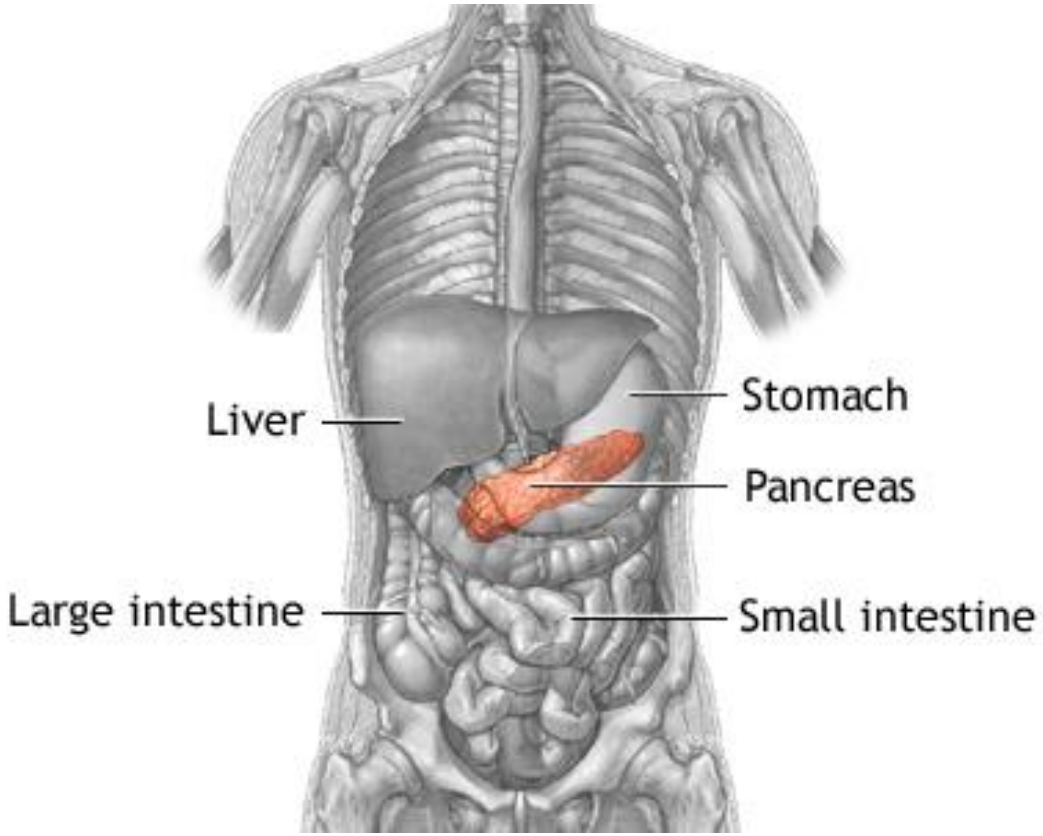
Determination of amylase in serum



Amal Alamri

- Amylase is an enzyme that helps digest carbohydrates. It is produced in the pancreas and the glands that make saliva. When the pancreas is diseased or inflamed, amylase releases into the blood.
- A test can be done to measure the level of this enzyme in a blood.
- Amylase in serum arise mainly from the pancreas (P-amylase) and the salivary gland (S-amylase). Serum P- amylase activity is a more sensitive and more specific test than total amylase for the detection of acute pancreatitis.

Why the Test is Performed

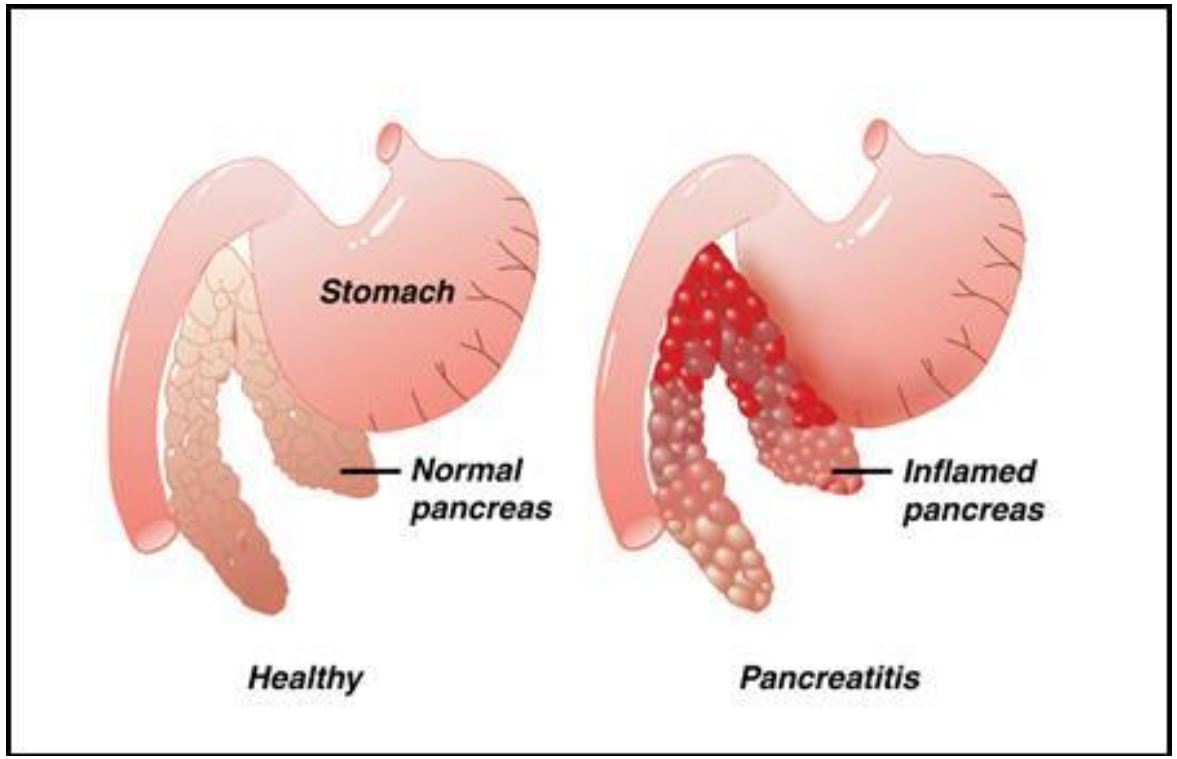
- This test is most often used to diagnose or monitor acute pancreatitis. It may also detect some digestive tract problems.

