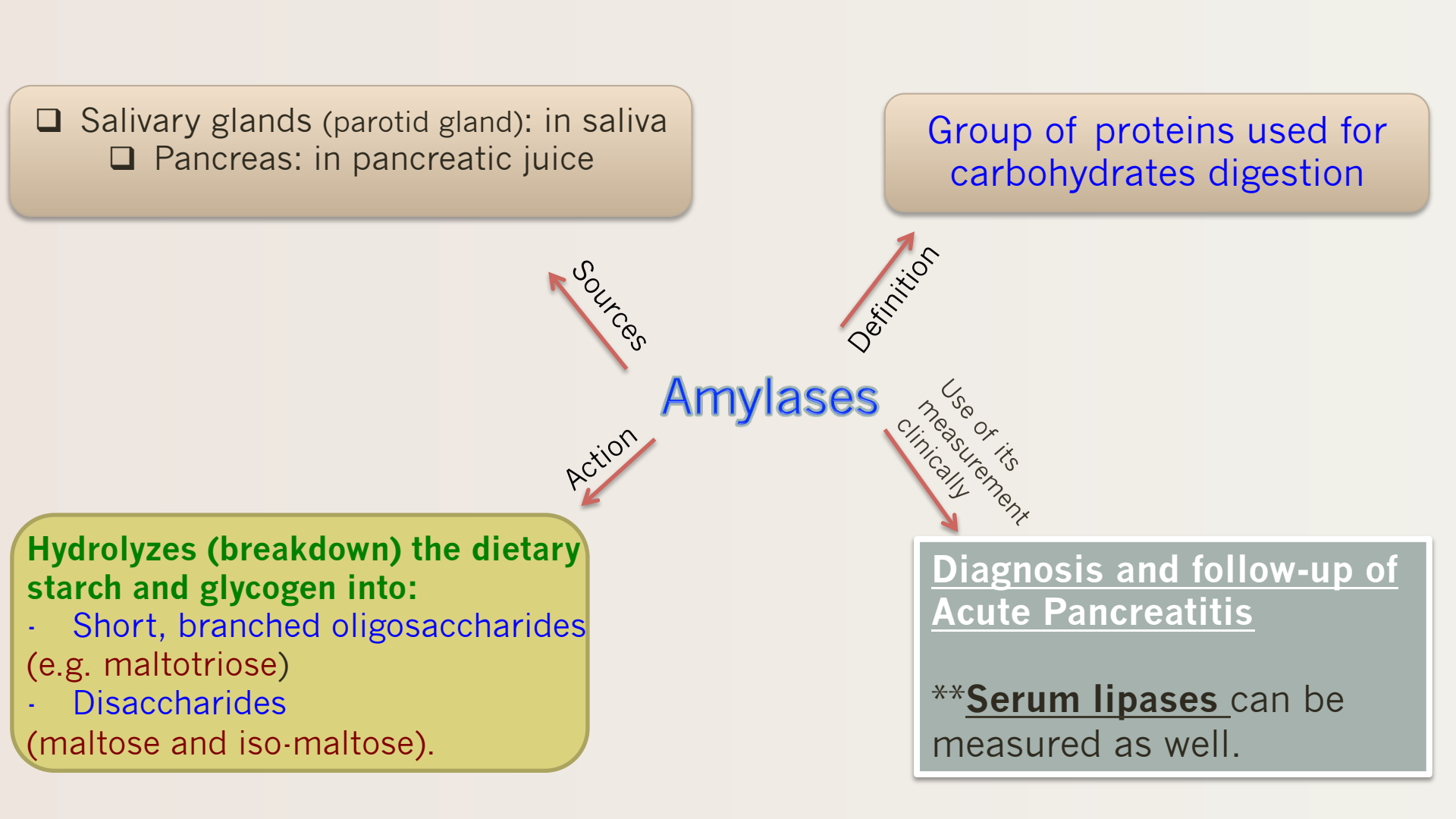


Biochemistry practical sessions.

- Serum amylases VS Serum lipases
- Precision VS Accuracy
- LFTs
- Bilirubin metabolism
- Sensitivity VS Specificity

Biochemistry433@hotmail.c


❑ Salivary glands (parotid gland): in saliva
❑ Pancreas: in pancreatic juice
Group of proteins used for carbohydrates digestion
Sources
Definition
Amylases
Action
Use of its measurement clinically
Hydrolyzes (breakdown) the dietary starch and glycogen into:
- Short, branched oligosaccharides (e.g. maltotriose)
- Disaccharides (maltose and iso-maltose).
Diagnosis and follow-up of Acute Pancreatitis
**Serum lipases can be measured as well.

Serum amylase vs. lipase for acute pancreatitis

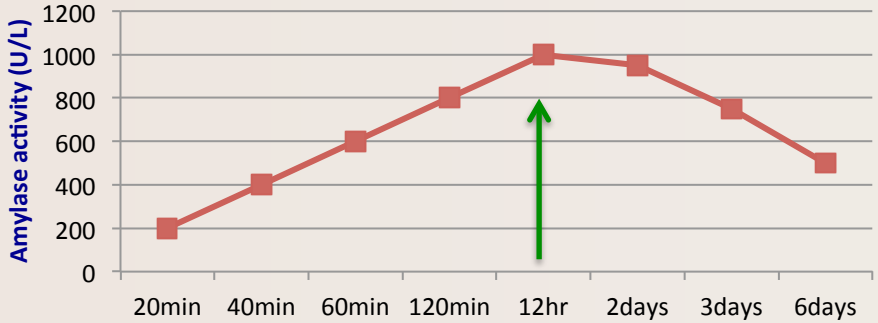
	Serum amylase	Serum Lipase
Levels start rising	2-12 hrs	4-8 hrs
Peak Levels	12-72 hrs	24 hrs
Levels return to normal	One week	8-14 days
Normal range:* adults < 60yrs	25-125 IU/L	10-140IU/L
Adults > 60 yrs	24-151 IU/L	18-181 IU/L

- **Values three times > than the normal range along with the clinical picture** (upper abdominal pain radiating to the back, nausea, vomiting ...etc) is considered **positive for acute pancreatitis.**

- Even though the specificity and sensitivity of serum amylase are lower than those of serum lipase, serum amylase is widely used for diagnosing pancreatitis because it is relatively inexpensive.

*Will be given in the question

Changes in serum amylase activity during course of acute pancreatitis



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

***The rising titer of amylase is more clinically significant than single high reading**

INTERPRETATION OF THE CURVE CHANGES:

When acute pancreatitis it leads to:

→ damage of the exocrine part of the pancreas.

→ release of the pancreatic enzymes into the circulation (α-Amylase is one them).

NOTE:

Elevated amylase levels, DOES NOT always refer o pancreatic disease.

It may also increase in :

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

** WHY DOES IT DECREASE BACK TO NORMAL:

- **The condition is self-limited**
- **The circulating amylase will be excreted in urine**
- **The circulating amylase will be degraded (protein turnover)**

SOURCES OF VARIATION IN TEST RESULTS

Analytical factors:	Biological factors:	Pathological factors:
e.g. Accuracy (reliability) precision (reproducibility)	e.g., sex, age, diet, drugs.	e.g., progression of the disease, complications
Related to the lab preparations	Related to the patient	Related to the disease

Accuracy Vs Precision

Accuracy

The reliability of the method in determining the true value of the analyte (amylase, Hb..etc)

reflects how close a measurement is to a known or accepted value.

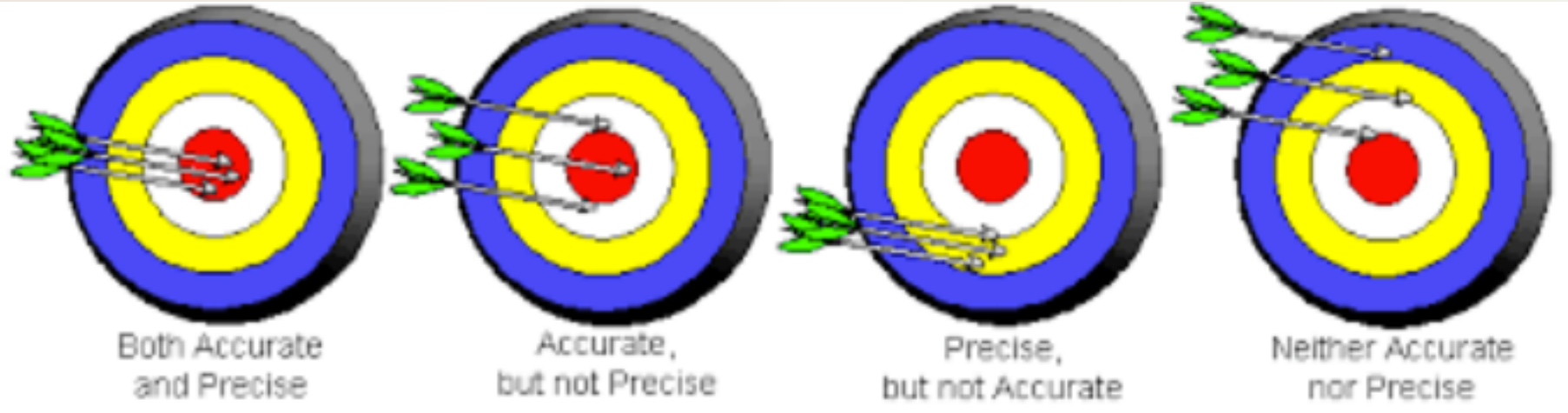
It is useful for comparison of original, gold standard method with other methods

