Pointe AST (SGOT) Reagent Set

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Intended Use
For the quantitative determination of Aspartate Aminotransferase (AST) in human serum using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

Clinical Significance
AST is widely distributed in tissues with the highest concentrations found in the liver, heart, skeletal muscle and kidneys. Diseases involving any of these tissues can lead to elevated levels of AST in serum. Following myocardial infarction, AST levels are elevated and reach a peak after 48 to 60 hours.
Hepatobiliary diseases such as cirrhosis, metastatic carcinoma and viral hepatitis can show increased levels of AST. Other disorders which can lead to an elevated level of AST are muscular dystrophy, dermatomyositis, acute pancreatitis and infectious mononucleosis.[1]

Method History
Karmen[2] developed a kinetic assay procedure in 1955 which was based upon the use of malate dehydrogenase and NADH. Optimized procedures were presented by Henry[3] in 1960 and Amador and Wacker[4] in 1962. These modifications increased accuracy and lowered the effect of interfering substances. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[5] published a recommended method based on optimized modifications in 1974. In 1976, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC)[6] proposed the addition of pyridoxal-5-phosphate to the reaction mixture to ensure maximum activity. The IFCC[7] published a recommended method that included P-5-P in 1978. The present method is based on IFCC recommendations but does not contain P-5-P since most specimens contain adequate amounts of this cofactor for full recovery of AST activity.[8,9,10]

Principle
$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{L-Glutamate}$$
$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+ + \text{H}_2\text{O}$$

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α-Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

Reagents
After combining R1 and R2, the reagent contains: L-aspartic acid 200mM, α-ketoglutaric acid 11mM, LDH (microbial) > 1000U/L, MDH (microbial) ≥800U/L, NADH >0.18mM, buffer, sodium azide 0.28%, stabilizers.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store the reagents at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use reagent if:
1. The initial absorbance at 340nm is below 0.800.
2. The reagent fails to meet stated parameters of performance.

Precautions
1. This reagent set is for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.28%) as a preservative. Do not in gest. 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.

Specimen Collection and Storage[11]
1. Non-hemolyzed serum is recommended. Red cells contain AST which can give falsely elevated results.
2. AST in serum is reported stable for ten days when refrigerated (2-8°C), two weeks when frozen (-20°C), and four days when stored at room temperature (15-30°C).

Interferences
1. A number of drugs and substances affect AST activity. See Young, et al.[12]
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]
3. Bilirubin to at least 18 mg/dl, and hemoglobin to at least 300 mg/dl, have been found to have a negligible effect on this procedure.

Materials Provided
AST (SGOT) Reagents R1 and R2

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Chemistry control, catalog number C7592-100

Test Parameters

Test:	AST	Chemistry:	Aspartate Aminotransferase
Chemistry No.:	203	Print Name:	AST
Reaction Type:	Kinetic	Reaction Direction:	Negative
Pri. Wave:	340 nm	Sec. Wave:	405 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	3 11
Unit:	U/L	Incubation Time:	3

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	6	uL	uL	uL	R1: 120	uL	uL
Decreased;		uL	uL	uL	R2: 30	uL	uL
Increased;		uL	uL	uL			

Linearity Range (Standard);	0-500		Linearity Limit:	0.3	
Linearity Range (Decreased):			Substrate Depletion:	5,000	
Linearity Range (Increased):			Mixed Blank Abs.:	-40000	40000
R1 Blank Abs.:	-40000	40000	On-board Stability:	30	Day (s)
Blank Response	-40000	40000	Reagent Alarm Limit:	5	
Twin Chemistry:					

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	U/L

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe
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Calibration Setup Parameters

Chem: AST
Calibration Setting
Math Model: K Factor
Factor: 4200.00 Replicates: 2
Acceptance Limits
Cal Time: 24 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	

Limitations

1. Samples with values above 500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.
2. Patients with severe vitamin B6 deficiency could have a decreased recovery of AST, presumably due to a lack of pyridoxal phosphate.[13]

Calibration

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Calculation (Example)

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$\text{AST (IU/L)} = \frac{\Delta\text{Abs./Min.} \times 1.10 \times 1000}{6.22 \times 0.10 \times 1.0} = \Delta\text{Abs./min.} \times 1768$$

Where ΔAbs./Min. = Average absorbance change per minute
1000 = Conversion of IU/ml to IU/L
1.10 = Total reaction volume (ml)
6.22 = Millimolar absorptivity of NADH
0.10 = Sample Volume (ml)
1.0 = Light path in cm

Example: If the average absorbance change per minute = 0.12 then 0.12 x 1768 = 212 IU/L

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal AST (SGOT) values. These controls should be run at least with every shift in which AST (SGOT) 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[13]

8 to 22 IU/L (30°C)
5 to 34 IU/L (37°C)
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

1. Linearity: 0-500 IU/L.
2. Comparison: A study was performed between the Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of 0.996 and a regression equation of y=1.069 x + 0.6. (n=50).
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[14]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
39.8	1.7	4.2	50.3	1.4	2.78
182.6	3.2	1.8	194.5	3.8	1.95

4. Sensitivity: The sensitivity for this reagent was investigated by reading the change in absorbance at 340nm for a saline sample and samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the AST (SGOT) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L AST activity gives a ΔAbs/Min. of 0.0004.

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders co., p 674 (1982).
2. Karmen, A., et al, J. Clin. Invest 34:126 (1955).
3. Henry, R.J., et al, Am. J. Clin. Path. 34:381 (1960).
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7. Expert Panel of Enzymes of the International Federation of Clinical Chemistry, Clin. Chem. 24:720 (1978).
8. Jung, K., Bohm, M., Enzyme 23:201 (1978).
9. Hafkenscheid, J.C.M., Dijit, C.C.M., Clin. Chem. 25/1:55 (1979).
10. Horder, M., Bowers, G.N., Jr., Clin. Chem. 23:551 (1977).
11. Henry, R.J., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, P882 (1974).
12. Young, D.S., et al, Clin. Chem. 21:1D (1975).
13. Kaplan, L.A., Pesce, A.J., Clinical Chemistry, St. Louis, C.V. Mosby, p.911-912 (1989).
14. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-A7561-100

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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CE

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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