The test may be done for

- Chronic pancreatitis
- Pancreatic pseudocyst

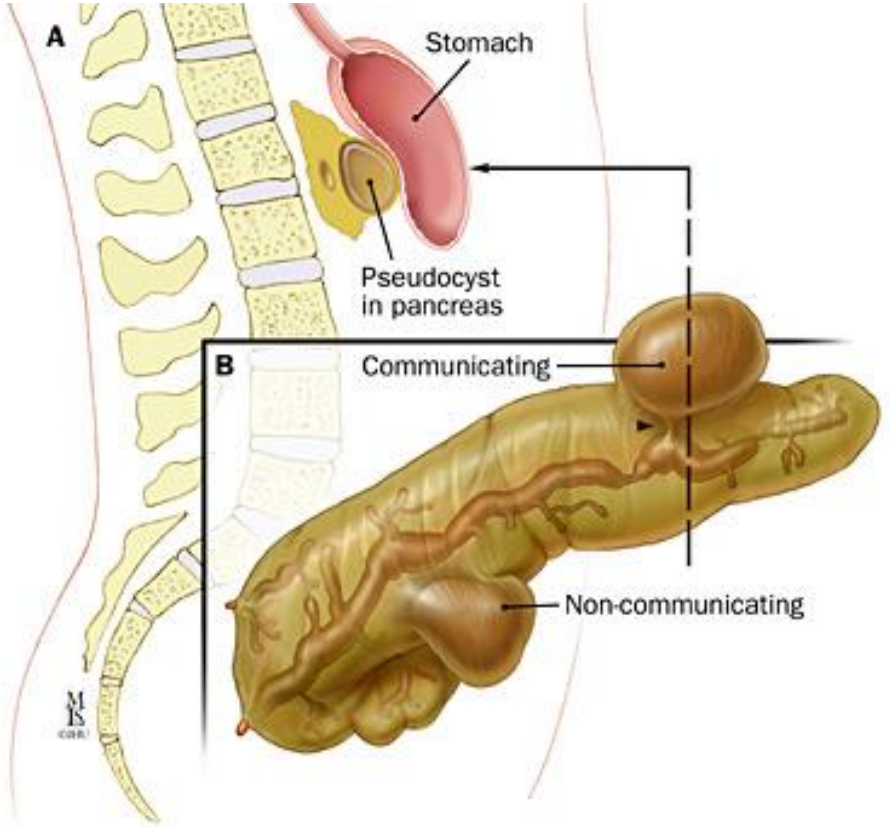
Chronic pancreatitis

Chronic pancreatitis is inflammation of the pancreas that does not heal or improve, gets worse over time, and leads to permanent damage.



Pancreatic pseudocyst

- A pancreatic pseudocyst is a fluid-filled sac in the abdomen, which may also contain tissue from the pancreas, pancreatic enzymes, and blood



RANGE OF EXPECTED VALUES

Serum : 16-108 U/L

Urine: 0 - 14 U/Hour

low values in Serum is may due liver diseases and pancreatic insufficiency

Principle:

Amylase hydrolyzed p-nitrophenyl D-maltoheptoside (PNPG7) to P-nitrophenylmaltotriose (PNPG3) and maltotetrose .

Glucoamylase hydrolyzes PNG3 to P-nitrophenylglycosie (PNPG1) and glucose .

Then PNPG1 is hydrolyzed by glycosidase to glucose and P-nitrophenol which produce a yellow color . The rate of increase in Ab is measured at 405nm and is proportional to the amylase activity in the sample.