Precision

The reproducibility of the method when it is run **repeatedly** under identical conditions

reflects how measurements are reproducible, even if they are far from the accepted value.

Precision and accuracy are independent



TO EXPLAIN:

- Each individual test has its own reference procedure and true values so, when we want to initiate a new procedure for a specific test, we compare its results to the true values of the REFERENCE METHOD, if the results are close to the true values then our new procedure is ACCURATE.
- If we have done a specific test many times using the same measurement and their result are close to each other (regardless their closeness to the true values) then this results will be PRECISE

مثل لاعب كرة القدم اللي دائما يضيع ركلات الجزاء يكون Precise لكنه NOT accurate لانه المفترض انها تكون في المرمى

Case 1

A 65-year-old retired school teacher is referred to KKUH by his general practitioner because of repeated abdominal pain and evidence of gallstones shown by ultrasound. On arrival to the hospital, he has upper abdominal pain, vomited once, and tenderness in the epigastrium. His vital signs are almost normal.
CBC results are shown below including serum levels of amylase & lipase.
Also LFTs have shown normal results.

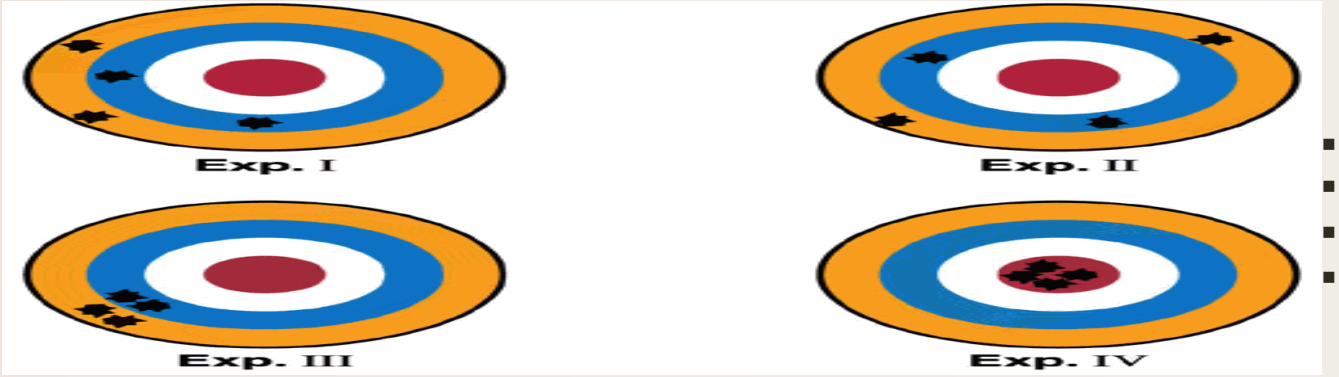
Based on the results, which organ do you think is the source of the pain?
PANCREAS
(Indicated by elevated levels of serum amylase & serum lipase)
What is the possible diagnosis?
Acute pancreatitis

Blood test	Patient's result	Normal range
Haemoglobin	135	115-160 g/L
WBC	12.5 x 10^9	4.0-11.0 x 10^9 /L
Platelets count	330 x 10^9	150-400 10^9 /L
Serum amylase	1100	25-125 U/L
Serum lipase	430	10-150 U/L
AST, ALT, ALP, bilirubin, albumin & PT are normal		

The doctor advised him to rest his pancreas (nothing per mouth).
1 week ago, biochemistry tests were done to follow up and the results showed normal serum amylase levels. What is your explanation for this ?

