AMYLASE

Introduction:

The determination of Amylase activity in serum and urine is most commonly performed for the diagnosis of acute pancreatitis. In acute pancreatitis, Amylase levels are elevated for longer periods of time in urine than in serum. Therefore, determining the ratio of the Amylase and Creatinine clearances is important in following the course of pancreatitis.

PRINCIPLE:

The enzymatic reaction sequence employed in the assay is as follows:

CNPG3 + α - Amylase ⟶ CNP + CNPG2 + Glucose + Maltotriose

REAGENT & STABILITY:

Amylase Reagent: Ready – To – Use

Avoid Contamination of Ready-To-Use Reagent. Always use fresh pipette tips. Keep always the caps tightly closed. The above Reagent is stable uptil the expiry date mentioned on the vial label.

SPECIMEN COLLECTION & STORAGE:

1. Unhemolyzed serum is the specimen of choice.
2. Plasma from heparin tubes may be used.
3. Other anticoagulants, such as Citrate and EDTA, bind to calcium, an ion needed for Amylase activity. Therefore, plasma with any anticoagulant other than heparin should not be used.
4. Urine specimens should be adjusted to a pH of 7 and kept refrigerated until assayed.
5. Amylase in serum and urine is reported to be stable for one week a Room Temperature (18^0 -25^0 C) and for several months when stored at 2^0 - 8^0 C and protected against evaporation and bacterial contamination.
6. Do not pipette by mouth. Avoid contamination of reagent with salivary Amylase. Amylase in saliva may be present at levels 1000 times higher than serum.

PROCEDURE:

WAVE LENGTH	: 405 nm
TEMPERATURE	: 37^0 C
CUVETTE	: 10 mm path length
FACTOR	: 3178
PRE-REACTION TIME	: 60 Sec.
REACTION TIME	: 180 Sec.

Pipette into cuvettes	Macro
Reagent	1000 µl
Sample	25 µl

Mix & incubate at 37^0C for 1minute. Read the initial absorbance and start timer immediately. Read again at one minute intervals for 3 minutes at 405 nm.

Incubate the reagents tubes at 37^0C before adding sample for at least five minutes.

CALCULATIONS:

Δ Abs. / min. x 3178 = U/L of Amylase

EXPECTED VALUES Serum **:** 25 to 98 U/L
Urine : < 470 U/L

LINEARITY **:** 2000 U/L

REFERENCES:

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5. Salt WB II, Schenker S, Medicine (1976, 55: 26)

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