

CONCEPT DIAGNOSTICS

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AMYLASE (COLORIMETRIC METHOD)

AMYLASE REAGENT SET

For quantitative determination of amylase in serum.

INTRODUCTION

Most colorimetric method used for assaying the activity of α -amylase are based on its breakdown of starch. This reaction primarily yields intermediate dextrans and maltose. The rate of the reaction is usually monitored by a) turbidimetric b) iodometric c) reductometric methods.¹ The iodometric method as later modified by Caraway is still considered to be one of the reference method for the amylase determination.² Our procedure is a slight-modification of the original Caraway method

PRINCIPLE

Starch is hydrolyzed in the presence of amylase to intermediate dextrans and maltose. Serum is incubated with buffered starch substrate at controlled temperature for 7 1/2 minutes and subsequently reacted with Iodine to produce blue color with reacted starch. The decrease in color, compared with that obtained in the absence of amylase, provides a measure of amylase activity.

CLINICAL SIGNIFICANCE

Serum amylase levels are elevated in acute pancreatitis, obstruction of pancreatic ducts (carcinoma, stone, stricture, duct sphincter spasm after morphine), mumps; occasionally elevated in the presence of renal insufficiency, diabetic acidosis, and with inflammation of the pancreas from a perforating peptic ulcer. Serum amylase levels are decreased in acute and chronic hepatitis, pancreatic insufficiency, occasionally in toxemia of pregnancy, and in barbiturate poisoning.³

REAGENTS (MATERIALS PROVIDED)

A. AMYLASE SUBSTRATE:

A solution of 0.04% starch, 0.85% sodium chloride, 0.86% benzoic acid and 2.7% disodium phosphate, adjusted to pH = 7.0.

B. AMYLASE COLOR REAGENT:

A solution of 193 mM potassium iodide and 11.9 mM potassium iodate in diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices.
2. Timer.
3. Test tube rack.
4. Spectrophotometer with temperature controlled cuvette.
5. Heating bath (37°).
6. Control serum.

WARNINGS AND PRECAUTIONS:

1. For in vitro diagnostic use.
2. Exercise the universal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT STORAGE

All reagents supplied with this procedure may be stored at room temperature. The reagents can be used until the expiration date indicated on the individual bottle.

REAGENT DETERIORATION

1. Physical Appearance:
Mold growth or the appearance of turbidity in the AMYLASE SUBSTRATE may indicate reagent deterioration and the product should be discarded.
2. Control Assays:
Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION AND HANDLING

Serum, free of bacterial and fungal contamination, is reportedly stable for one week at room temperature and several months at 4 - 8°C, however, it is generally recommended that the specimen be refrigerated if there is a delay between acquisition of the specimen and analysis.

PROCEDURE (MANUAL ENDPOINT METHOD)

1. Place 0.5ml of AMYLASE SUBSTRATE into test tubes labeled "blank", "control", "unknown", etc. and place in 37°C heating bath for 3-5 minutes.
2. Using timed intervals, add 0.010 ml (10 μ l) of sample to its respective tube, mix by gently swirling and immediately return to heating bath for exactly 7 1/2 minutes.
3. Remove tubes from 37°C heating bath and immediately add 4.0 ml of distilled water, then add 0.5 ml of AMYLASE COLOR REAGENT. Mix by gently inversion (4-5 times). **DO NOT REVERSE ORDER OF REAGENT ADDITION.**
4. Let all tubes stand at room temperature for 15 minutes.
5. Set wavelength of spectrophotometer at 590 nm and zero instrument with distilled water. Read and record absorbance of all tubes. (Wavelength range: 580-630).

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

PROCEDURE NOTES

One amylase unit is defined by Caraway (2) as the amount of enzyme that will hydrolyze 10 mg of starch in 30 minutes to a state at which no color is produced by the addition of iodine; therefore, in 7 1/2 minutes with a 0.01 ml sample, 0.25 mg of starch is required per reaction mixture.