1) Due to the self recovery mechanism
2) The circulating amylase has been excreted in urine
3) The circulating amylase has been degraded

Q1) Mark the following pictures with their right interpretation:



- Precise and accurate
- Precise but not accurate
- Least precise
- Neither accurate nor precise

ANSWERS:

Precise and accurate	(IV)
Precise but not accurate	(III)
Least precise	(II)
Neither accurate nor precise	(I, II)

Q2) A 3 groups of students measures the mass of a product from the same chemical reaction. The groups recorded data of 8.83 g, 8.84 g and 8.82 g. The known mass of the product from that reaction is 8.70g.

What is your comment on the group values? Why ?

Their values are precise but NOT Accurate, because their values are close to each other but they are far from the standard (true) value which is 8.70 gm

Liver Function tests (LFTs)

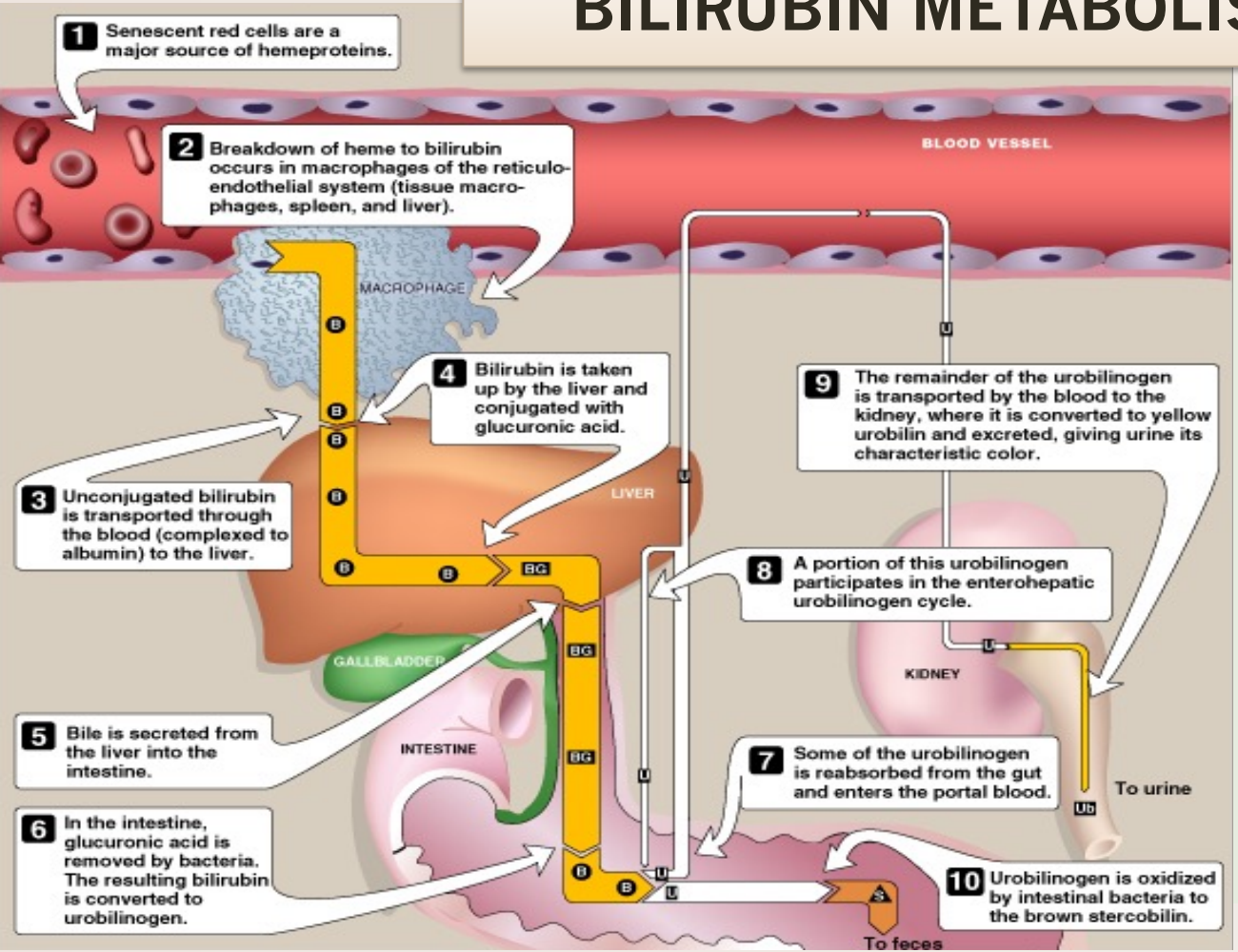
Liver chemistry test	Clinical implication of abnormality
Alanine aminotransferase	Hepatocellular damage
Aspartate aminotransferase	Hepatocellular damage
Bilirubin	Cholestasis, impaired conjugation, or biliary obstruction
Alkaline phosphatase	Cholestasis, infiltrative disease, or biliary obstruction
Prothrombin time	Synthetic function
Albumin	Synthetic function
γ-glutamyltransferase	Cholestasis or biliary obstruction
Bile acids	Cholestasis or biliary obstruction

