ASPARTATE AMINOTRANSFERASE (AST/GOT) Kinetic

AST reagent is for the in- vitro quantitative determination of AST activity in human serum or plasma on both automatedand manual systems

REF: V/OT05.010	50 test	REF: V/OT04.025	100 test
REF: V/OT02.025	50 test	REF: V/OT04.050	200 test
REF: V/OT10.010	100 test	REF: V/OT05.050	250 test
REF: V/OT05.020	100 test		

CLINICAL SIGNIFICANCE [1]

The AST is a cellular enzyme, is found in highest concentration in heart muscle, the cells of the liver, the cells of the skeletal muscle and in smaller amounts in other weaves.
Although an elevated level of AST in the serum is not specific of the hepatic disease, is used mainly to diagnostic and to verify the course of this disease with other enzymes like ALT and ALP.
Also it is used to control the patients after myocardial infarction, in skeletal muscle disease and other.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

METHOD PRINCIPLE [3,4]

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxaloacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

The rate of decrease in concentration of NADH, measured photometrically is proportional to the catalytic concentration of AST present in the sample.

REAGENT COMPOSITION

R1:Buffer enzyme	
Tris buffer PH 7.7	80mmole/L
L-asparatat	>240mmole/L
MDH	>450U/L
LDH	>1200U/L
NaoH	220mmole/L
Sodium Azide	8mmole/L
R2: Coenzyme	
NADH	0.18mmole/L
2-Oxoglutarate	18mmole/L
Sodium Azide	8mmole/L

PRECAUTIONS AND WARNING

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** GOT reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie AST reagents (R1) and (R2) contain sodium azide which may reactwith copper or lead plumbing.
Preparing the working solution according to the number of tests required by mixing 4 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2), e.g. 400 µl R1 +100 µl R2.
All reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2-8ºC.Working solution is stable for 4 weeks at specified temperature.

Deterioration
The **Lab.Vie** AST reagent normally clear. Do not use **Lab.Vie** AST reagent if it is turbid or if the absorbance is less than 0.1 at 340nm.

SPECIMEN COLLECTION AND PRESERVATION [1]

Serum or Plasma
Anticoagulants currently in use like EDTA, oxalate and fluoride do not affect the results. Hemolysis interferes with the assay.

SYSTEM PARAMETERS

Wavelength	340 nm (334 – 365 nm)
Optical path	1Cm
Assay type	Kinetic
Direction	Decrease
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:10 1 ml 100µl
Temperature	37º c to 30ºc
Equilibration time	60 Sec
Read time	1-3 mins
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU High 2.5 AU
Sensitivity	5.0 U/L
Linearity	400 U/L

ASSAY PROCEDURE

	Macro	*Semi-micro*
Working Reagent	1.0 ml	500µl
Specimen	100µl	50µl

Mix, read initial absorbance after 60 seconds, and start timer simultaneously. Read again after 1, 2 and 3 minutes.
Determine the mean absorbance change per minute (ΔA/min).

CALCULATION

To calculate the AST/GOT activity uses the following formulae:
U/l = 1780 x ΔA 334 nm /min
U/l = 1746 x ΔA 340 nm /min
U/l = 3235 x ΔA 365 nm /min
µKat/L=IU/60

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie** technical support.

PERFORMANCE CHARACTERISTICS

Precision

	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	32.6	133	33.1	135.5
SD. mg/dl	1.3	1.3	1.5	1.42
CV. %	4.08	0.97	4.25	1.13

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie** AST (4+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera.
Correlation of 0.991 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity
The reaction is linear up to AST concentration of 400 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

INTERFERING SUBSTANCES [3,5]

Hemolysis
Postive interference because AST released from erythrocytes
Hemoglobin
Positive interference above 150µmol/L
Turbidity
No interference
Total bilirubin
Negative interference above 20mg/dL.
Icterus
No significant interference.
Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.
Anticoagulants
Citrate and fluoride inhibit the enzyme activity
Drugs
Calcium desolate and doxycycline HCL cause artificially low AST values at the tested drug level.

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

EXPECTED VALUES [2,5]

	30ºC	37ºC
Newborns	25-75 U/L	39-117 U/L
Infant	15-60 U/L	23-94 U/L
Adult	8 - 20 U/L	13 -31 U/L

Temperature conversion factor

To correct results to other temperatures multiply by:

Assay temperature	Conversion Factor to		
	20ºC	**30ºC**	**37ºC**
20ºC	1.00	1.37	2.08
30ºC	0.73	1.00	1.54
37ºC	0.48	0.65	1.00

These values are for orientation purpose; each laboratory should establish its own reference range.

DYNAMIC RANGE

5 - 400U/L

REFERENCES

1. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
2. Clinical guide to laboratory test4th ed.N.W.TIETZ(2006)p-154-159
3. IFCC Method for L- Aspartate aminotranferase.Jcli,chem.,clin.Biochem.(1986),24,p.497- 510
4. Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1112-116.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		
	Expiration Date		
	Manufactured by		

Ismailia – Free zone, Ismailia – Egypt
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