



Swemed DIAGNOSTICS

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:

α -Amylase uses a chromogenic substrate Gal-G2-CNP which by the reaction of \square - Amylase breaks down to release 2-chloro-4-Nitrophenol (CNP). The release of 2-chloro-4-Nitrophenol can be detected spectrophotometrically at 405 nm to measure of α -Amylase activity.

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 up to 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 at Room Temperature (18° - 25° C) and for several months when stored at 2° - 8° 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° C
FACTOR	: 1628
PRE-REACTION TIME	: 60 Sec.
REACTION TIME	: 120 Sec.
INTERVAL	: 30
No. of readings.	: 4

Pipette into cuvettes	Macro
Reagent	1000 μ l
Sample	50 \square μ l
Mix well and read the initial absorbance after 1 minute and subsequently 3 more readings with 30 seconds interval at 405nm. Calculate the mean absorbance change per minute.	

CALCULATIONS:

Δ Abs / min. x 1628 = U/L of Amylase

EXPECTED VALUES Serum: 35- 140 U/L

LINEARITY : 1200U/L

REFERENCES:

1. Ransor, JHC, Curr. Prob. Surg 1979. 16:1
2. Blair HE, U.S. Patent No.4, 649. 108
3. Genzyme data File 1995
4. Chavez RG etal, U.S. Patent 4
5. Salt WB II, Schenker S, Medicine (1976, 55: 26)