Amylase (Powder)

1.0 INTENDED USE

This reagent is intended for the quantitative determination of a-Amylase activity in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

The classic method of determining a-Amylase activity is enzymatic hydrolysis of a starch substrate followed by product analysis. The saccharogenic analysis method (10.2,10.3) measures the quantity of reducing sugars formed. There are difficulties associated with this method such as inconsistent results due to variations in substrate preparation and treatment.

Other methods of analysis include the dye-starch substrate methods (10.4) and the amyloclastics methods (10.5). Each has drawbacks (10.6,10.7). Modified saccharogenic methods have been introduced recently which utilize a defined oligosaccharide substrate. These substrates produce colorimetric products when coupled with p-nitrophenyl. Wallenfels et al (10.11) introduced p-nitrophenylglycosides as defined substrates for a-amylase determination in a procedure that eliminates interference from endogenous glucose and pyruvate.

2.2 TEST PRINCIPLE

The present procedure is based on modifications of Wallenfels, using as substrate a silyl-blocked p-nitrophenyl-a-D-malthoheptaoside to reduce spontaneous degradation of the substrate by a-glucosidase and glucoamylase. The test is performed in a kinetic mode with a very short lag time and offers much greater stability that previous amylase methodologies.

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          a-amylase
PNPG7  --------------------> PNPG3 + maltotetraose
          glucoamylase
PNPG3  --------------------> PNPG1 + glucose
          a-glucosidase
PNPG1  --------------------> p-nitrophenol + glucose
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a-Amylase hydrolyzes p-nitrophenyl-a-D-malthoheptaoside (PNPG7) to p-nitrophenylmaltotriose (PNPG3) and maltotetraose. Glucoamylase hydrolyzes PNPG3 to p-nitrophenylglycose (PNPG1) and glucose. Then PNPG1 is hydrolyzed by a-glucosidase to glucose and p-nitrophenol, which produces a yellow color. The rate of increase in absorbance is measured at 405nm and is proportional to the a-amylase activity in the sample.

2.3 CLINICAL SIGNIFICANCE

Assays for the a-Amylase activity are of interest for the evaluation of pancreatic function of the diagnosis of pancreatic disease. The greatest elevation in serum a-Amylase activity is seen in acute pancreatitis and obstruction of pancreatic duct (10.1).

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. It is known that many of the common anticoagulants inhibit a-Amylase activity (10.1). Therefore a-Amylase assays should be performed on serum samples.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-50 µl. Call Biotron's technical service department at 1-800-595-8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Amylase in serum is reported stable for one week at room temperature (18-26°C) and for two months when stored refrigerated at 2-8°C. (10.1)

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS (10 X 5 ml)
(6.X50 ml)

Reagents necessary for the determination of a-Amylase are included in the kit.

4.1 REAGENT

a-Amylase reagent contains, after reconstitution with deionized water:

p-nitrophenyl-a-D-malthohexaoside	0.7 mM
a-glucosidase (microbial)	≥ 20,000 U/L
glucoamylase (microbial)	≥ 10,000 U/L
sodium chloride	50 mM
calcium chloride	5 mM
buffer pH 6.7 ± 0.1	
sodium azide as preservative	0.01%

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact. This reagent contains sodium azide (0.01%) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

4.3 REAGENT PREPARATION

4.3.1

Each reagent vial is reconstituted with 10ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.2

Each reagent vial is reconstituted with 50ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label. The working reagent is stable for 10 days at room temperature (18°-26°C) or 60 days when refrigerated (2°-8°C.)

Do not use if the absorbance of the reagent is greater than 0.600 when measured at 405nm against water in a cuvette with a 1cm path length or if the reagent fails to meet stated parameters of performance.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 A spectrophotometer or colorimeter capable of reading absorbance accurately at 405 nm.
4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.
4.5.3 Test tubes capable of holding 2 ml.
4.5.4 Pipettes capable of delivering 1 ml and 20 µl.
4.5.5 Timer with 30 second increments.
4.5.6 Constant temperature heat source which can be adjusted to 37° C.
4.5.7 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	405 nm
Temperature	37° C
Pathlength	1 cm
Mode	Kinetic
Reaction Time	2 min.
Sample Volume	20 µl
Reagent volume	1.0 ml
Total Volume	1.20 ml
Sample to reagent ratio	1/50

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

No reagent calibration is necessary as this method is standardized by means of the molar absorptivity of p-nitrophenyl taken as 8.5 at 405nm under the test conditions described.

