Liquid Amylase (CNPG3) Reagent Set

Intended Use

For the quantitative kinetic determination of α-amylase activity in human serum using the Mindray BS-200 analyzer.

Clinical Significance

The determination of amylase activity in serum is most commonly performed for the diagnosis and treatment of diseases of the pancreas.

Method History

Amylase was first measured quantitatively by an iodometric method introduced by Wohlegemuth in 1908.[1] Somogyi introduced a procedure in 1938 that 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 included long incubation times, endogenous glucose interference, and unstable reaction colors resulting in poor reproducibility and reliability.

Rinderknecht et al introduced a dye-coupled starch method in 1967[5] that was relatively simple to perform. However, the procedure used an insoluble substrate, lacked linearity, and still required centrifugation or filtration.

Turbidimetric procedures have been introduced[6] that are relatively fast but they require special instrumentation and have difficulty producing stable and reproducible starch solutions.

Several enzymatic procedures have been suggested[7,8] including one that used the defined substrate maltotetraose.[9] These methods represented significant improvement in amylase measurement, but were still subject to relatively long pre-incubation times, possible endogenous glucose interference, and a series of other potential interferences with the formation of NADH.[10]

Wallenfels et al[11] introduced p-nitrophenylglycosides as defined substrates for α-amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. A variety of coupling enzymes have been used to hydrolyze the short chain oligosaccharides resulting from the amylase activity in the specimen. Unfortunately, these coupling enzymes contained residual amylase activity that adversely affected the stability of these reagents.

The present method is based on the use of a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The reaction of amylase with this substrate results in the formation of 2-chloro-p-nitrophenol, that can be measured spectrophotometrically at 405nm. This reaction proceeds very rapidly, no coupling enzymes are required, and the reaction is not readily inhibited by endogenous factors.

Principle

$$\text{10 CNPG3} \xrightarrow{\alpha\text{-amylase}} \text{9 CNP + CNPG2 + 9G3 + G}$$

α-Amylase hydrolyzes the 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) to release 2-chloro-nitrophenol and form 2-chloro-p-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of increase in absorbance is measured at 405 nm and is proportional to the α-amylase activity in the sample.

Reagents

MES Buffer, pH 6.0$\pm$0.1, 2-Chloro-p-Nitrophenyl-α-D-Maltotrioside 1.8 mM, Sodium Chloride 350 mM, Calcium Acetate 6 mM, Potassium Thiocyanate 900 mM, Sodium Azide 0.1% (See 'Precautions').

Reagent Preparation

The reagent is provided as a ready-to-use liquid. No preparation is required.

Reagent Storage

1. Store reagent at 2-8°C.
2. The reagent is stable until the expiration date if stored as directed.

Reagent Deterioration

Do not use if:

1. The absorbance of the working reagent is greater than 0.600 when measured at 405 nm against water in a cuvette with a 1 cm path length.
2. The reagent fails to meet stated parameters of performance.
3. The reagent is turbid or displays other evidence of bacterial contamination.

Precautions

1. This reagent kit is intended for *in vitro* diagnostic use only.
2. This reagent contains potassium thiocyanate. POISON. Do not ingest.
3. This reagent contains sodium azide (0.1%) as preservative. Do not ingest. 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. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[12]

Specimen Collection and Handling

1. Unhemolyzed serum is the specimen of choice. Specimens should be collected as per NCCLS document H4-A3.[13]
2. Anticoagulants, such as Citrate and EDTA, bind calcium that is needed for amylase activity. Plasma with these anticoagulants should not be used.
3. Amylase in serum is reported stable for one week at room temperature (18-25 °C) and for two months when stored refrigerated at 2-8 °C.[14]

Interferences

1. A number of drugs and substances affect the determination of amylase.[15,16] Young et al have published a comprehensive list of such substances.[17]
2. Macroamylase in the specimen can cause a measured hyperamylasemia, that could lead to a false diagnosis of acute pancreatitis. However, no clinical symptoms are usually associated with macroamylasemia.[18]
3. Bilirubin (30mg/dl) and hemoglobin (500mg/dl) have each been found to have a negligible effect on this procedure.
4. Lipemic samples up to 1000 mg/dl have been reported to have no effect on serum amylase determinations.[19]

Materials Provided

Amylase (CNPG3) reagent.

Materials Required but not Provided

1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry control, catalog number C7592-100

Mindray BS-200 Test Parameters

Test :	AMY	R1 :	200
No.:	004	R2 :	0
Full Name:	Amylase	Sample Volume:	5
Standard No.:		R1 Blank:	
Reac. Type:	Kinetic	Mixed Rgt. Blank:	
Pri. Wave:	405nm	Linearity Range:	0 - 2000
Sec. Wave:		Linearity Limit:	0.2

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Test Parameters (continued)

Direction:	Increase	Substrate Limit:		
Reac. Time:	3 / 11	Factor:	3178	
		Compensate: Slope 1.0	Intercept: 0	
Incuba. Time:	0	□ Prozone check		
Unit:	U/L	q1: q2:	q3: q4:	
Precision:	Integer	PC:	Abs:	

Calibration Parameters

Rule:		Calibrator 1:
Sensitivity:		Calibrator 2:
Replicates:	2	Calibrator 3:
Interval (day):		Calibrator 4:
Difference Limit:		Calibrator 5:
SD:		Calibrator 6:
Blank Response:		
Error Limit:		
Coefficient:	0	

Limitations
1. Samples that exceed the linearity limit (2000 U/L) should be diluted with an equal volume of saline, re-assayed and multiply the result by two.
2. Macroamylase in the specimen can cause a measured hyperamylasemia, that could lead to a false diagnosis of acute pancreatitis. However, no clinical symptoms are usually associated with macroamylasemia.[18]

Calibration
The procedure is standardized by means of the millimolar absorptivity of 2-chloro-p-nitrophenol that is 12.9 at 405 nm under the test conditions described.

Calculations (Example)

$$\frac{\Delta\text{Abs./min} \times \text{TV} \times 1000}{\text{MMA} \times \text{SV} \times \text{LP}} = \text{U/L } \alpha\text{-amylase in sample}$$

Where: ΔAbs./min = Absorbance difference per minute
TV = Total assay volume (1.025 ml)
1000 = Conversion of U/ml to U/L
MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
SV = Sample volume (0.025 ml)
LP = Light path (1 cm)

$$\frac{\Delta\text{Abs./min} \times 1.025 \times 1000}{12.9 \times 0.025 \times 1.0} = \Delta\text{Abs./min} \times 3178 = \text{U/L } \alpha\text{-amylase}$$

Example: If ΔAbs./min = 0.03, then 0.03 x 3178 = 95 U/L

NOTE: To convert to SI Units (nKat/L) multiply the U/L value by 16.67.

Quality Control
The validity of the reaction should be monitored by use of control sera with known normal and abnormal amylase values. These controls should be run at least with every working shift in which amylase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values
Serum: 25-125 U/L for a similar kinetic method.[20] 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.

Performance
1. Linearity: 0-2,000 U/L
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.999 and the linear regression equation was y=0.963 x + 1.7 (n=33).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[21]

Within Run (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
50.4	2.3	4.5	64.6	2.0	4.7
537.0	17.0	3.2	425.6	12.1	2.8

4. Sensitivity: The sensitivity for the Amylase reagent was investigated by reading the change in absorbance per minute at 405nm for a saline sample, and a serum with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Liquid Amylase reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L amylase activity gives a ΔAbs./min of 0.0003.

References

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