

ASAT/AST/GOT (COLORIMETRIC)



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 manual systems.

METHOD

Colorimetric method according to Reitman and Frankel¹.

BACKGROUND

Aspartate aminotransferase (glutamate oxaloacetate transaminase) belongs to the group of transaminases, which catalyze the conversion of amino acids to the corresponding α -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 onset².

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 obtained¹. The series of reactions involved in the assay system is as follows:

- The amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of α -oxoglutarate yielding oxaloacetate and L-glutamate.
- Oxaloacetate formed is measured in its derivative form, 2,4-dinitrophenylhydrazone.



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	mol/l

• Reagent Preparation & Stability

The pyruvate standard (R₁), the buffer (R₂) and the color reagent (R₃) are ready for use and stable up to the expiry date given on label when stored at 2–8°C. The sodium hydroxide reagent (R₄) should be completed to 1 liter before use.

SPECIMEN

Serum is the only accepted specimen. Avoid hemolysis.

Specimen Preparation & Stability For serum specimen

Separate serum from clot/cells within 8 hours at room temperature or 48 hours at 2–8°C. AST activity is stable at 2–8 °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 °C
Zero adjustment	against reagent blank
Specimen	Serum

“Transaminase activities in some sera are stimulated by high concentrations of aldehydes, ketones, or oxoacids. Measurement against a sample blank instead of reagent blank is highly recommended to avoid the risk of finding such artifacts”.

Method (1) using table

	Blank	Specimen
R ₂	0.5 ml	0.5 ml
incubate at 37°C for 5 minutes		
Specimen	100 μ l
Dist. H ₂ O	100 μ l
Mix, incubate for exactly 30 minutes at 37°C .		
R ₃	0.5 ml	0.5 ml
Mix, incubate for exactly 20 minutes at 20-25 °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.

- Label tubes for standard curve from 1 to 10.
- Pipette 200 μ l of distilled water in all 10 standard tubes.
- Add R₂ (buffer reagent) and R₁ (pyruvate standard) to the respective tubes as following:

Tube #	Pyruvate Standard (ml)	DI H ₂ O (ml)	Buffer R ₂ (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 R₃ (color reagent) in all tubes, and incubate at 20 - 25°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/I	Absorbance	U/I
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59
0.070	23	0.150	67
0.080	27	0.160	76
0.090	31	0.170	89

● **For Method (2)**

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

Tube No.2	06 U/I	Tube No.7	31 U/I
Tube No.3	11 U/I	Tube No.8	37 U/I
Tube No.4	16 U/I	Tube No.9	44 U/I
Tube No.5	20 U/I	Tube No.10	52 U/I
Tube No.6	25 U/I		

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

Ordinate: Absorbance

Abscissa: Activity in U/I

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

Units

One Wróblewski unit (WU) of GOT or GPT is defined as the amount of enzyme that will form 4.82 x 10⁻⁶ µmole of Glutamate/min (25°C).

To convert international units (U/I), into WU multiply results by 2.07

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

● **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.

Youngs³ 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 & PRECAUTION

- A Vitro 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.

Method Comparison

Comparison studies were carried out using a similar commercially available GOT reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The results obtained did not show systematic differences when compared with other commercial reagents.

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/I.

LINEARITY

Linearity










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

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

BIBLIOGRAPHY

1. **Reitman, S, and Frankel, S.(1957):** Amer. J. Clin. Path. 28: 56.
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.

SYMBOL DECLARATION

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

ORDERING INFORMATION

REF	SIZE
1221	2 X 60 ml
1222	2 X 100 ml

Manufactured in Egypt by:

Drugs: Vitro Scient
www.vitroscent.com

Technical Support:

+202 26224468,
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+202 26218944,
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