BECKMAN COULTER

AU US

Instructions For Use

AMY
AMYLASE

REF OSR6006 4 x 10 mL R1
OSR6106 4 x 40 mL R1

For *in vitro* diagnostic use only.

For Rx use only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of α-Amylase in human serum and urine on Beckman Coulter AU analyzers.

SUMMARY AND EXPLANATION

α-Amylase (EC 3.2.1.1) is found primarily in the pancreas and salivary glands. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis.[1]

Amylase activity in serum tends to increase rapidly after an attack of pancreatitis and may be demonstrated as early as six to eight hours after its onset. Levels stay elevated for one to three days and then return rapidly to normal, reflecting the efficient renal clearance of the enzyme.

Decreased amylase levels have been found in abscesses of the liver, acute hepatocellular damage, cirrhosis, cancer of the liver and bile duct and cholecystitis.[1]

Amylase is a relatively small protein and is therefore filtered readily into the urine. The enzyme can be found in increased concentrations in the urine for longer periods of time than in the serum. An amylase content determined on a 2-hour urine collection is an excellent test for detecting pancreatitis.

METHODOLOGY

This Amylase procedure utilizes 2-chloro-4-nitrophenyl-α-D-maltotrioside ($CNPG_3$) as substrate.[2] This substrate reacts directly with α-Amylase and does not require the presence of ancillary enzymes. The release of 2-chloro-4-nitrophenol (CNP) from the substrate and the resulting absorbance increase per minute is directly related to the α-Amylase activity in the sample. The resulting increase in absorbance can be measured spectrophotometrically at 410/480nm.

$$CNPG_3 \xrightarrow{\alpha\text{-Amylase}} CNP + CNPG_2 + Glucose + Maltotriose$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Amylase is stable in serum for one week when stored at 15 - 25°C and for up to one month when stored at 2 - 8°C.[3]

In urine, an acid pH may make the enzyme less stable; therefore, pH should be adjusted to approximately 7.0 before storage.

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum or heparinized plasma free from hemolysis is the recommended specimen. Separate from blood cells as soon as possible.

Non-acidified urines with random or timed collections are valid specimens.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

α-Amylase Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

MES (pH 6.05)	36.1 mmol/L
Calcium Acetate	3.60 mmol/L
NaCl	37.2 mmol/L
Potassium Thiocyanate	253 mmol/L
$CNPG_3$	1.63 mmol/L
Also contains preservative.	

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

EQUIPMENT AND MATERIALS

For AU400/400^e/480, AU640/640^e/680, AU2700/5400/AU5800 and DxC 700 AU Beckman Coulter Analyzers.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The α-Amylase reagent is ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 8°C.
2. The opened reagent is stable for 30 days when stored in the refrigerated compartment of the analyzer.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

Calibration of this amylase procedure is based upon the theoretical extinction coefficient for $CNPG_3$, which has a molar absorptivity of 11,320 at 410/480 nm.

QUALITY CONTROL

During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU analyzer User Guide/Instructions For Use (IFU). Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Appropriate qualified urine controls should be established and utilized during urine analysis.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedure can be found in the User Guide/IFU appropriate to the Beckman Coulter AU analyzer.

RESULTS INTERPRETATION

Automatically printed out for each sample in U/L at 37°C.

REPORTING RESULTS

EXPECTED RESULTS

Serum:[4] 29 - 103 U/L

The following urine reference interval values are based on spontaneously voided samples.

Urine Male:[5]	16-491 U/L
Urine Female:[5]	21-447 U/L

Expected values may vary with age, sex, diet, and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

1. Do not pipette the reagent or sample by mouth to avoid salivary amylase contamination. Laboratory studies have shown that chelating agents such as the anticoagulants citrate and EDTA will interfere with this method.[6]
2. Results of studies[7] show that the following substances interfere with this amylase procedure.

Serum

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 20 mg/dL Bilirubin
Hemolysis:	No significant interference up to 250 mg/dL Hemolysate
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid serum samples.

Eltrombopag and its metabolites may interfere with this assay causing erroneously low patient results.

Urine

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 50 mg/dL Ascorbate
Bilirubin:	No significant Interference up to 40 mg/dL or 684 µmol/L Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[8] for a compilation of reported interferences with this test.

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

The following data was obtained using the Amylase Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Amylase procedure is linear from 10 to 2,000 U/L for serum determinations and to 1,500 U/L for urine determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

SENSITIVITY

Typical change in absorbance per minute for 1 U/L of amylase is 0.11 mAbsorbance.

METHODS COMPARISON

Reference [9]

Serum

Patient samples were used to compare this Amylase Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	DxC 700 AU
X Method	AU5800
Slope	0.960
Intercept	0.8
Correlation Coeff. (r)	1.0000
No. of Samples (n)	121
Range (U/L)	12 - 1912

Urine

Urine samples were used to compare this Amylase Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	DxC 700 AU
X Method	AU5800
Slope	0.984
Intercept	0.7
Correlation Coeff. (r)	0.9999
No. of Samples (n)	131
Range (U/L)	11-1453

PRECISION

Reference [9]

Estimates of precision, based on CLSI recommendations[10], are consistent with typical performance. The within run precision for serum samples is less than 5% CV and total precision is less than 10% CV. Assays of control sera and pooled urine were carried out and data reduced following CLSI guidelines above.

Serum

N = 100	**Within-run**		**Total**	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
102.7	0.8	0.8	2.1	2.0
412	3.1	0.8	7.7	1.9

Urine

N = 100	Within run		Total	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
29.9	0.5	1.7	0.9	2.9
136.1	1.2	0.9	3.6	2.7

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
AMY1U	Amylase (Serum)
AMY1U	Amylase (Urine)

Setting Sheet Footnotes

User defined

* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.

AU5800 Serum: Ψ Parameters specific factors provided by service engineer for each ring

AU5800 Urine: Ψ Instrument MB factor – set by engineer, specific for each instrument cuvette wheel.

Ψ System Factor

REVISION HISTORY

Revised GHS section

Revised Interferences section.

Preceding version revision history

Correct error in Spanish Language

REFERENCES

1. Friedman, R.B. and Young, D.S., Effects of Diseases on Clinical Laboratory Tests, 3rd Edition, AACC Press, Washington, DC, 1997.
2. Chavez, R.G., et al; `An aromatic substituted glycoside', American patent 4,963,479 1986.
3. Tietz, N.W., Clinical Guide to Laboratory Tests, 2nd Edition, W.B. Saunders, 1990.
4. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
5. Junge W, Wortmann W, Wilke B, Waldenstrom J, Weittenhiller A, Finke J, Klein G, Development and evaluation of assays for the determination of total and pancreatic amylase at 37°C according to the principle recommended by the IFCC. Clin Biochem 2001;34:607-615.
6. Henry, R.J., Cannon, D.C. and Winkelman, J.W., Clinical Chemistry, Principles & Techniques, 2nd Edition, Harper & Row, 943 - 949; 1974
7. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-P, 1986.
8. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
9. Data is on file for specific AU analyzers.
10. CLSI/NCCLS Evaluation Protocol, EP5-T2, 1992.

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