ASAT/AST/GOT

(KINETIC)

SINGLE REAGENT

INTENDED USE

Vitro AST reagent is intended for the in vitro quantitative determination of aspartate aminotransferase (EC 2.6.1.2) activity in serum on both automated and manual systems.

METHOD

Kinetic UV method according to IFCC specifications.

Liquid stable reagent.

BACKGROUND

Aspartate aminotransferase (glutamate oxaloacetate transaminase) belongs to the group of transaminases, which catalyze the conversion of amino acids to the corresponding □-keto acids via the transfer of amino groups; they also catalyze the reverse process. AST is commonly found in human tissue. Although heart muscle is found to have the most activity of the enzyme, significant activity has also been seen in the brain, liver, gastric mucosa, adipose tissue, skeletal muscles, and kidneys. AST is present in both cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of AST is that from the cytoplasm, with a smaller amount coming from the mitochondria. Severe tissue damage results in more of the mitochondrial enzyme being released. Elevated AST levels are found in hepatopathies, muscular dystrophy, and damage to internal organs. Increased levels of AST however are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis, and obstructive jaundice. Following a myocardial infarction, serum levels of AST are elevated and reach a peak 48 to 60 hours after onset[1].

ASSAY PRINCIPLE

In 1955, Karmen et al described the first kinetic determination of AST activity in serum[2], using a coupled reaction of malate dehydrogenase (MDH) and NADH. This assay system was critically evaluated and optimized in 1960 by Henry et al.[3]. A modification of this method incorporates lactate dehydrogenase into AST assay mixtures in order to accelerate the lag phase by exhaustion of endogenous ketoacids[4]. Vitro AST reagent is based on the recommendation of the IFCC[5]. The series of reactions involved in the assay system are as follows:

1. The amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate.
2. Oxaloacetate is reduced to malate by MDH present in the reagent with the simultaneous oxidation of NADH to NAD.

L-Asparte+2-Oxoglutarate $\xleftrightarrow{AST/GOT}$ **oxalacetate+L-Glutamate**

Oxalacetate+NADH+H$^+$ $\longleftrightarrow$ **L-Malate+NAD$^+$**

The rate of oxidation of the coenzyme NADH is proportional to the AST activity in the specimen. It is determined by measuring the decrease in absorbance at 334 / 340 / 365 nm correspondingly. Lactate dehydrogenase is included in the reagent to convert endogenous pyruvate in the specimen to lactate during the lag phase prior to measurement.

EXPECTED VALUES

	Male	Female
25^{0}C	Up to 18 U/l Up to 0.30 □kat/l	Up to 15 U/l Up to 0.25 µkat/l
30^{0}C	Up to 25 U/l Up to 0.42 □kat/l	Up to 21 U/l Up to 0.35 µkat/l
37^{0}C	Up to 37 U/l Up to 0.62 □kat/l	Up to 31 U/l Up to 0.52 µkat/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the cholesterol results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R$_1$	Tris buffer pH 7.8	80	mmol/l
	L-Aspartate	240	mmol/l
	LDH	900	U/l
	MDH	600	U/l
	NADH	0.18	mmol/l
	2-Oxoglutarate	12	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

Serum, EDTA or heparinized plasma. Avoid hemolysis

Specimen Preparation & Stability For serum specimen

Non-hemolyzed specimen is the specimen of choice[6]. Separate serum/plasma from clot/cells within 8 hours at room temperature or 24 hours at 2–8^{0}C.

AST activity is stable at 2-8 ^{0}C for 7 days. Freezing of the samples is not recommended[7].

PROCEDURE

- **Manual Procedure**

Wavelength	340,334,365 nm
Cuvette	1 cm light path
Temperature	25, 30 or 37 ^{0}C
Zero adjustment	against air

Pipette into test tube or cuvette	
Reagent	1 ml□
Serum or plasma	100 µl

Mix, incubate for 1.0 minute, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

- **Automated Procedure**

User defined parameters for different autoanalyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the AST/GOT activity by using the following formulae:

U/l = 1780 × ΔA 334 nm/min

U/l = 1746 × ΔA 340 nm/min

U/l = 3235 × ΔA 365 nm/min

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{\sum \times SV \times LP}$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*∑	millimolar absorptivity of NADH
LP	Cuvette path length in cm.
1000	Conversion of U/ml to U/l.

***** millimolar absorptivity of NADH at:

334 nm= 6.18,

340 nm= 6.22, and

365 nm= 6.40

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- **Unit conversion**
  U/l x 16.67 x 10^{-3} = µkat/l
- **Temperature correction**
  Multiply the result by 1.31 if the assay performed at 25^{0}C but is to be reported at 30^{0}C.
  Multiply the result by 1.91 if the assay performed at 25^{0}C but is to be reported at 37^{0}C.
  Multiply the result by 1.43 if the assay performed at 30^{0}C but is to be reported at 37^{0}C.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established AST/GOT values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.

If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Fluoride and citrate inhibit the enzyme activity. The only accepted anticoagulants are heparin and EDTA.
- **Bilirubin:**
  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- **Drugs:**
  Young[8] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
- **Haemolysis:**
  Any erythrocyte contamination elevates result, since AST activity in erythrocytes is fifteen times higher than in normal sera.
- **Lipemia:**
  Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

WARNING & PRECAUTION

- Vitro AST reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid or if the absorbance is less than 1.0 at 340 nm.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	17	135
SD (U/l)	0.72	1.05
CV (%)	4.26	0.77

Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	17.2	131
SD (U/l)	0.7	2.2
CV (%)	4.68	1.76

Method Comparison

Comparison studies were carried out using a similar commercially available AST(GOT) reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.
Number of sample pairs 64
Range of sample results 9 - 204 U/L (0.150 - 3.41 µkat/L)
Mean of reference method results 47 U/l
Mean of AST(GOT) results 39 U/l
Slope 0.84
Intercept -0.92 U/L
Correlation coefficient 0.999

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable AST/GOT activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 2 U/l or 0.03 □kat/l

LINEARITY

When run as recommended, the assay is linear up to 450 U/l or 7.46 □kat/l.

If result exceeds 450 U/l or 7.46 □kat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1511	2 x 25 ml
1512	5 X 20 ml

Manufactured in Egypt by:	Between Day

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