E.g. Hepatitis

Generally they are used to : Asses hepatic function & Detect hepatic injury

BILIRUBIN METABOLISM

VERY IMPORTANT



- **Bilirubin** is a yellow bile pigment produced from the degradation of **Heme**; which is one of the breakdown products of RBCs.

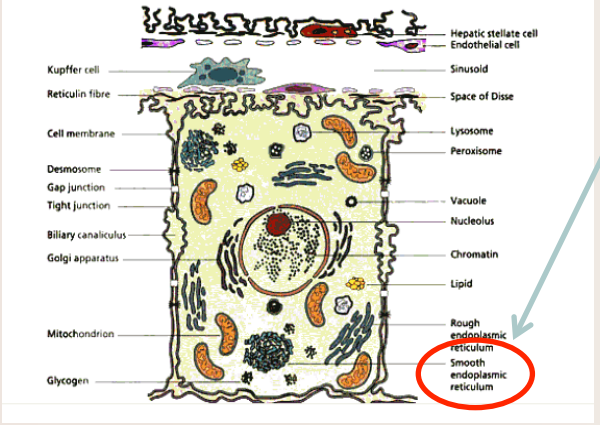
1. **Hb (in senescent RBC)=> (RES)**
2. **In RES : (Heme) → BILIRUBIN (FREE)**

3. **Unconjugated (free) Bilirubin forms a complex with Albumin and transported to the Liver**

4. **Bilirubin is conjugated to Glucuronic Acid in Hepatocytes by the enzyme ‘‘Glucoronyl transferase’’**

5. **Conjugated-bilirubin is secreted into the bile => Small intestine.**

Fate of Bilirubin in small intestine (Next slide)



- **The conjugation of Bilirubin happens intracellularly in the Smooth Endoplasmic Reticulum**
- The conjugation reaction is carried by the enzyme **(Bilirubin Glucuronyl Transferase**
- Examples of clinical conditions due to congenital deficiency this enzyme:
  - **Crigler-Najjar syndrome**
  - **Gilbert syndrome**

**FATES OF BILIRUBIN IN THE INTESTINE:

In small in testine: Bacteria removes Glucuronic acid from conjugated bilirubin.
⇒Bilirubin becomes unconjugated(free) & Bacteria convert it to **Urobilinogen**

- ✓ 70% of UBG is oxidized to Stercobilin (by intestinal bacteria) → **Excreted in Feces**
- ✓ 20% of UBG → Reabsorbed into portal blood
  => **Participate in Entero-Hepatic circulation of Urobilinogen.**
- ✓ 10% of UBG → Excreted in Urine & oxidized to Urobilin
  → Gives Urine its characteristics

Why is Bilirubin Conjugated ?

Conjugated

- Water soluble
- Loosely bound to albumin
- Filtered through renal glomeruli and excreted in urine
- Non-toxic
- Present in low concentration in the blood

Unconjugated

- Insoluble in water
- Tightly complex to albumin
- Not filtered through renal glomeruli, is not excreted in urine
- Toxic substance
- The chief form of bilirubin in the blood

Case 3

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.

What is the most likely type of elevated form of bilirubin in this case?

Conjugated & unconjugated bilirubin

In this case, why did the level of conjugated and unconjugated bilirubin elevate?

Damage of hepatocyte → either decreasing in the ability of the conjugation or the ability to secrete the bilirubin into the bile.

