CONCEPT DIAGNOSTICS
ONTARIO, CALIFORNIA 91761 USA

CRESTLINE SCIENTIFIC CORPORATION
2902 Finlandia cor Copernico Streets, San Isidro Village, Makati City
Telefax No.: 887-5777 / 887-2150

AMYLASE REAGENT SET (KINETIC METHOD)

INTENDED USE
For the quantitative kinetic determination of amylase activity, using manual or automated procedures, in serum and urine.

METHOD HISTORY
Amylase was first measured quantitatively by an iodometric method introduced by Wohlegemuth in 1908.[1] Somogyi introduced a procedure in 1938 which standardized the amounts of starch and iodine.[2] His work became the basis for the widely-used Amyloclastic and Saccharogenic methods introduced in 1956[3] and 1960[4] respectively. Disadvantages of these methods include long incubation times, endogenous glucose interference, and unstable reaction colors resulting in poor reproducibility and reliability.

Several procedures have been suggested[5,6,7,8], including one which uses the defined substrate maltotetraose.[9] This methods represent significant improvement in amylase measurement but are still subject to relatively long preincubation times, possible endogenous glucose interference, and a series of other potential interferences with the formation of NADH.[10]

Wallenfels et a1[11] introduced p-Nitrophenylglycosides as defined substrates for amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. The present procedure is based on modifications of Wallenfels, using as substrate p-Nitrophenyl-D-Maltoheptaoside (PNPG7) with the terminal glucose blocked to reduce spontaneous degradation of the substrate by glucosidase and glucoamylase.[12] The test is performed in a kinetic mode with a very short lag time and offers much greater stability than previous amylase methodologies.

PRINCIPLE

$$\text{PNPG7} \xrightarrow{\text{amylase}} \text{PNPG3} + \text{Maltotetraose}$$

$$\text{PNPG3} \xrightarrow{\text{glucoamylase}} \text{PNPG1} + \text{Glucose}$$

$$\text{PNPG1} \xrightarrow{\text{glucosidase}} \text{p-Nitrophenol} + \text{Glucose}$$

Amylase hydrolyzed p-Nitrophenyl D-maltoheptaoside (PNPG7) to p-Nitrophenylmaltotriose (PNPG3) and maltotetraose. Glucoamylase hydrolyzes PNPG3 to p-Nitrophenylglycoside (PNPG1) and glucose. Then PNPG1 is hydrolyzed by glucosidase to glucose and p-Nitrophenol, which produces a yellow color. The rate of increase in absorbance is measured at 405 NM and is proportional to the amylase activity in the sample.

CLINICAL SIGNIFICANCE
The determination of amylase activity in serum and urine is most commonly performed for the diagnosis of acute pancreatitis. In acute pancreatitis, amylase levels are elevated for longer periods of time in urine than in serum. Therefore, determining the ratio of the amylase and creatinine clearances is important in following the course of the pancreatitis.[13]

REAGENTS (MATERIALS PROVIDED)
(Concentrations refer to reconstituted reagents): p-Nitrophenyl D-Maltoheptaoside 0.9 mM, Glucosidase (yeast) 25,000 IU/L, Glucoamylase (Rhizopus Sp.) 10,000 IU/L , Sodium Chloride 50 mM, Calcium Chloride 5 mM, Buffer 50 mM, pH 6.9 ± 0.1.

PRECAUTIONS
1. Reagents are intended for In Vitro diagnostic use.
2. Exercise universal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION
Dissolve contents of each vial in volume of deionized water indicated on vial label. Avoid pipetting by mouth to prevent contamination of the reagent by salivary amylase. After the addition of water, stopper vial and immediately mix several times by inversion. DO NOT SHAKE.

REAGENT STORAGE
1. Store dry reagent refrigerated (2 - 8°C). Reagent is stable until the expiration date shown on the label.
2. Reconstituted reagent is stable for at least ten days at room temperature (18 - 25°) and at least 30 days refrigerated (2 - 8°C).

REAGENT DETERIORATION
1. Discard vial if the absorbance of the reconstituted reagent is greater than (0.70) when measured at 405 nm against water in a cuvette with a 1 cm path length.
2. Discard vial if the dry reagent exhibits caking due to possible moisture penetration, does not dissolve completely upon reconstitution or if the solution appears turbid.

SPECIMEN COLLECTION AND STORAGE
1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T.
2. After blood collection, promptly separate serum from clot. This unhemolyzed serum is the ideal specimen, but plasma from Heparin tubes may be used.
3. Other anticoagulants, such as Citrate and EDTA, bind calcium, an ion needed for amylase activity. Therefore, plasma with any anticoagulant other than Heparin should not be used.[13]
4. Urine specimens should be collected for 24 hours period, adjusted to a pH of 7 with either 0.1N NaOH or 0.1N HCl and kept refrigerated until assayed. There is no need to add preservatives to the urine samples.[7]
5. Amylase in serum and urine is reported stable for one week at room temperature (18 - 25°C) and for several months when stored refrigerated at 2 - 8°C and protected against evaporation and bacterial contamination.[14]

INTERFERENCES
1. Macroamylasemia increases the activity of pancreatic amylase in serum.[9,20]
2. A number of diseases affect the determination of amylase.[16]
3. Young et al.[15] have listed certain drugs and other substances which are known to interfere with amylase activity.
4. Lipemia, Hemoglobin, and Lipid will increase amylase values.[7]
5. Insulin and some bacteria will also increase amylase activity.[16]

MATERIALS PROVIDED

Amylase reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices.
2. Test tubes/rack.
3. Timing device.
4. Heating block/bath (37°C).
5. Spectrophotometer capable of reading at 405 NM (400-420 NM). The cuvette compartment should be temperature controlled to maintain temperature (37°C) during the assay.
6. Serum controls.

