Amylase (AMY7) - Serum, Plasma, Fluids
Beckman UniCel DxC Systems

Technical Procedure 3106

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For *In Vitro* Diagnostic Use Only

Principle

Intended Use

AMY7 reagent, in conjunction with UniCel® DxC 800 System(s), is intended for quantitative determination of total amylase activity in human serum, plasma and fluids.

Clinical Significance

Amylase measurements are used primarily in the diagnosis and treatment of pancreatitis.

Acute pancreatitis is characterized when α–amylase serum levels are four to six times the upper reference limit for the analyte. Generally, these levels start to rise about five to eight hours after the onset of symptoms, reach a peak between 12 and 72 hours and return to normal by the third or fourth day. Increased amylase in plasma may be an indicator of salivary trauma, mumps or renal failure.

Methodology

AMY7 reagent is used to measure amylase activity by an enzymatic rate method.(1) In the reaction, amylase cleaves 4,6-ethylidene(G1)-4-nitrophenyl(G7)-α-(1→4)-D-maltoheptaoside (Ethyledene Protected Substrate = EPS) and subsequent hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 30 parts reagent. The system monitors the change in absorbance at 410 nanometers. This change in absorbance is directly proportional to the activity of AMY7 in the sample and is used by the System to calculate and express the total AMY7 activity.

Use of this product will result in assay values which are compatible with the methods recommended by the International Federation of Clinical Chemistry (IFCC).(2)

Chemical Reaction Scheme

$$\text{EPS} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{4,6-ethylidine-G x} + \text{4 nitrophenol-G (7-x)}$$

$$\text{4 nitrophenol-G (7-x)} + \text{(7-x)}\ H_2O \xrightarrow{\alpha\text{-glucosidase}} \text{(7-x) Glucose} + \text{4-nitrophenoxide}$$

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers
Original specimen container or 13 x 75 Clear Cap BD tube (min = 0.3 mL) for fluids

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(3) Freshly drawn serum, plasma or fluids are the preferred specimens. Acceptable anticoagulants are listed in *Procedural Notes* section of this chemistry information sheet. Whole blood is not recommended for use as a sample. When handling samples, avoid contamination from sneezing and/or saliva.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.(4)

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.(4)

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the *Primary Tube Sample Template*.

Criteria for Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items: **Kit Reorder # A71607**

Two AMY7 Reagent Cartridges (2 x 200 tests)

Volumes per Test

Sample Volume	7 µL
ORDAC Sample Volume	3 µL
Total Reagent Volume	210 µL
Cartridge Volumes	
A	175 µL
B	35 µL
C	-----

Reactive Ingredients

Reagent Constituents

α-glucosidase (microorganism)		9700 U/L
4,6-ethylidene(G1)-4-nitrophenyl (G7)-α-(1→4)-D-maltoheptaoside Protected Substrate = EPS)	(Ethyledene	11 mmol/L
Sodium Chloride		87 mmol/L
Calcium Chloride		0.08 mmol/L
Magnesium Chloride		12.6 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

NOTICE

Avoid all contact with reagent. Sweat and saliva contain α-amylase. It is recommended that gloves be worn when handling the reagent cartridges. Use caution when recapping reagent cartridges. Reagent Mixture and Starter Reagent caps must not be interchanged or reagent contamination will occur.

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

Materials Needed But Not Supplied With Reagent Kit

At least two levels of control material
Saline

Reagent Preparation

No preparation is required.
Date and initial cartridge and document in reagent log before loading each new cartridge.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

Reagent Storage and Stability

AMY7 reagent when stored unopened at +2°C to +8°C will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 21 days at +2°C to +8°C unless the expiration date is exceeded.
DO NOT FREEZE.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

Calibration is not required.

Traceability

AMY7 assay is traceable to IFCC primary reference method.

Quality Control

At least two levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProce- dures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\ControlIFUs*). Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	+2°C to +8°C*
MAS ChemTrak 3	+2°C to +8°C*

*Controls are received frozen and stored at –15°C to –25°C. Bottles of controls in use are thawed and stored at +2°C to +8°C and are good for 14 days.

Testing Procedure

1. If necessary, load the reagent onto the system.
2. Program samples and controls for analysis.
3. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

Reporting Results

Equivalency between the SYNCHRON LX and UniCel DxC 800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

Reference Intervals

The following reference intervals were taken from literature.(6) UCDMC adult reference interval was determined from a normal study.

Reference Intervals

Intervals	Sample Type	Conventional Units	S.I. Units
Literature	Serum or Plasma	28 - 100 U/L	0.47 - 1.67 µkat/L
	Urine (Male)	16 - 491 U/L	0.27 - 8.18 µkat/L
	Urine (Female)	21 - 447 U/L	0.35 - 7.45 µkat/L
UCDMC	Serum or Plasma	33 - 130 U/L	0.55 - 2.17 µkat/L

Refer to References (7, 8, 9) for guidelines on establishing laboratory-specific reference intervals

Conversion factor from conventional units(U/L) to S.I. Units (µkat/L) is 0.01667

A reference interval for fluids has not been determined by UCDHS. This result may be less accurate than for the usual sample type, and should be interpreted in the context of the patient's clinical condition.

Critical Values

None

Procedural Notes

Anticoagulant Test Results

1. The following anticoagulants were assessed by Deming regression analysis with 52 paired human serum and plasma samples. Values of serum (X) ranging from 8.0 to 1203.3 U/L were compared with the values for plasma (Y) yielding the following results.

Compatible Anticoagulants[a]

Anticoagulant	Level Tested for In Vitro Interference	Deming Regression Analysis
Lithium Heparin	14 Units/mL	Y = 0.996X + 1.21, r = 1.000
Sodium Heparin	14 Units/mL	Y = 1.000X + 0.85, r = 1.000

Refer to References (7,8,9) for guidelines on establishing laboratory-specific reference intervals.

Limitations

None identified

Interferences

1. The following substances were tested for interference with this methodology:

Interferences

Substance	Source	Level Tested	Observed Effect
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[a]
Bilirubin (Total)	Porcine	5.7 mg/dL DBIL	≤ +8.6 U/L or 7%
Hemoglobin	RBC Hemolysate	250 mg/dL	≤ -8.6 U/L or 7%
Lipemia	Intralipid[b]	500 mg/dL	NSI
	Human	Serum Index 11	NSI
Ascorbic Acid	NA[c]	200 mg/dL	NSI
Glucose	NA	2000 mg/dL	NSI

[a] NSI = No Significant Interference (within ±6.0 U/L or 7%).

[b] Registered trademarks are the property of their respective owners

[c] NA = Not applicable

2. Samples showing evidence of hemolysis may cause decreased results.

3. For interferences caused by drugs, disease and preanalytical variables refer to References (10, 11, 12).

Performance Characteristics

Analytical Measurement Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical ranges:

Analytical measurement Range (AMR)

Sample Type	Conventional Units	S.I. Units
Serum/Plasma/Fluid	5 – 1200 U/L	0.08 – 20.00 µkat/L
Serum/Plasma/Fluid (ORDAC)[a]	1000 – 2000 U/L	16.67 – 33.34 µkat/L

[a] Overrange Detection and Correction. Refer to the UniCel DxC 800 System Instructions For Use (IFU) manual for more details on this function.

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units	S.I. Units
Serum/Plasma/Fluid	5 – diluted result U/L (dilute no more than x201)	0.08 – diluted result µkat/L (dilute no more than x201)

Samples with concentrations below the AMR and CRR (5 U/L) will be reported as "**< 5 U/L**".
Samples with concentrations greater than the AMR should be diluted with saline and reanalyzed.
If a numerical result cannot obtained with a x201 dilution, result as **>240,000 U/L**.

If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for AMY7 determination is 5 U/L (0.08 µkat/L).

Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods as determined by Beckman.

Serum or Plasma (in the range of 10 to 1089 U/L):

Y (SYNCHRON DxC Systems)	= 1.081X - 3.4
N	= 83
MEAN (SYNCHRON DxC Systems)	= 211.8
MEAN (UDR on SYNCHRON DxC)[a]	= 199.1
CORRELATION COEFFICIENT (r)	= 1.0000

[a] A product of Thermo Fisher Scientific Inc.

Urine (in the range of 9.7 to 534.4 U/L):

Y (SYNCHRON DxC Systems)	= 1.039X - 0.70
N	= 78
MEAN (SYNCHRON DxC Systems)	= 192.8
MEAN (UDR on SYNCHRON DxC)	= 186.2
CORRELATION COEFFICIENT (r)	= 0.9997

Refer to References (13) for guidelines on performing equivalency testing.

Precision

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD		Changeover Value[a]		%CV
			µkat/L	U/L	µkat/L	
Within-run	Serum/Plasma/Urine	3.0	0.05	85.7	1.43	3.5
	Serum/Plasma/Urine (ORDAC)	NA	NA	NA	NA	10.0
Total	Serum/Plasma/Urine	4.5	0.08	85.7	1.43	5.3
	Serum/Plasma/Urine (ORDAC)	NA	NA	NA	NA	15.0

[a] When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

[b] NA = Not applicable.

Precision established at UCDMC

Type of Precision	Sample Type	n	Mean (U/L)	1 SD (U/L)	%CV
DxC800-4118 Within-run	MAS ChemTrak 1	20	95	0.9	0.9
	MAS ChemTrak 3	20	478	2.8	0.6
DxC800-4427 Within-run	MAS ChemTrak 1	20	94	1.4	1.5
	MAS ChemTrak 3	20	472	4.8	1.0
DxC800-4449 Within-run	MAS ChemTrak 1	20	94	1.4	1.5
	MAS ChemTrak 3	20	472	4.8	1.0

Type of Imprecision	Sample Type	No. Systems	No. Data Points	Test Mean Value (U/L)	SD	%CV
DxC800-4118 Day to Day	MAS ChemTrak 1	1	86	95	1.1	1.2
	MAS ChemTrak 3	1	88	480	4.0	0.8
DxC800-4427 Day to Day	MAS ChemTrak 1	1	68	96	1.5	1.6
	MAS ChemTrak 3	1	139	482	5.9	1.2
DxC800-4449 Day to Day	MAS ChemTrak 1	1	95	95	1.4	1.5
	MAS ChemTrak 3	1	98	479	5.6	1.2

Comparative performance

Comparative performance data for the SYNCHRON DxC System evaluated using the CLSI/NCCLS Approved Guideline EP5-A2 appears in the table below.(14)

CLSI/NCCLS EP5-A2 Precision Estimate Method

Type of Imprecision	Sample Type	No. Systems	No. Data Points[a]	Test Mean Value (U/L)	EP5-A2 Calculated Point Estimates	
					SD	%CV
Within-run	Serum/Plasma Level 1	1	80	78.7	1.0	1.3
	Serum/Plasma Level 2	1	80	913.6	4.5	0.5
	Serum/Plasma (ORDAC)	1	80	1775.4	8.7	0.5
	Urine Level 1	1	80	56.5	0.7	1.2
	Urine Level 2	1	80	168.4	0.9	0.5
	Urine (ORDAC)	1	80	1181.0	6.0	0.5
Total	Serum/Plasma Level 1	1	80	78.7	0.8	1.1
	Serum/Plasma Level 2	1	80	913.6	5.5	0.6
	Serum/Plasma (ORDAC)	1	80	1775.4	11.0	0.6
	Urine Level 1	1	80	56.5	0.7	1.2
	Urine Level 2	1	80	168.4	1.1	0.7
	Urine (ORDAC)	1	80	1181.0	55.5	4.7

[a] The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

References

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