AMYLASE (CNPG3)

INTENDED USE
Bioline Amylase is used for the quantitative determination of α-amylase activity in serum, plasma or urine.

CLINICAL SIGNIFICANCE
The determination of amylase activity in serum, plasma 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.

METHOD AND PRINCIPLE
Substrate Galactose-Glucose-Glucose-Chloronitrophenol (Gal-G_2-α-CNP) is hydrolyzed by α-amylase to $G_2$and CNP stoichiometrically. The rate of CNP formation due to substrate hydrolysis by α-amylase is proportionally correlated with α-amylase activity which is measured by following the rate of absorbance increase at 405 nm.
The system monitors the change in absorbance at 405 nm. This change in absorbance is directly proportional to the activity of α -amylase in the sample.

$$\text{Gal-G}_2\text{-α-CNP} + \text{H}_2\text{O} \xrightarrow{\text{α-amylase}} \text{Gal-G}_2 + \text{CNP}$$

REAGENT COMPOSITION
Gal-G_2-α-CNP: 0.3mmol/L
$CaCl_2$: 10 mmol/L
Phosphate buffer: 50 mmol/L
Also non-reactive chemicals for optimal system performance.

WARNINGS AND PRECAUTIONS.
1. For *invitro* diagnostic use.
   CAUTION: Invitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.

REAGENT PREPARATION
Amylase reagent is ready to use.

REAGENT STORAGE AND STABILITY
α-Amylase reagent when stored at 2ºC to 8ºC is stable until the expiration date showed on the bottle label. DO NOT FREEZE.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity maybe a sign of contamination.
2. The working reagent has an absorbance of 0.70 or greater when measured against water at 405 nm.

SPECIMEN COLLECTION AND HANDLING
1. The test can be performed on serum, plasma. For serum, blood is drawn into a tube which does not contain anticoagulant and allow clotting. The serum is then separated from the clot. A maximum limit of two hours from the time of collection is recommended.
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum and plasma should be stored at 2ºCto8ºC. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48hours, samples should be frozen at-15ºCto-20ºC.Frozensamples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
3. It is recommended that urine assays be performed within 2 hours of collection Due to the instability of amylase in acidic urine; the pH of the specimen should be adjusted to the alkaline range and stored at4ºC. For timed specimens, the collection container should be kept in the refrigerator or on ice during the timed period.
4. For plasma, add whole blood directly into a tube containing anticoagulant. Acceptable anticoagulants are listed in the "LIMITATIONS" section.

INTERFERENCE
1. Samples showing evidence of hemolysis should not be used.
2. Lipemic samples >3+ should be ultra-centrifuged and the analysis performed on the infranate.
3. Pyruvate at level of 2 mg/dL may cause decreased results.
4. On this method, refer to the work of Young for a review of drug and comprehensive list of substances effect on a-Amylase level.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZERS
Wavelength 405nm
Temperature 37ºC

	Test
Reagent	1000µL
Sample	20µL

Mix and aspirate into the analyzer, after 60 Second of delay measure the change of optical density per minute(ΔOD/min.) during next 90Sec.

CALCULATION
AMYLASE Activity = ΔAbs/min X 3806

LIMITATIONS
1. The anticoagulants Potassium Oxalate, Sodium Fluoride, Sodium Citrate and EDTA were found to be incompatible with this method.
2. The anticoagulants Ammonium Heparin, Sodium Heparin and Lithium Heparin were found to be compatible with this method.

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established Amylase values maybe used for quality control. The assigned value of the control material must be confirmed, by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrumental function, or procedural errors.

EXPECTED VALUE
Serum or Plasma: 40-126 IU/L

PERFORMANCE CHARACTERISTICS

Analytical Range: 2.5-2000 IU/L. For samples above the linearity range dilute and rerun. Multiply the result with the dilution factor to get correct Amylase value.

Accuracy: Comparison study yielded correlation coefficient of 0.98 with a regression equation of y=0.99X -1.1.

Precision: Within Run precision for Amylase Reagent Set was determined. Two commercial human serum were assayed on biochemistry analyzer for 25 times.

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Sample	Sample1	Sample2
N	25	25
Mean (IU/L)	78	380
Standard Deviation (IU/L)	3.1	12.5
Coefficient of Variation (%)	3.9	3.2

Within Run precision: Two commercial human serum were assayed on biochemistry analyzers five times per day for five days for the total of 25 values.

Sample	Sample1	Sample2
N	25	25
Mean (IU/L)	81	379
Standard Deviation (IU/L)	2.9	10.6
Coefficient of Variation (%)	3.5	2.8

GENERAL TECHNICAL PARAMETER

Mode	**Kinetic**
Wavelength (Filter)	**405nm**
Reaction Direction	**Increasing**
Sample Vol.	**20µL**
Reagent Vol.	**1000µL**
Delay Time/Lag Time	**60Seconds**
Measuring Time	**90Seconds**
Interval Time	**30 seconds**
No. of Reading	**3**
Reagent Blank Abs. (Max)	**NMT 0.700**
Calibration Method	**Factor**
Factor	**3806**
Linearity	**2000 IU/L**
Decimal Places	**1**
Temp.	**37°C**
Unit	**IU/L**
Ref.Low	**40 IU/L**
Ref.High	**126 IU/L**

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