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

LFT	Patient's result	Normal range
Bilirubin	83	0-19 μ mol/L
AST	72	0-40 IU/L
ALT	59	0-50 IU/L
APL	175	0-120 IU/L
GGT	109	0-50 IU/L

Why does bilirubin get conjugated in the liver?

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)

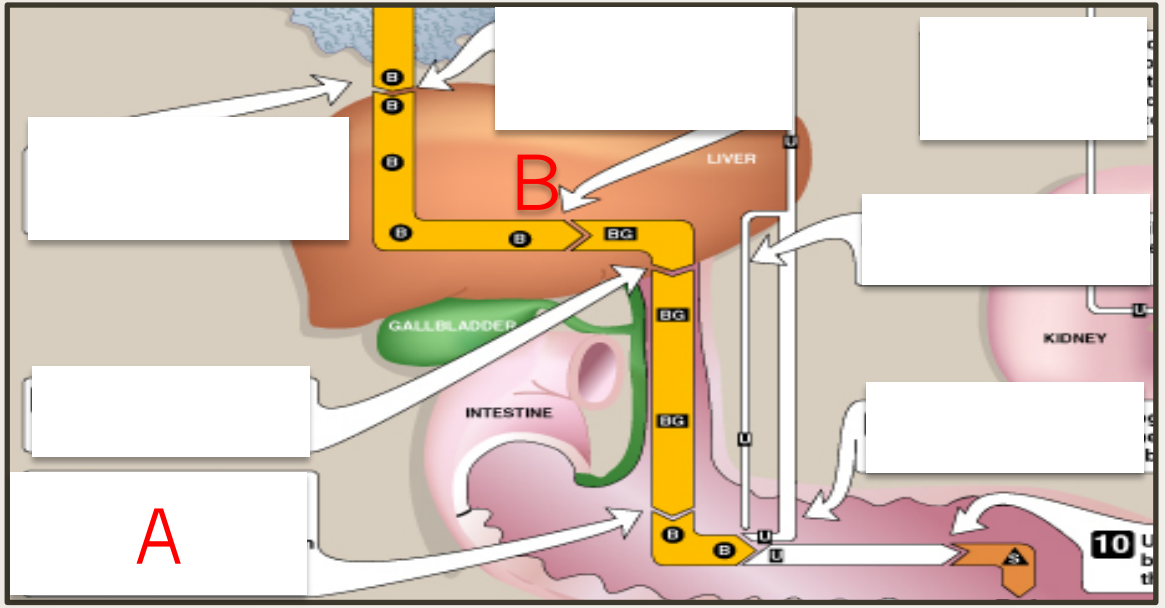
Crigler-Najjar syndrome & Gilbert syndrome

Describe the role of the intestine in excretion of the bile (A in the picture)

Bilirubin is deunconjugated by the bacteria which results in transforming bilirubin to Urobilinogen.

Bilirubin is found in what form In picture B ?

Conjugated bilirubin



Sensitivity

A Sensitive **test helps RULE OUT disease (when the result is negative).**

- Sensitivity rule out or "**Snout**"

(To understand) :

Sensitivity answers the following question:

- If a person has a disease, how often will the test be positive (true positive rate)?
- 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 RULES IN a disease with a high degree of confidence (when the result is positive).

Specificity rule in or "**Spin**"

(To understand) :

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

VERY IMPORTANT

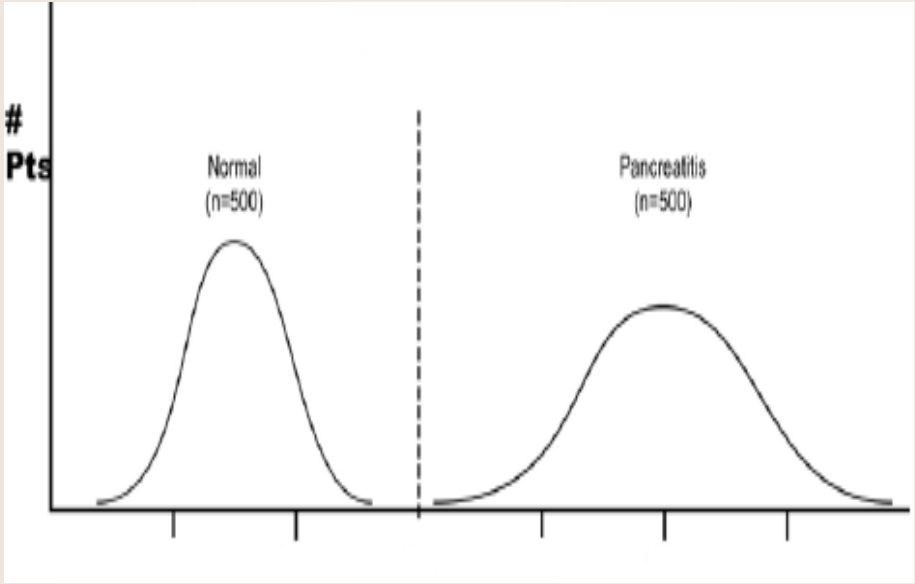
Test	Disease	
	Positive	Negative
Positive	True Positive **(TP)**	False Positive **(FP)**
Negative	False Negative **(FN)**	True Negative **(TN)**

Sensitivity = $\frac{TP}{TP+FN}$ X 100

Specificity= $\frac{TN}{TN+FP}$ X 100

An ideal diagnostic lab test results for many subjects (normal and patients)

A perfect test for acute hepatitis:



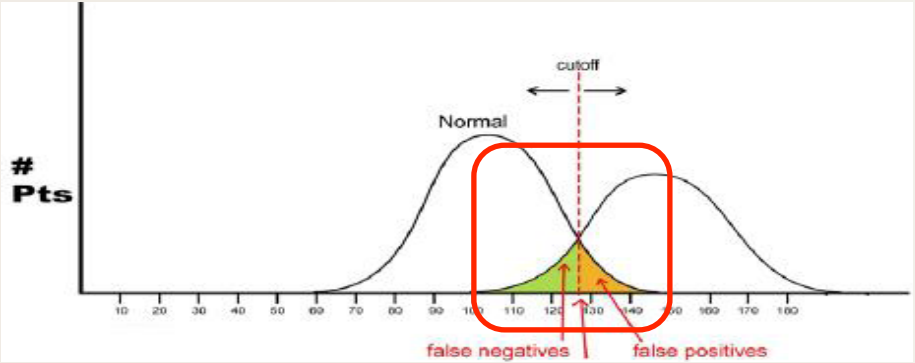
The test identifies ALL patients with disease and All subjects without disease 100% of the time.

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.

There are some normal subjects who will have a positive results (False positives)

There are some patients who will have negative results (False negatives)



Example

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:

1.The sensitivity 2.The specificity

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)

Test	Disease	
	+	-
+	TP	FP
-	FN	TN

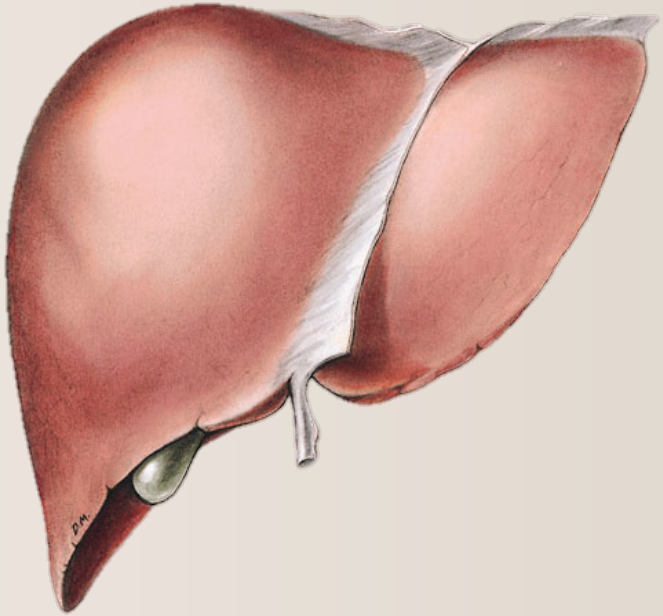
→

Test	Disease	
	+	-
+	440	50
-	60	450

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 = 440/500 = 0{,}88 \times 100 = \mathbf{88\%}$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100 = 450/500 = 0.9 \times 100 = \mathbf{90\%}$$

Good luck for you all, we hope that helped !



Special thanks for our great academic leaders

Done by:

Mohammed alnafisah

Basmah aldeghaither

Ahmed Alhussien