PROCEDURE (AUTOMATED)

Application procedures for the determination of amylase activity using Amylase reagent are available for various automatic instrument.

PROCEDURE (MANUAL)

1. Reconstitute reagent according to instructions.
2. Pipette 1.0 ml of reagent into tubes labeled "Control", "Patient" etc. DO NOT PIPETTE BY MOUTH.
3. Pre-warm all tubes at 37ºC for at least 3 minutes.
4. Zero spectrophotometer with water at 405 nm. Add 0.025ml (25 µl) of sample and read after 15 seconds.
6. Continue reading every 30 seconds for 2 minutes.
7. Determine the mean absorbance difference per minute (ΔAbs./min.).
8. Multiply the ΔAbs./min. by 4824 to obtain result in IU/L.

CALIBRATION

The procedure should be standardized by means of the millimolar absorptivity of Nitrophenol which is 8.5 at 405 nm under the test conditions described for each new lot.

QUALITY CONTROL

The validity of the reaction should be monitored by use of control serum with known normal and abnormal amylase values for each assay.

CALCULATIONS

$$\frac{\Delta\text{Abs./min. x T.V. x 1000}}{\text{M.M.A. x S.V. x L.P.}} = \text{IU/L amylase in sample}$$

WHERE:

ΔAbs./min.	=	Absorbance difference per minute
T.V.	=	Total assay volume (1.025 ml)
1000	=	Conversion of IU/ml to IU/L
MMA	=	Millimolar absorptivity of p-Nitrophenol (8.5)
S.V.	=	Sample volume (0.025 ml)
L.P.	=	Light path (1 cm)

$$\frac{\Delta\text{Abs./min. x l.025 x 1000}}{\text{8.5 x 0.025 x 1.0}} = {}_{\Delta}\text{Abs./min. x 4824}$$

$$= \text{IU/L amylase}$$

Example: If ΔAbs./min. = 0.03 then 0.03 x 4824 = 146 IU/L

SI Units

To convert to SI Units (nkat/L) multiply the IU/L value by 16.67

LIMITATIONS

1. Samples that exceeded the linearity limit (1,500 IU/L) should be diluted with an equal volume of saline and rerun. Multiply the result by two.
2. Misleading increases in the activities of amylase and pancreatic amylase in the serum of a patient with Macroamylasemia.

EXPECTED VALUES [21, 22]

Serum: up to 96 IU/L Urine: 18 to 330 IU/L

It is recommended that each laboratory establish its own expected range.

PERFORMANCE

1. Linearity: 1,500 IU/L
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 5 IU/L.
3. Comparison: A comparison study was performed between this method and another manufacturer's with the same methodology in urine and serum. The results are indicated below. Urine samples of 70 range from 60 IU/L to 1000 IU/L and serum samples of 71 range from 30 IU/L to 900 IU/L.

Sample	Correlation Coefficient	Sample Size	Regression Equation
Serum	0.99	71	Y= 0.97X + 0.78
Urine	0.99	70	Y= 0.96X + 1.99

4. Precision Studies: done according to NCCLS guidelines

Within Run (N= 21)

Mean	SD	C.V. %
60.6	2.4	4.0
414	8.9	2.1

Run to Run (N=20)

Mean	SD	C.V. %
57.2	1.1	2.0
409	6.2	1.5

REFERENCES

1. Wohlegemuth, *J. Bio Chem.*, 29:1 (1908).
2. Somogyi, M., *J. Bio. Chem*., 125:399 (1938).
3. Street, H.V., Close J.R., *Clin. Chem. Acta,* 1:256 (1956).
4. Henry, R.J., Chiamori, N., *Clin. Chem*., 6:434 (1960).
5. Rinderknecht, H.P., et al., *Experentia,* 23:805 (1967).
6. Zinteerhofer, L., et al., *Clin. Chem. Acta,* 43:5 (1973).
7. Tietz, N.W., et al., *Abs. of Proc. of Int'l Seminar and Workshop on Enzymology*, Chicago, I11. (May 1972).
8. Schiwara, H.W., *Artzl. Lab*, 17:430 (1971).
9. Pierre, K.J., et al., *Clin. Chem*., 22:1219 (1976).
10. Kaufman, R.A., Tietz, N.W., *Clin. Chem*., 26/7:851 (1980)
11. Wallenfels, K., et al., *Carbohydrate Research,* 61:359 (1978).
12. Blair, H.E., *U.S. Patent Pending*
13. Tietz, N.W., *Textbook of Clinical. Chemistry*., Philadelphia, W.B. Saunders Company, pp. 725-734 (1986).
14. Demetriou, J., et al., *Clinical Chemistry*: Principles and Technics, 2nd Ed., (Henry, R.J., et al eds.), Hagerstown (MD) Harper & Row (1974).
15. Young, D.S., *Effects of Drugs on clinical Laboratory Tests*, 3rd Ed. 3-34 AACC Press, Washington D.C. (1990).
16. Friedman, R.B., *Effects of Disease on clinical Laboratory Tests*, 2nd Ed. 3-28. AACC Press, Washington D.C. (1989).
17. Young, D.S., et al., *Clin. Chem*., 21:ID (1975).
18. Henry, R.J., et al., *Clinical Chemistry*: Principles and Technics 2nd Ed., Hagerstown (MD), Harper & Row, Ch. 13 (1974).
19. Baeber, E.T., et al., *Clin. Chem*., 36:1124 (1990).
20. Cobbaert, C. *Clin. Chem*., 37:2159 (1991).
21. Davidson, et al., Evaluation of Pancreatic Amylase. EPS Method for Use with Urine Samples, *Clin. Chem*. 38:976-977 (1992).
22. Junge, et al., *Clin Biochem*., 22:109 (1989).

Date revised: 8/94