AST/ASAT/GOT

Colorimetric determination of serum GOT

REF. GOT-MC – 02100 (2 X 100 ml)

INTENDED USE

NS Biotec AST reagent is intended for the in vitro quantitative determination of aspartate aminotransferase on manual systems.

CLINICAL SIGNIFICANCE

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 levels of transaminases are indicative of myocardial infarction, 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[2].

ASSAY PRINCIPLE

Colorimetric methods based on formation of the chromogenic dinitrophenylhydrazone of oxaloacetate are in wide use. However, the accuracy of these methods is limited, and since dinitrophenylhydrazine reacts with **α** -ketoglutarate as well as oxaloacetate, high reagent blanks are obtained[1]. The series of reactions involved in the assay system is as follows:

1. The amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of □-oxoglutarate yielding oxaloacetate and L-glutamate.
2. Oxaloacetate formed is measured in its derivative form, 2.4-dinitrophenylhydrazone.

L-Aspartate + α-Oxoglutarate $\xleftrightarrow{\text{AST/GOT}}$ **Oxaloacetate +L-Glutamate**

The intensity of the color produced is directly proportional to the enzyme activity. It is determined by measuring the increase in absorbance at 530 – 550 nm.

EXPECTED VALUES

Serum Up to 12 U/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 AST results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Pyruvate standard	2.0	mmol/l
R_2	Phosphate buffer pH 7.4	100	mmol/l
	L-Aspartate	100	mmol/l
	α-oxoglutarate	4.0	mmol/l
R_3	2,4-dinitrophenyl-hydrazine	2.0	mmol/l
R_4	NaOH	4.0	N

- **Reagent Preparation & Stability**

The pyruvate standard (R_1), the buffer (R_2) and the color reagent (R_3) are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C. The sodium hydroxide reagent (R_4) should be completed to 1 liter before use.

SPECIMEN

Serum is the only accepted specimen. Avoid hemolysis.

- **Specimen Preparation & Stability**

Separate serum from clot/cells within 8 hours at room temperature or 48 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.

PROCEDURE

- **Manual Procedure**

Wavelength	546 nm (530 – 550)
Cuvette	1 cm light path
Temperature	37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum

Method (1) using table

	Blank	Specimen
R_2	0.5 ml	0.5 ml
incubate at 37^{0}C for 5 minutes		
Specimen Dist. H_2O	…… 100 µ l	100 µ l ……
Mix, incubate for ***exactly 30 minutes at 37^{0}C.***		
R_3	0.5 ml	0.5 ml
Mix, incubate for ***exactly 20 minutes at 20-25 ^{o}C***		
NaOH (0.4 N)	5.0 ml	5.0 ml

Mix, read the absorbance of specimen ($A_{specimen}$) against reagent blank after 5 minute.

The color development is stable for 60 minutes.

Obtain the activity of AST activity in serum specimen from the table (p.2).

Method (2) using standard curve

To plot a standard curve for GOT, dilute 1.5 ml of pyruvate standard with 4.5 ml of GOT buffer immediately before use.

- **Standard curve.**

1. Label tubes for standard curve from 1 to 10.
2. Pipette 200 µl of distilled water in all 10 standard tubes.
3. Add **R_2** (buffer reagent) and **R_1** (pyruvate standard) to the respective tubes as following:

Tube #	Pyruvate Standard (ml)	DI H2O (ml)	Buffer R2 (ml)
1	0.00	0.2	1.00
2	0.05	0.2	0.95
3	0.1	0.2	0.9
4	0.15	0.2	0.85
5	0.2	0.2	0.8
6	0.25	0.2	0.75
7	0.3	0.2	0.7
8	0.35	0.2	0.65
9	0.4	0.2	0.6
10	0.45	0.2	0.55

4. Pipette 1000 µl of **R_3** (color reagent) in all tubes, and incubate at 20-25^{0}C for *exactly 20 minutes.*
5. Pipette 10 ml of 0.4 N NaOH in all tubes. Mix, read the absorbance of against reagent blank after 5 minutes.
6. The color development is stable for one hour.

- **Assay.**

As same as Method (1) exactly but obtain the activity of AST activity in serum specimen from standard curve.

CALCULATION

- **For Method (1)**

Obtain the activity of AST in the serum specimen from the following table:

Absorbance	U/l
0.020	**7**
0.030	**10**
0.040	**13**
0.050	**16**
0.060	**19**
0.070	**23**
0.080	**27**
0.090	**31**
0.100	**36**
0.110	**41**
0.120	**47**
0.130	**52**
0.140	**59**
0.150	**67**
0.160	**76**
0.170	**89**

- **For Method (2)**

The absorbances of the increasing amounts of pyruvate standard correspond to the following transaminase activities in U/l.

Tube No.2	**6 U/l**
Tube No.3	**11 U/l**
Tube No.4	**16 U/l**
Tube No.5	**20 U/l**
Tube No.6	**25 U/l**
Tube No.7	**31 U/l**
Tube No.8	**37 U/l**
Tube No.9	**44 U/l**
Tube No.10	**52 U/l**

The standard curve is obtained by plotting the measured absorbances against the transaminase activities in U/l.

Ordinate: Absorbance
Abscissa: Activity in U/l

Obtain the activity of AST in the serum specimen from the standard curve.

LINEARITY

When run as recommended, the assay is linear up to absorbance 0.17 or 89 U/l

If result exceeds 89 U/l, specimen should be diluted 1+9 with 0.9% NaCl solution and reassayed. Multiply the result by 10.

SENSITIVITY

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

When run as recommended the sensitivity of this assay is 7 U/l.

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 NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **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:**
  Youngs[3] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Hemoglobin:**
  No interference from hemoglobin up to a level of 500 mg/dl.
- **Hemolysis:**
  Any erythrocyte contamination elevates result, since AST activity in erythrocytes is fifteen times higher than in normal sera.
- **Lipemia:**
  No relevant interference.
- **Pyruvate:**
  High levels of serum pyruvate may interfere with assay performance.

WARNING & PRECAUTIONS

- NS Biotec reagent is for in vitro diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed.
- Incubation time and temperature are vital factor, instructions must be followed exactly.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Don't use the reagent if it is turbid.

BIBLIOGRAPHY

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2. **Henry, JB, (1974):** Clinical Diagnosis and Management by Laboratory Methods. W.B. Saunders and Co., Philadelphia, PA. p 361.
3. **Young, Ds (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

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	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By