Amilokit

For amylase determination in serum or urine

SUMMARY

Amylase, produced in the exocrine pancreas, and the salivary glands, splits the α 1-4 glycosidic bonds of polysaccharides (starch and glycogen).

Serum amylase is increased in patients with acute pancreatitis reaching its highest values between 24 to 30 hours after onset and returning to normal levels during the 24 to 48 following hours. In this case urinary output of the enzyme is also increased, and hyperamylasuria lasts 3 to 5 days, after serum activity has reached normal levels.

It is also possible to find increased values in any case of acute abdome or surgical operations on sites close to the pancreas. Both bacterial parotiditis and mumps, are also related to increases in serum amylase levels.

PRINCIPLE

Buffered starch substrate is incubated with sample thus producing enzymatic hydrolysis. This is stopped by addition of iodine reagent which, at the same time, develops color with the remaining non-hydrolyzed starch. Decrease of color compared with a control substrate (without sample) is the measure of enzymatic activity. Results are expressed in Amylolytic Units (Smith & Roe)/dl (AU/dl) comparable to Sac-charogenic Units (Somogyi)/dl.

PROVIDED REAGENTS

A. Reagent A: 500 mg/l starch solution buffered at pH 7.0 with 0.1 mol/l phosphate buffer in 0.15 mol/l NaCl.
B. Reagent B: 0.01 eq/l iodine solution in 0.02 mol/l HCl.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.
Before using the **Reagent A**, gently shake for a few seconds to homogenize any eventual starch deposit in the bottom of the vial.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at room temperature (< 25ºC) until expiration date shown on the box. Once opened, stable one year in refrigerator (2-10ºC).

INSTABILITY OR DETERIORATION OF REAGENTS

Control readings must not show variations greater than 10%. Any sudden drop in such values is a sign of substrate contamination with saliva.

SAMPLE

Serum or urine
a) Collection: if serum is used, collect in the usual way.
Urine should be collected as it indicates below: patient must empty his bladder and discard this urine. Bladder must be emptied again after two hours and all this urine must be collected. This 2-hour diuresis sample should be diluted to 200 ml with water. Perform the test using 20 ul of this dilution, thus the results are expressed in Amylolytic Units/hour. Due to great diuresis variations in acute cases compatible with a pancreatitis diagnosis, amylasuria detection on simple urine samples is not effective, thus hourly urine output should be tested.
b) Additives: not required.
c) Known interfering substances: enzymatic activity is inhibited by citrates and oxalates. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: if assay cannot be immediately performed, samples may be kept up to 1 week in refrigerator (2-10ºC) without loss of activity.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 640 nm in spectrophotometer or in photocolorimeter with red filter (610-660 nm).
- Temperature: 37ºC
- Reaction time: 7.1/2 minutes
- Sample volume: 20 ul
- Final reaction volume: 10 ml

PROCEDURE

In two test tubes labeled C (Control), and U (Unknown) place:

	C	U
Reagent A	1 ml	1 ml

Place tubes in water bath at 37°C for some minutes. Then add:		
Sample	-	20 ul
Incubate tubes at 37°C. After exactly 7.1/2 minutes add:		
Reagent B	1 ml	1 ml
Mix by gently shaking. Remove tubes from water bath. Add:		
Distilled water	8 ml	8 ml
Mix by inversion. Read tubes in colorimeter with red filter or in spectrophotometer at 640 nm, setting instrument to zero O.D. with distilled water.		

STABILITY OF FINAL REACTION

Final reaction color is stable 60 minutes, therefore absorbance should be read within this period. Whether room temperature is above 25°C, readings should be performed within first 20 minutes.

CALCULATIONS

$$\text{Amylase (UA/dl)} = \frac{C - U}{C} \times 1000$$

Units: an Amylolytic Unit (AU) is the amount of enzyme contained in 100 ml sample, which can hydrolyze 10 mg starch in 30 minutes under assay conditions described. In this procedure, 20 ul sample are incubated with 0.5 mg starch, contained in 1 ml Substrate 1 during 7 and a half minutes, equivalent to incubating 100 ml serum with 10000 mg starch during 30 minutes. If all the starch were hydrolyzed, amylase activity of the sample would be 1000 AU/dl. To get amylase activity units the fraction of starch splitted should be multiplied by 1,000.

REFERENCE VALUES

Clinical condition	Serum (AU/dl)	Urine (AU/hour)
Normal	< 120	< 260
Acute pancreatitis	300 to 12,000	over 900
Chronic pancreatitis	up to 200	over 300
Parotiditis	200 to 350	350 a 750
Parotiditis with pancreatic involvement	over 350	over 750
Acute abdominal pain (without pancreatitis)	normal	normal

It is recommended that each laboratory establishes its own reference values.

PROCEDURE LIMITATIONS

Saliva contamination should be avoided since salivary amylase activity is 700 times higher than that of serum. Substrate should be measured with 5 ml pipette or 1 ml pipette with double calibration mark. Pipettes should not be blown even slightest contamination with saliva definitely damages substrate.

Color developed in **Amilokit** reaction is not blue due to iodine excess: this excess and HCl of Iodine Reagent B are needed to ensure complete inhibition of enzyme reaction and color stability, as well as to avoid protein interference in the color reaction.

PERFORMANCE

a) Dynamic range: if amylase activity is higher than 600 AU/dl repeat assay diluting sample 1/10 with distilled water or saline.

b) Detection limit: depends on the photometer used. In spectrophotometer at 640 nm (with 1 cm optical length square cuvettes, $\pm$ 2 nm reproducibility, stray light $\leq$ 0.5% pathlength $\leq$ 8 nm), minimal detectable change of activity is 2 AU/dl for 0.001 O.D.

c) Reproducibility: when replicates of the same sample were assayed on the same day the following results were obtained:

Serum

Level	S.D.	C.V.
85 AU/dl	$\pm$ 1.1 AU/dl	1.2 %
240 AU/dl	$\pm$ 2.5 AU/dl	1.1 %

Urine

Level	S.D.	C.V.
160 AU/dl	$\pm$ 0.9 AU/dl	0.6 %
930 AU/dl	$\pm$ 3.2 AU/dl	0.3 %

WIENER LAB PROVIDES

- 25-40 tests (Cat. Nº 1021001).

REFERENCES

- Huggins, C. & Russell, P.S. - Ann. Surg. 128:668 (1948).
- Sachar, L.E. - Am. J. Clin. Path. 22:117 (1952).
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- Van Loon, E.J.; Likins, M.R. & Seger, A.J. - Am. J. Clin. Path. 22:1134 (1952).
- Tietz, N. - Fundamentals of Clinical Chemistry - W.B. Saunders Co - (1970).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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