PNPG7 —AMYLASE—> PNPG3 + Maltotetrose

PNPG3 —GLUCOAMYLASE—> PNPG1 + Glucose

PNPG1 —GLUCOSIDASE—> p-Nitrophenol + Glucose

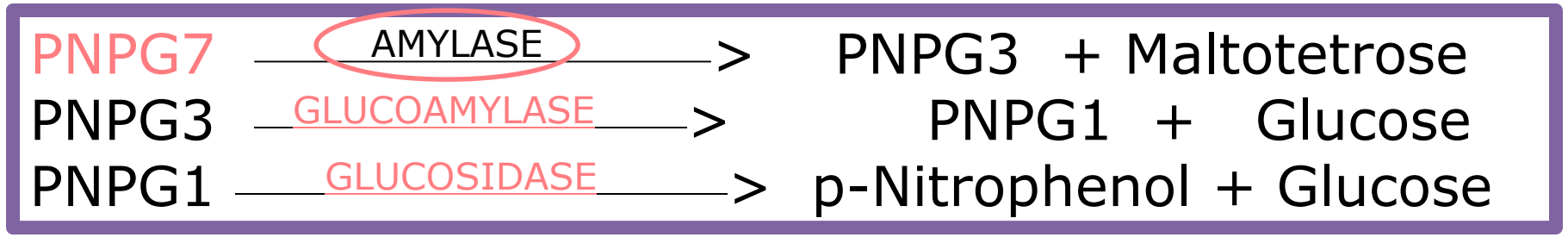
MATERIALS

CEMICALS:

- **AMYLASE SUBSTRATE (PNPG7):**

p-Nitrophenyl D-Maltoheptoside ,Glucosidase , Glucoamylase , SodiumChloride 50 mM, Calcium Chloride and Buffer , pH 6.9 ± 0.01.

- **2 SERUM SAMPLES**



MATERIALS

GLASSWARE:

1. Accurate pipetting devices.

2. Test tubes / rack

3. Timing device.

4. Heating block /bath (37 ^{o}C).

5. Spectrophotometer capable of reading at 405 nm (400-420 nm).

The cuvette compartment should be temperature controlled to maintain temperature (37 ^{o}C) during the assay.

METHOD

CHEMICALS	SAMPLE 1
AMYLASE SUBSTRATE (PNPG7):	1.0 ml
Pre-warm at 37oC for 5 minutes and add:	
Sample1	0.025 ml

1. Mix and incubate at 37oC for 90 seconds and read the absorbance at 405 nm against distilled water.

2. Continue readings every 30 seconds for 2 minutes and determine ΔA/Min.

RESULTS

ABSORBANCE AT 405	
A 0 S	
A 30 S	
A 60 S	
A 90 S	
A 120 S	

CALCULATIONS

Amylase Activity in TEST (U/L)= ΔA/Min x 4824

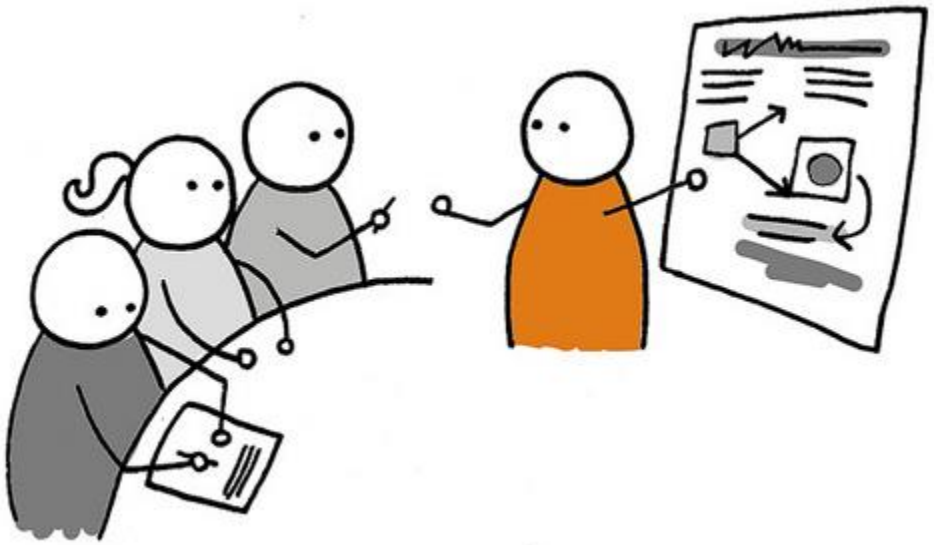
ΔA/Min = (ΔA1+ ΔA2)÷2

ΔA1= (A 60 s – A30 s)+(A30 s-A0 s)

ΔA2= (A 120 s – A90 s)+(A90 s-A60 s)

DISCUSSION

Comment on the concentration of amylase in sample.



REFRENCES

- UDI **AMYLASE (COLOR/KINETIC) KIT**
- http://www.nlm.nih.gov/medlineplus/ency/article/003464.htm
- **Lecture Notes: Clinical Biochemistry** Geoffrey Beckett, Simon W. Walker, Peter Rae
- http://heartsfortheclass.blogspot.com/2012/07/bio-202-endocrine.html