

AMYLASE FL

AM F120 CH	12 x 10 ml
AM F245 CH	12 x 20 ml

SUMMARY OF TEST

Assays of amylase activity in serum and urine are largely of use in the diagnosis of diseases of the pancreas and in the investigation of pancreatic function. In acute pancreatitis, a transient rise in serum amylase activity occurs within 2 to 12 h of the onset; levels return to normal by the third or fourth day. A four- to six-fold elevation in amylase activity above the reference limit is usual, with maximal levels attained in 12 to 72 h. In acute pancreatitis associated with hyperlipemia, serum amylase activity may be normal; the spuriously normal amylasemia may be unmasked either by serial dilution of the serum. A significant amount of the serum amylase is excreted in the urine, and therefore elevation of serum activity is reflected in the rise of urinary amylase activity. Urine amylase, as compared with serum amylase, appears to be more frequently elevated, reaches higher levels, and persists for longer periods. The urinary clearance of amylase is markedly increased in acute pancreatitis; in quiescent chronic pancreatitis, both serum and urine activities are usually subnormal. In some important groups of nonpancreatic disorders, reasons for hyperamylasemia are known. Although about 25% of the serum amylase is normally eliminated in the urine, in renal insufficiency the serum amylase activity is increased up to two-fold of the upper reference limit and in proportion to the extent of renal impairment. Neoplastic hyperamylasemia is an increasingly recognized entity for which clinical chemists must be constantly alert. Additionally, tumors of the lung and serous tumors of the ovary can produce hyperamylasemia with elevations as high as 50 times the upper reference limit. Both kinds of tumor can produce pleural effusion. The amylase activities in these effusions can be greater than 200 times the upper reference limit for serum. Salivary gland lesions caused by infection, irradiation, obstruction, surgery, and tumor have all been reported as producing a significant S-type hyperamylasemia. Mumps (infective parotitis) and maxillofacial surgery can cause a two-fold elevation, and salivary gland irradiation can produce a transient 9- to 18-fold elevation of serum amylase activity. Biliary tract diseases such as cholecystitis can cause up to four-fold elevations of the serum amylase activity as a result of either primary or secondary pancreatic involvement. It has been estimated that some 200 methods for the assay of amylase have been described. The present method is based on a chromogenic substrate (2-chloro-4-nitrophenyl- α -D-maltotriose) and it is very simple. It does not need ancillary enzymes, it is fast and could be easily adapted to almost all analyzers.

PRINCIPLE OF THE METHOD

The enzyme α -amylase (EC 3.2.1.1, 1,4 α -D-glucose glucanohydrolase) hydrolyzes the 2-chloro-4-nitrophenyl- α -D-maltotriose (CNP3) to release 2-chloro-4-nitrophenol and form 2-chloro-4-nitrophenyl- α -D-maltoside (CNP2), maltotriose (G3) and glucose (G). The rate of formation of the 2-chloro-4-nitrophenol can be detected spectrophotometrically at 405 nm to give a measurement of α -amylase activity in the sample.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

DO NOT PIPETTE BY MOUTH!

Reagent A **F120: 12 x 10 ml (liquid) blue cap**
F245: 12 x 20 ml (liquid) blue cap

Composition: CNP-G3 2.3 mM, NaCl 350 mM, calcium acetate 6 mM, potassium thiocyanate 600 mM, Good's buffer pH 6.0 100 mM, stabilizers and non-reactive components.

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Use reagent ready to use.

Stability: up to expiration date on labels at 2-8°C.

Stability since first opening of vials: \geq 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma (heparinate only).

Amylase is stable in serum and plasma sample up to 2 months at 2-8°C.

TEST PROCEDURE

Wavelength:	405 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	25 μ l
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 3 readings at 60 seconds intervals. Calculate the $\Delta A/min$.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the $\Delta A/min$ by the factor as it is indicated.

Calculation in U/l: $\Delta A/min \times 3178$

Calculation in μ kat/l: $U/l \times 0.0167 = \mu$ kat/l

EXPECTED VALUES

Serum - plasma: < 96 U/L ($< 1.60 \mu$ kat/l)
Urine: < 480 U/L ($< 8.00 \mu$ kat/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH **QUANTINORM CHEMA** **10 x 5 ml**
with normal or close to normal control values

QP 0050 CH **QUANTIPATH CHEMA** **10 x 5 ml**
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH **AUTOCAL H** **10 x 3 ml**

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 3000 U/l.

If a $\Delta A/min$ of 0.500 is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 3 U/l.

Interferences

no interference was observed by the presence of:

hemoglobin \leq 500 mg/dl
bilirubin \leq 50 mg/dl
lipids \leq 1000 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	71.30	1.34	1.90
sample 2	190.80	1.55	0.80

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	74.62	1.72	2.30
sample 2	190.51	3.42	1.80

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Amylase Chema = x
Amylase competitor = y
n = 107

y = 0.88x + 8.39 r = 0.987

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.








REFERENCES

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MANUFACTURER

Chema Diagnostica
Via Padre Vincenzo Pellegrini 3
60035 Jesi (AN) - ITALY - EU
phone +39 0731 213360
fax +39 0731 213361
e-mail: mail@chema.com
website: http://www.chema.com

SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions