BECKMAN COULTER

SYNCHRON® System(s) Chemistry Information Sheet

AMY Amylase

REF 442775

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

AMY reagent, in conjunction with SYNCHRON LX® System(s), UniCel® DxC 600/800 System(s) is intended for the quantitative determination of total amylase activity in human serum, plasma or urine. Use of this product, in conjunction with the SYNCHRON® Systems Enzyme Validator Set, will result in assay values which are compatible with the methods recommended by the International Federation of Clinical Chemistry (IFCC).[1]

CLINICAL SIGNIFICANCE

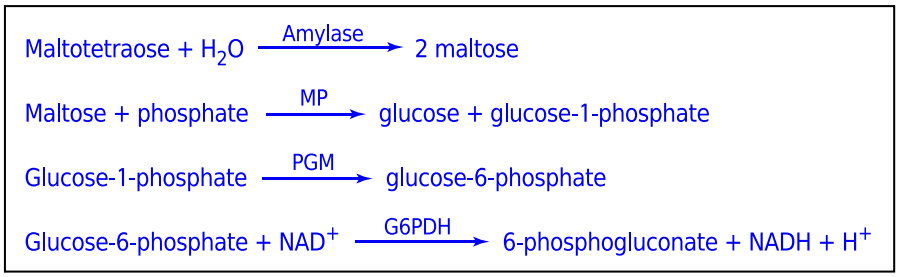
Amylase measurements are used primarily in the diagnosis and treatment of pancreatitis.

METHODOLOGY

AMY reagent is used to measure amylase activity by an enzymatic rate method.[2] In the reaction, amylase catalyzes the hydrolysis of the defined substrate, maltotetraose, to maltose. The rate of formation of maltose is measured through the use of three coupled reactions catalyzed by maltose phosphorylase (MP), β-phosphoglucomutase (PGM), and glucose-6-phosphate dehydrogenase (G6PDH) which results in the production of reduced β-nicotinamide adenine dinucleotide (NADH) from β-nicotinamide adenine dinucleotide (NAD).

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 21 parts reagent. The system monitors the change in absorbance at 340 nanometers. This change in absorbance is directly proportional to the activity of AMY in the sample and is used by the System to calculate and express the total AMY activity.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[3] Freshly drawn serum or plasma are the preferred specimens. Freshly collected urine may also be used for testing. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

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]
3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container should be kept in the refrigerator or on ice during the timed period. No preservative is required.[5]

Additional specimen storage and stability conditions as designated by this laboratory:

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 for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two AMY Reagent Cartridges (2 x 200 tests)

VOLUMES PER TEST

Sample Volume	12 µL
ORDAC Sample Volume	3 µL
Total Reagent Volume	250 µL
Cartridge Volumes	
A	238 µL
B	– –
C	12 µL

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Maltotetraose	7.9 mmol/L
NAD	3.0 mmol/L
Maltose Phosphorylase	5.5 KIU/L
β-Phosphoglucomutase	1.8 KIU/L
Glucose-6-phosphate dehydrogenase	5.5 KIU/L

Also non-reactive chemicals necessary for optimal system performance.

> ⚠ CAUTION
>
> 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).

Avoid skin contact with reagent. Use water to wash reagent from skin.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

At least two levels of control material
Saline

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

AMY 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 30 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems Enzyme Validator Set (Levels 1 and 2)

Calibration is not required. Calibration is recommended if patient results compatible with the IFCC method are desired.

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems Enzyme Validator Set when stored unopened at -15°C to -20°C will remain stable until the expiration date printed on the label. Once opened, resealed calibrators are stable for 60 days at -15°C to -20°C unless the expiration date is exceeded.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[6]

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have a valid calibration in memory before controls or patient samples can be run.
2. Under typical operating conditions the AMY reagent cartridge must be calibrated one time and also with certain parts replacement or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 Systems *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.
4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. 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 appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

The Synchron System(s) amylase (AMY) reagent has different affinities for human amylase and porcine amylase. Control materials consisting of porcine amylase do not perfectly mimic the performance of the reagent with patient samples.

Due to these different affinities, noticeable lot-to-lot shifts with animal based control materials may occur. To aid you in ensuring consistent reagent quality for human samples, you may run several known patient samples (including elevated

samples) on both the new and old lots. Alternatively, you may contact the Clinical Support Center at 1-800-854-3633 from the United States and Canada or your local Beckman Coulter Representative for the results of human patient samples performed during manufacture.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

The SYNCHRON® System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.[7]

The reference interval for serum when using Enyzme Validator (based on the IFCC method) is 28 to 100 U/L (0.42 to 1.68 µkat/L).[8] Multiply the AMY result by a factor of 2 when reporting IU/L or µkat/L.

Table 2.0 Reference intervals (non-calibrated)

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS		S. I. UNITS
Literature	Serum or Plasma	27 – 131 U/L	13.5 – 65.5 IU/L	0.23 – 1.10 µkat/L
	Urine (timed)	1 – 17 U/h	0.5 – 8.5 IU/h	0.01 – 0.14 µkat/h
Laboratory	Serum or Plasma	36 – 128 U/L	18 – 64 IU/L	0.30 – 1.07 µkat/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS		S. I. UNITS
Laboratory				

Refer to References (9,10,11) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

1. If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Table 3.0 Acceptable Anticoagulants[a]

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	AVERAGE PLASMA-SERUM BIAS (U/L)
Ammonium Heparin	29 Units/mL	NSI[b]
Lithium Heparin	29 Units/mL	NSI
Sodium Heparin	29 Units/mL	NSI

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b NSI = No Significant Interference (within ±6.0 U/L or 7%).

2. The following anticoagulants were found to be incompatible with this method:

Table 4.0 Incompatible Anticoagulants[a]

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	PLASMA-SERUM BIAS (U/L)[b]
EDTA	3.0 mg/mL	-64.0

Table 4.0 Incompatible Anticoagulants, Continued

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	PLASMA-SERUM BIAS (U/L)[b]
Potassium Oxalate/Sodium Fluoride	4.0 / 5.0 mg/mL	±34.0
Sodium Citrate	6.6 mg/mL	-37.0

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b Bias is based on worst case instead of average. Plus (+) or minus (-) signs in this column signify positive or negative bias.

LIMITATIONS

None identified.

INTERFERENCES

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

Table 5.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[a]
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[b]
Hemoglobin	RBC hemolysate	50 mg/dL	+7 U/L
Lipemia	Intralipid[c]	500 mg/dL	NSI

a Plus (+) or minus (-) signs in this column signify positive or negative interference.
b NSI = No Significant Interference (within ±6.0 U/L or 7%).
c Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

2. Pyruvate at a level of 2 mg/dL may cause decreased results.
3. Refer to References (12,13,14) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

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

Table 6.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS		S.I. UNITS
Serum/Plasma/Urine	5 – 800 U/L	2.5 – 400 IU/L	0.04 – 6.68 µkat/L
Serum/Plasma/Urine (ORDAC)[a]	600 – 2400 U/L	300 – 1200 IU/L	5.01 – 20.04 µkat/L

a Overrange Detection and Correction. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for more details on this function.

Samples with activities exceeding the high end of the analytical range should be rerun with ORDAC enabled or diluted with saline and reanalyzed.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 7.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for AMY determination is 5 U/L or 2.5 IU/L (0.04 µkat/L).

EQUIVALENCY

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

Serum or plasma (in the range of 10 to 789 U/L):

Y (SYNCHRON LX Systems)	= 1.017X - 0.89
N	= 77
MEAN (SYNCHRON LX Systems)	= 162.8
MEAN (SYNCHRON CX 4CE)	= 161.0
CORRELATION COEFFICIENT (r)	= 0.9999

Serum (in the range of 14 to 563 IU/L) using Enzyme Validator:

Y (SYNCHRON LX Systems)	= 0.979X + 3.7
N	= 142
MEAN (SYNCHRON LX Systems)	= 100.7
MEAN (IFCC Formulation)	= 99.0
CORRELATION COEFFICIENT (r)	= 0.9974

Urine (in the range of 5 to 396 U/L):

Y (SYNCHRON LX Systems)	= 1.000X - 0.21
N	= 73
MEAN (SYNCHRON LX Systems)	= 133.4
MEAN (SYNCHRON CX7 DELTA)	= 133.7
CORRELATION COEFFICIENT (r)	= 0.9996

Refer to References (15) for guidelines on performing equivalency testing.

PRECISION

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

Table 8.0 Precision Values

TYPE OF PRECISION	SAMPLE TYPE	1 SD			CHANGEOVER VALUE[a]			% CV
		U/L	IU/L	µkat/L	U/L	IU/L	µkat/L	
Within-run	Serum/Plasma/Urine	3.0	1.5	0.03	85.7	42.9	0.72	3.5
	Serum/Plasma/Urine (ORDAC)	NA[b]	NA	NA	NA	NA	NA	10.0
Total	Serum/Plasma/Urine	4.5	2.3	0.04	85.7	42.9	0.72	5.3
	Serum/Plasma/Urine (ORDAC)	NA	NA	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.

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[16] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 9.0 NCCLS EP5-T2 Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (U/L)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	46.59	1.76	3.79
	Serum	Control 2	1	80	378.70	3.32	0.88
Total	Serum	Control 1	1	80	46.59	1.87	4.02
	Serum	Control 2	1	80	378.70	3.63	0.96

a The point estimate is based on the pooled 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 SYNCHRON LX Systems or UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Siekmann, L., et al., "Standard Operating Procedure for the Measurement of Catalytic Concentration of Enzymes; Alpha-amylase", *Clin. Chem. Lab. Med.*, (in preparation).

2. Pierre, K. J., Tung, K. K., Nadj, H., *Clin. Chem.*, 22:1219 (1976).

3. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).

4. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).

5. National Committee for Clinical Laboratory Standards, *Routine Urinalysis and Collection, Transportation and Preservation of Urine Specimens*, Tentative Guideline, NCCLS publication GP16-T, Villanova, PA (1992).

6. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).

7. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1995).

8. Junge, W., et al., "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.*, 34 pp 607 615 (2001).

9. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).

10. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).

11. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).

12. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).

13. Friedman, R. B., Young, D. S.,*Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).

14. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).

15. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).

16. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

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