

Intended Use

For the quantitative kinetic determination of α -amylase activity in human serum using the Mindray BS-480 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 1956³ 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 1967⁵ that was relatively simple to perform. However, the procedure used an insoluble substrate, lacked linearity, and still required centrifugation or filtration.

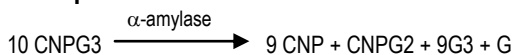
Turbidimetric procedures have been introduced⁶ that are relatively fast but they require special instrumentation and have difficulty producing stable and reproducible starch solutions.

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 al¹¹ 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



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 and Stability

Store reagent at 2-8°C. The reagent is stable until the expiration date if stored as directed. Once placed in the refrigerated reagent carousel (2-10°C), reagent is stable for 30 days.

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 and Hazards

1. This reagent kit is intended for *in vitro* diagnostic use only.
2. 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.
3. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)¹²

Hazards:

Hazard Classifications: Hazardous to the aquatic environment, long-term hazard, Category 4

Hazard Statements: H413: May cause long lasting harmful effects to aquatic life.

Precautionary Statements: **Prevention:** P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection.

Response: P391: Collect spillage. Hazardous to the aquatic environment. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents to an approved waste disposal plant. **Refer to the Safety Data Sheet for this product (SDS-AMY600) available at www.medtestdx.com.**

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



Signal Word: Warning

Liquid Amylase (CNPG3) Reagent Set

- 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
- Bilirubin (30mg/dl) and hemoglobin (500mg/dl) have each been found to have a negligible effect on this procedure.
- 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

- Mindray BS-480 Analyzer and Operation Manual
- Chemistry control, catalog number CHEQ480

Limitations

- 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.
- 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.

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

- Assay Range: 1-2,000 U/L
- Comparison: A study was performed between the Mindray BS-480 and a similar analyzer and method, resulting in the following:

Method	Amylase
N	85
Mean Amylase (U/L)	127.2
Range (U/L)	9-1856
Standard Deviation	257.0
Regression Analysis	$y = 0.964x - 6.5$
Correlation Coefficient	0.9981

- Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.²¹

Sample	Within Day			Sample	Total		
	LOW	MID	HIGH		LOW	MID	HIGH
N	20	20	20	N	40	40	40
Mean	237.9	679.9	1918.5	Mean	242.9	558.6	1988.0
Standard Deviation	0.9	6.8	6.1	Standard Deviation	6.3	17.2	37.6
Coefficient of Variation (%)	0.4%	1.0%	0.3%	Coefficient of Variation (%)	2.6%	3.1%	1.9%

- Sensitivity: 2SD limit of detection (95% Conf) = 1 U/L

References

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CHEMISTRY PARAMETERS

Chem:	AMYL	No.:	204	Sample Type:	Serum
Chemistry:	Amylase			Print Name:	AMYL
Reaction Type:	Kinetic			Reaction Direction:	Positive
Pri Wave:	412			Sec Wave:	
Unit:	U/L			Decimal	0
Blank Time:	0	0		Reaction Time:	19 33
	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	3.0 ul	--- ul	--- ul	R1:	120 ul --- ul
Decreased:	--- ul	--- ul	--- ul	R2:	--- ul -- ul
Increased:	--- ul	--- ul	--- ul	R3:	--- ul -- ul
	<input type="checkbox"/> Sample Blank	<input checked="" type="checkbox"/> Auto Rerun		R4:	--- ul --- ul
<u>Slope/Offset Adjustment</u>					
	Slope: 1	Offset: 0			

Linearity Range (Standard)	1	2000	Linearity Limit:	0.2
Linearity Range (Decreased)	---	---	Substrate Depletion:	25000
Linearity Range (Increased)	---	---	Mixed Blank Abs:	
R1 Blank Abs:	---	---	Uncapping Time	
Blank Response:	---	---	Reagent Alarm Limit:	
Twin Chemistry:			<input type="checkbox"/> Enzyme Linear Extension	
<input type="checkbox"/> Prozone Check		<input type="radio"/> Rate Check	<input type="radio"/> Antigen Addition	
Q1:	Q2:	Q3:	Q4:	
PC:	ABS:			

Liquid Amylase (CNPG3) Reagent Set

CALIBRATION PARAMETERS

Calibrator Definition						
Calibrator:	*	Lot No.:	*			
Exp Date:	*					
Carousel		Pos				
Sample Carousel 1	*					
Sample Carousel 2						
Sample Carousel 3						
Reagent/Calibration						
<u>Calibrator</u>	<u>Pos</u>	<u>Lot No</u>	<u>Exp Date</u>	<u>Chem</u>	<u>Conc</u>	<u>Unit</u>
Water	W	*	*	AMYL	0	U/L
Calibration Setup						
Chem:	AMYL					
<u>Calibration Settings</u>						
Math Model:	K Factor					
Factor:	3178	Replicates:	1			
<u>Acceptance Limits</u>						
Cal Time:	*	Hour				
Slope Diff:	---	SD:	---			
Sensitivity :	---	Repeatability:	---			
Deter Coeff:	---					
<u>Auto Calib.</u>						
<input type="checkbox"/> Bottle Changed	<input type="checkbox"/> Lot Changed	<input type="checkbox"/> Cal Time				

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF AMY480



Manufactured for MedTest DX
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IVD

Symbol Key

Use by (YYYY-MM-DD)	Lot and batch code	Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	In vitro diagnostic medical device	