Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 14721 R1 1 x 10 ml
For 12 tests R2 1 x 2 ml

Pancreatic α-Amylase

Kinetic colorimetric, according to IFCC recommendations

Liquid Reagents

Test principle

$$5\ \text{ethyliden-G}_7\text{-PNP} + 5H_2O \xrightarrow[\alpha\text{-Amylase}]{\text{Pancreatic}} 2\ \text{ethyliden-G}_5 + 2G_2PNP + 2\text{ethyliden-G}_4 + 2G_3PNP + \text{ethyliden-G}_3 + G_4PNP$$

$$2\ G_2PNP + 2G_3PNP + G_4PNP + 14H_2O \xrightarrow{\alpha\text{-Glycosidase}} 5PNP + 14\text{Glucose}$$

Two types of α-Amylases are present in serum sample; the pancreatic type, and the salivary type. The activity of pancreatic α-Amylase is important for diagnosing acute pancreatic. Determination of pancreatic α-Amylase is based on inhibition of the activity of salivary α-Amylase by two different monoclonal antibodies, and the cleavage of ethyliden-G7-PNP (EPS) by pancreatic α-Amylase followed by hydrolysis of all degradation products to PNP by the aid of α-Glycosidase. The color intensity of the PNP formed, measured calorimetrically at 405 nm, is directly proportional to P-α-amylase activity in the sample.

Concentrations in the test

Reagent R1		
HEPES Buffer, pH = 7.15	52.5	mmol/L
Magnesium chloride	12.6	mmol/L
Sodium chloride	188	mmol/L
α-Glucosidase	≥ 3.2	KU/L
Monoclonal antibodies	≥ 79	mg/L
Preservative		
Reagent R2		
HEPES Buffer, pH = 7.15	52.5	mmol/L
Ethyliden-G7PNP (EPS)	22	mmol/L
Stabilizer, preservative, detergent		

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Mix 5 volumes of reagent R1 with 1 volume of reagent R2.
Stability: 2 weeks at 2 - 8 °C.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized plasma.
2. Anticoagulants, such as citrate and EDTA, bind calcium that is needed for amylase activity. Plasma with these anticoagulants should not be used.
3. Amylase in serum is reported to be stable for 1 week at 20 - 25°C, and 1 month at 2 - 8 °C.
4. Urine: Dilute (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.
5. Amylase in urine is reported to be stable for 2 days at 20 - 25°C, and 10 days at 2 - 8°C.
   Stability may decrease in urine samples with pH < 5.

Calibrator

MediCal U Cat .No 15011

Quality control

Meditrol N Cat .No 15171
Meditrol P Cat .No 15181

Procedure

Wavelength	Hg 405 nm
Spectrophotometer	405 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – increase

Assay : Incubate working reagent at 37 °C before use:

Sample	30 µl
Working Reagent	1000 µl
Mix, incubate at 37°C for exactly 3 min. Read change in the absorbance per 1 min for 3 min. Determine the mean absorbance change per 1 min (ΔA/min).	

Calculation

P-α-Amylase activity (U/L) = ΔA/min X Factor

Factor = 3270

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{\text{Conc.}_{\text{Clibrator}}}{\Delta/\text{min}_{\text{Calibrator}}}$$

Linearity

Up to 1000 U/L.
If the result exceeds 1000 U/L repeat the test using diluted serum (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

Interference

1. A number of drugs and substances affect the determ nation of amylase. Young et al have published a comprehensive list of such substances.
2. Macroamylase in the specimen can cause a measured hyperamylasemia that could lead to a false diagnosis of acute pancreatitis. However no clinical symptoms are usually associated with macroamylasemia.
3. Bilirubin up to 20 mg/dl and hemoglobin up to 500 mg/dl have demonstrated to have a negligible effect on this procedure.
4. Lipemic samples up to 1000 mg/dl have been reported to have no effect on serum amylase determination.
5. EDTA plasma gives approximately 10 % lower values.

Precautions

1. Avoid contamination of pipette and reagent with saliva, sweat and skin contact due to the presence of α-amylase.
2. Avoid ingestion.
3. Reagents are acidic solutions, flush with water when contact occurs.
4. Reagents contain sodium azide. Don't swallow. Avoid any contact with skin and mucous membranes. May react with lead and copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Reference range

Serum

Adults	< 53	U/L

Urine

Random Urine	< 325	U/L

References:

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6. Tietz, N. W. Textbook of clinical chemistry, Philadelphia, W.B. Saunders Company, pp. 725 - 734 (1986).
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Medichem Middle East Aleppo, Syria, E-mail-mdchemme@scs-net.org