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# ASPARTATE AMINOTRANSFERASE (AST/GOT)

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

BS.1/OT05.010.0050	50 test	BS.1/OT02.025.0050	50 test
BS.1/OT10.010.0100	100 test	BS.1/OT04.025.0100	100 test
BS.1/OT05.020.0100	100 test	BS.1/OT04.050.0200	200 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  $\alpha$ -ketoglutarate forming glutamate and oxaloacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

Aspartate + 
$$\alpha$$
- Ketoglutarate  $\longrightarrow$  Glutamate +Oxaloacetate

Oxaloacetate + NADH + H $^+$   $\longrightarrow$  Malate + NAD $^+$ 

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 L-asparatat MDH LDH	80mmole/L >240mmole/L >450U/L >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.

**\$56:** dispose of this material and its container at hazardous or special waste collection point.

**\$57:** use appropriate container to avoid environmental contamination.

\$61: avoid release in environment.

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

# REAGENT PREPARATION, STORAGE AND STABILITY

**Bioscien** AST reagents (R1) and (R2) contain sodium azide which may react with 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  $2-8^{\circ}$ C. Working solution is stable for 4 weeks at specified temperature.

## Deterioration

The *Bioscien* AST reagent normally clear. Do not use 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)	
Ü	1	
Optical path	1Cm	
Assay type	Kinetic	
Direction	Decrease	
Sample Reagent Ratio	1:10	
e.g: Reagent volume	1 ml	
Sample volume	100µl	
Temperature	37° c to 30°c	
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 (ΔΑ/min).

#### **CALCULATION**

To calculate the AST/GOT activity uses the following formulae:

 $U/I = 1780 \quad x \quad \Delta A \ 334 \ nm \ /min$   $U/I = 1746 \quad x \quad \Delta A \ 340 \ nm \ /min$   $U/I = 3235 \quad x \quad \Delta A \ 365 \ nm \ /min$   $\mu Kat/L = IU/60$ 

# **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 **Bioscien** 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.	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 *Bioscien* 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  $5.0\,\mathrm{U/L}$ .

## 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 (resultx6).

## **INTERFERING SUBSTANCES (3,5)**

### Hemolysis

Positive 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)**

	30°C	37°C
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	C	onversion Factor	rto
temperature	20°C	30°C	37°C
20°C	1.00	1.37	2.08
30°C	0.73	1.00	1.54
37°C	0.48	0.65	1.00

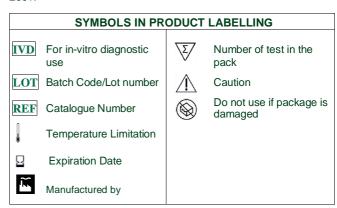
These values are for orientation purpose; each laboratory should establish its own reference range.

#### DYNAMIC RANGE

5 - 400U/L

#### **REFERENCES**

- Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
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- 3. IFCC Method for L- Aspartate aminotranferase. Jcli, chem., clin. Biochem. (1986), 24, p. 497-510
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