Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 14711 R1 1 x 20 ml
For 24 tests R2 1 x 4 ml

α-Amylase

Kinetic colorimetric method, according to IFCC recommendations

Liquid Reagents

Test principle

5 ethyliden-G_7-PNP+ 5H_2O $\xrightarrow{\alpha\text{-Amylase}}$ ethyliden-G_3 + G_4PNP
+2 ethyliden-G_4 + 2G_3PNP
+2ethyliden-G_5 + G_2PNP

2 G_2PNP+2G_3 PNP+G_4PNP+14 H_2O $\xrightarrow{\alpha\text{-Glucosidase}}$ 5PNP+14Glucose

α-Amylases catalyse the hydrolytic degradation of the ligosaccharide ethyliden-G7-PNP to release G2PNP, G3PNP, and G_4PNP.
These fragments are then completely hydrolyzed to p-Nitrophenol and glucose by α-Glycosidase. The rate of increase in absorbance of PNP, measured calorimetrically at 405 nm, is proportional to total 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
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 volume 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

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

Factor = 3270

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

$$F = \frac{Conc_{calibrator}}{\Delta A / \min_{Calibrator}}$$

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 determination of amylase. Young et al have published a comprehensive list of such substances.[3]
2. Macro amylase in the specimen can cause a measured Hyper amylasemia that could lead to a false diagnosis of acute pan creatitis. However no clinical symptoms are usually associated with macro amylasemia.
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.

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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	< 100	U/L

Urine

Random Urine	< 460	U/L

References

1. Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC method for α-Amylase. Clin Chem Lab Med 1998, 36(3): 185 - 203.
2. Tietz, N. W., et. al. Abs. of Proc. Of Int'l Seminer and workshop on Enzymology, Chicago, IL (May 1972).
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
4. Tietz , N.W. chinical Guide to laboratory tests., Phladelphia , W.B.Saunders company ,p.54 (1983)
5. Tietz, N. W. Textbook of clinical chemistry, Philadelphia, W.B. Saunders Company, pp. 725 - 734 (1986).
6. Tietz , N. W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders Company, p. 54 (1983).