Cat. No. 4-576

DIAGNOSTIC KIT FOR DETERMINATION OF α-AMYLASE ACTIVITY

HC – AMYLASE EPS

INTRODUCTION

α-Amylases are hydrolytic enzymes which hydrolyze 1,4-α-glucosidic bond in starch and other similar polysaccharides to maltose and other oligosaccharides. Several types of amylases can be distinguished, depending on the organ they are originating from. α-amylase is the most commonly measured in the diagnosis of acute pancreatitis, when its activity in serum is very high. Elevation of α-amylase activity in serum is also accompanied by increased excretion of enzyme in urine which can last longer than in the blood. Because of that activity in α-amylase in urine is used as a indicator of acute pancreatitis. Hyperamylasemia occurs also in chronic pancreatitis, failures of kidneys, lungs, diseases of the salivary glands, cerebral traumas, surgical interventions and macroamylasemia. To confirm pancreatic specificity it is recommended to determine also other pancreas specific enzyme like lipase.

METHOD PRINCIPLE

Enzymatic colorimetric method, with EPS substrate, in accordance to recommendations of IFCC – International Federation of Clinical Chemistry and Laboratory Medicine (modified IFCC method).
α-Amylase catalyzes hydrolysis of substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α,D-maltoheptaoside (EPS, Ethylidene Protected Substrate). Ethylidene group prevents the substrate from breaking down because of exo-enzymes activity, therefore in absence of α-amylase no increase of absorbance is observed.
α-Amylase hydrolyses the substrate into smaller fragments which are acted upon by α-glucosidase, causing the ultimate release of chromophore p-nitrophenol (pNP) and glucose.

$$\text{4,6-ethylidene-pNP-G7 (EPS)} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{4,6-ethylidene-Gx} + \text{p-nitrophenylo-G (7-x)}$$

$$\text{p-nitrophenylo-G (7-x)} + \text{(7-x)}\ H_2O \xrightarrow{\alpha\text{-glucosidase}} \text{p-nitrophenol} + \text{(7-x) glucose}$$

Increase of absorbance related to formation of p-nitrophenol is proportional to the α-amylase activity in sample and is measured spectrophotometrically at 405 nm wavelength.

REAGENTS

Package

1-Reagent	2 x 48.5 ml
2-Reagent	2 x 12.2 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Prevent the reagents from microbiological contamination and from saliva and sweat α-amylase. Protect from direct light. The reagents must be clear, do not use if turbid.

Concentrations in the test

HEPES buffer, pH 7.2	52.5 mmol/l
sodium chloride	87 mmol/l
magnesium chloride	12.6 mmol/l
calcium chloride	0.075 mmol/l
α-glucosidase	≥ 4kU/l
4,6-ethylidene-pNP-G7 (EPS)	> 4 mmol/l
stabilizers and preservatives	

Warnings and notes

- Product for in vitro diagnostic use only.
- A slight yellow colour of 2-Reagent does not influence the reagent performance.

SPECIMEN

Serum or plasma collected on heparin, free from hemolysis, urine.
Do not use anticoagulants: EDTA, citrates and oxalates as they inhibit amylase activity.
Serum / plasma can be stored for 7 days at 20-25ºC or for one month at. 4ºC.
Urine can be stored for 2 days at 20-25ºC or for 10 days at 4-8ºC. Amylase is very unstable in acid urine. Adjust pH to alkaline range before storage.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

The reagents are ready to use.
These reagents may be used in automatic analyser Hitachi 911/912.
Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:

1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.
4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

REFERENCE VALUES [5]

serum / plasma	28 – 100 U/l	0.47 – 1.7 µkat/l
urine	≤ 460 U/l	≤ 7.7 µkat/l

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum or CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended. **Calibrator and 0.9% NaCl** should be used for calibration.
The calibration curve should be prepared every 8 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 1.0 U/l (0.017 µkat/l).
- **Linearity:** up to 2000 U/l (33.3 µkat/l).
  If amylase activity exceeds 2000 U/l, dilute the sample with 0.9% NaCl 1:10 and repeat the assay. Multiply the result by the dilution factor.

- **Specificity / Interferences**
  Haemoglobin up to 0.156 g/dl, bilirubin up to 20 mg/dl, ascorbate up to 62 mg/l, triglycerides up to 1250 mg/dl and glucose up to 2000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	67.6	0.42	0.62
level 2	380.2	2.30	0.60

Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	68.5	1.19	1.73
level 2	381.5	3.42	0.90

- **Method comparison**

A comparison between amylase values determined at Hitachi 912 (y) and at Advia 1650 (x) using 125 samples gave following results:
y = 1.011 x - 2.8995 U/l;
R = 0.9998 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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5. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 100-104, (2006).
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MANUFACTURER

PZ CORMAY S.A.
22 Wiosenna Street,
05-092 Łomianki, POLAND
tel.: +48 (0) 22 751 79 10
fax: +48 (0) 22 751 79 14
http://www.cormay.pl

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