STABILITY OF ENDPOINT REACTION

The final color produced in the reaction is stable for about 30 minutes.

CALIBRATION

By definition, the Caraway Unit for the amylase activity is that amount of enzyme that will digest 10 mg starch in 30 minutes at 37°C. From the above reaction conditions, complete digestion of starch would require:

$$\frac{1,000 \text{ m}}{2,000 \text{ ml}} \times \frac{0.5 \text{ ml}}{10 \text{ mg}} \times \frac{30 \text{ min.}}{7 \frac{1}{2} \text{ min.}} \times \frac{100}{0.01 \text{ ml}} = 1,000 \text{ U/dl}$$

Calculation of the unknown then is based on the fractional decrease in starch multiplied by 1,000 U/dl, the activity present if all starch is digested. Since the reaction becomes non-linear when about one-half substrate is used, linearity is limited to 500 U/dl.

QUALITY CONTROL

Fresh control sera with known normal and abnormal values should be run per each assay to monitor the validity of the reaction.

CALCULATION OF RESULTS

Use the absorbance readings of the UNKNOWN(S) and BLANK to calculate the amylase as follows: (A = Absorbance)

$$\frac{A(\text{BLANK}) - A(\text{UNKNOWN})}{A(\text{BLANK})} \times 1,000 = \text{Amylase Activity in UNKNOWN (U/dl)}$$

EXAMPLE OF CALCULATION:

(blank) absorbance = 0.73
(unknown) absorbance = 0.62

$$\frac{0.73 - 0.62}{0.73} \times 1,000^* = 151 \text{ U/dl}$$

*1,000 = maximum activity of enzyme needed to reduce all the starch present under conditions of the procedure (see Procedure Notes)

PROCEDURE LIMITATIONS

1. Highly icteric or lipemic sera may produce false values in this method so that serum blanks are recommended (see Procedure Notes)
2. Most anticoagulants, i.e., EDTA, sodium fluoride, citrate, and oxalates reportedly produce erratic results due to binding of calcium ions which are required for maximum activation.¹
3. Abnormally low concentrations of protein in the sample reportedly produce erratic results.³
4. The injection of morphine reportedly causes a temporary rise in serum amylase levels for up to 24 hours, as does the ingestion of large amounts of alcohol or the administration of thiazide diuretics.⁴

EXPECTED VALUES⁴

40 - 180 U/dl

Since the expected values are affected by age, sex, diet, and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Linearity: Amylase values exceeding 500 U/dl should be re-run on dilution.
2. Sensitivity: Typically, a decrease of 0.001 A (i.e. from 0.730 to 0.729) would represent about 1.4 U/dl under the above assay conditions.
3. Comparison: Studies done manually between this procedure and a similar procedure using forty-seven patients samples yielded a correlation coefficient of 0.97 with a regression equation of $Y = 0.98X - 4.0$ (N= 47).
4. Precision:

	Within Run (N= 20)	
<u>Mean (mg./dl)</u>	<u>S.D.</u>	<u>C.V.(%)</u>
83	6.7	8.1
290	12.5	4.3

	Run to Run (N= 20)	
<u>Mean (mg./dl)</u>	<u>S.D.</u>	<u>C.V.(%)</u>
84	8.3	9.9
284	27.2	9.5

REFERENCES

1. Henry, R.J., *Clinical Chemistry: Principles and Technics*, Harper & Row, NY, p 469 (1964).
2. Caraway, W.T., *Am. J. Clin. Path.* 32:97-99 (1959).
3. Tietz, N.W., *Text Book of Clinical Chemistry*, W.B. Saunders, Philadelphia. p. 726 (1986).
4. Faulkner, W.R. and Meites S., *Selected Methods for the Small Clinical Chemistry Laboratory*, Vol. 9, AACC. Washington, p. 91 (1982).

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