5.4 PROCEDURE

5.4.1 Prepare the required volume of working reagent (see 4.3 Reagent Preparation Section.)
5.4.2 Pipette 1.0ml of reagent into tubes labeled "control", "patient", etc. DO NOT PIPETTE BY MOUTH.
5.4.3 Pre-incubate all tubes at 37°C for at least five minutes.
5.4.4 Zero spectrophotometer with water at 405nm.
5.4.5 Add 0.020ml (20µl) of sample and read after 30 seconds.
5.4.6 Record the absorbance at 30 second intervals for 2 minutes.

5.5 CALCULATION AND RESULTS

5.1.1 Amylase U/L =

$$\frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{8.5 \times \text{light path (cm)} \times \text{sample volume (ml)} \times 1.6} = \Delta A/\text{min} \times 3750$$

ΔA/min = change in absorbance per minute
assay volume = 1.2 (ml)
1000 = converts U/ml to U/L
8.5 = absorbance coefficient of p-nitrophenyl at 405 nm
lightpath = 1 (cm)
sample volume = 0.02 (ml)
1.6 = conversion factor from 37 to 25°C
3750 = factor derived from constants in the equation
Example:

$$\text{Amylase U/ L} = \frac{0.019 \times 1.02 \times 1000}{8.5 \times 1 \times 0.02} = 0.019 \times 3750 = 71 \text{ U/L}$$

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES

The range of expected values is:

0 - 93 U/L (37 degrees C)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 LINEARITY

This procedure is linear through 2500 U/L beyond which the specimen should be diluted with an equal volume of saline. Reassay the specimen and multiply the results by 2.

6.3 MEDICAL ALERT VALUES (10.10)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.4 LIMITATIONS OF PROCEDURE

Young (10.8) gives a list of drugs and other substances that interfere with the determination of amylase activity.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.
A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

No routine reagent calibration is necessary as this method is standardized by means of the molar absorptivity of p-nitrophenyl taken as 8.5 at 405nm under the test conditions described.
The results obtained when measuring the activity of a kinetic reaction are based on the change in absorbance per minute. In order to accurately monitor and report this reaction rate, the operating parameters of the spectrophotometer (wavelength, temperature of the reaction and timing of the test) must be known and controlled.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run		
Mean (U/L)	SD (U/L)	CV (%)
48	± 0.6	1.3
492	± 2.6	0.5

Between-Run		
Mean (U/L)	SD (U/L)	CV (%)
51	± 2.0	3.9
489	± 7.9	1.6

9.2 CORRELATION

A correlation study was done comparing this method (y) and a similar comparative method (x). The samples ranged from 15 to 771 U/L. The study yielded a regression curve of y = 0.98 x -0.5 with a correlation of 0.999.

9.3 LINEARITY

Linearity: This procedure is linear through 2500 U/L beyond which the specimen should be diluted with an equal volume of saline. Reassay the specimen and multiply the results by 2.

9.4 SENSITIVITY

A change in absorbance of 0.001 ΔA/min at 405nm at 37° C corresponds to 6.0 U/L.

10.0 REFERENCES

10.1 Tietz, N.W., (Editor) Fundamentals of Clinical Chemistry, W.B. Saunders Company, Philadelphia (1982).
10.2 Somogyi, M., J. Biol. Chem., 125:399 (1938).
10.3 Henry, R.J., Chiamori, N., Clin, Chem., 6:434 (1960).
10.4 Klein, B., Foreman, J., Searry, R., Clin. Chem., 16:32 (1970).
10.5 Somogyl, M., Clin. Chem., 6:23 (1960).
10.6 Kaufman, R.A., Tietz, N.W., Clin. Chem., 26:846 (1980).
10.7 Rauscher, E., Neumann, U., Schaich, E., von Bulow, S., Wahlefeld, A.W., Clin. Chem., 31:14 (1985).
10.8 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
10.9 Tietz, N.E., (Editor) Clinical Guide to Laboratory Tests, W.B. Saunders Company, Philadelphia (1983) p. 54.
10.10 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704
10.11 Wallenfels, K., et al, Carbohydrate Research 61:359 (1978).