Spectrum For Diagnostic Industries

Aspartate aminotransferase (AST/GOT) UV-Kinetic (1+1)

IVD

REF.	Pack size
174 04 025	(4 X 25 ml) 100 tests

Intended Use

AST reagent is intended for the in-vitro quantitative, diagnostic determination of AST in human serum on both automated and manual systems.

Introduction

The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues such as myocardial infarction, viral hepatitis and muscular dystrophy. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the sera of patients with coronary and hepatobiliary diseases.

Method

Kinetic method according to the International Federation of Clinical Chemistry (IFCC).

Principle

The series of the reactions involved in the assay system is as follows:

1. The amino group is enzymatically transferred by AST present in the sample from L-aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

2. Oxaloacetate in presence of NADH and malate dehyrogenase (MDH), is reduced to L-malate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD.

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+

3. Addition of lactate dehydrogenase (LDH) to the reagent is necessary to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

Sample pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

Reagents

Buffer reagent

Tris buffer (pH 7.7)	80 mmol/L
L- Aspartate	240 mmol/L
MDH	>450 U/L
LDH	>1200 U/L
Sodium Hydroxide	220 mmol/L
Sodium Azide	8 mmol/L

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

Coenzyme

NADH	> 0.18 mmol/L
2 – Oxoglutarate	18 mmol/L
Sodium Azide	8 mmol/L

Reagents preparation, storage and stability

Prepare working solution as following:

Working solution can be prepared by adding equal volumes from R1 and R2; Stability: 2 days at 2 – 8 oC.

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 ^{o}C.
Once opened, the reagent is stable for 2 months at the specified temperature.

Deterioration

Do not use liquizyme AST reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm.
Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Precautions and Warnings

Do not ingest or inhalate. 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.

Specimen collection and preservation

Use non-hemolyzed serum. Heparin and EDTA are the only acceptable anticoagulants. The biological half-life of AST in serum is 17 hours.
Stability: 1 day at 15 – 25 ^{o}C; 7 days at 4 - 8^{o}C;
12 weeks at -20 ^{o}C

Procedure

Wavelength	340 nm
Optical path	1 cm
Assay type	Kinetic
Direction	decrease
Sample : Reagent Ratio	1 : 10
Temperature	37 ^{o}C or 30 ^{o}C
Equilibration time	30 seconds
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU High 2.5 AU

Working solution	1.0 ml (or add 0.5 ml R1+ 0.5 ml R2)
Specimen	100 µl

Mix, read initial absorbance after 30 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 use the following formulae:

U/L = 1746 x ΔA 340 nm /min

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run.

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).

Interference

Hemolysis
Erythrocyte contamination elevates results, since AST activities in erythrocytes are 15 times higher than those in normal sera.

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 dobesilate and doxycycline HCL cause artificially low AST values at the tested drug level.

Expected values

37 °C	Females	up to 31 U/L	(up to 0.52 µKat/L)
	Males	up to 37 U/L	(up to 0.62 µKat/L)
30 °C	Females	up to 21 U/L	(up to 0.35 µKat/L)
	Males	up to 25 U/L	(up to 0.42 µKat/L)

Temperature conversion factor is 1.37(25 ⟶ 30 °C) and 2.04 (25 ⟶ 37 °C).

Performance characteristics

A comparison between AST (1+1) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.98 was obtained.

Precision

Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (U/L)	32.6	133
SD	1.3	1.3
CV%	4.08	0.97

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	33.1	135.5
SD	1.5	1.42
CV%	4.25	1.13

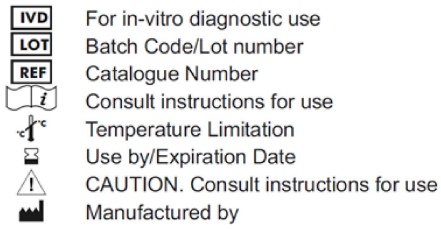
Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct 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. refer to special instructions/safety data sheets.

References

1. ECCLS. Determination of the catalytic activity concentration in serum on L- aspartate aminotransferase (EC 2.6.1.1,AST) Clin Chem. 1989;20:204-211.
2. Henry RJ, et al. Am j Clin Path 1960 :34:381
3. Young DS. Effects of drugs on clinical laboratory tests.Third edition.

SYMBOLS IN PRODUCT LABELLING



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