GOT/AST - L

SCE recommended method for quantitative determination of aspartate aminotransferase (AST) activity in serum and plasma

IVD

ORDER INFORMATION

REF	Kit size
GA4910 00	5x40 + 1x20 ml
GA4911 00	10x40 + 2x20 ml
KL4910 00	8x50 + 8x5 ml
BK4910 00	2x(80+8 ml)

INDICATION

Measurement of the activity of serum aminotransferases (formerly called transaminases) is indicated in the diagnosis of acute hepatic disorders and in monitoring their evolution. Increased aspartate aminotransferase (AST) levels, however, can occur in connection with damages of hearts or skeletral muscle as well as of liver perenchyma. In patients with myocardial infarction, there is an increased AST concentration in blood due to its rapid release into the by the damaged cells; therefore it is an important clinical parameter for the evaluation of this pathology.

METHOD PRINCIPLE

Optimized UV test according to SCE (Scandinavian Committee on Enzymes) recommendations.
The principle of the method is based on the following enzymatic reactions:

$$\text{L-Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{L-Glutammate} + \text{Oxalacetate}$$

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

Decrease in absorbance value at 340 nm, due to the oxidation of NADH to NAD^+, is directly proportional to the AST activity in the sample.

COMPOSITION

REAGENT A:

TRIS	28 mmol/l
EDTA-Na_2	5.68 mmol/l
L-Aspartate	284 mmol/l
MDH	≥ 800 U/l
Sodium azide	2 g/l

Harmful (x_n) R28-32; S1/2-28-45-60-61

REAGENT B:

2-Oxoglutarato	68 mmol/l
NADH	1.12 mmol/l
Sodium azide	0.095 g/l

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 10 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 2 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 5 days at 15-25 °C or 28 days at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, (heparin or EDTA) plasma.
Do not use haemolysed samples because haemolysis can cause falsely positive results. Do not use anticoagulants containing ammonium salts (ex. ammonium heparin).

Loss of activity within 3 days: at 2-8 °C < 8%
at 15-25 °C < 10%
Stability at - 20 °C at least 3 months.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known GOT/AST activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	340 nm (334 nm, 365 nm)
Optical path	1 cm
Reaction	Kinetic (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Sample
Reagent A	1000 µl
Sample	100 µl
Mix and incubate at 37 °C for 5 minutes, then add:	
Reagent B	100 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Sample
Working reagent	1000 µl
Incubate at 37 °C for 5 minutes, then add:	
Sample	100 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Note

- Reaction volumes can be proportionally changed.
- For values upper than 440 U/l dilute samples 1+9 with saline solution and multiply result by 10.

CALCULATION OF RESULTS

Activity (U/l) = ΔA/min x factor (f) indicated in the following table:

Bireaegent procedure

340 nm	f = 1905
334 nm	f = 1945
365 nm	f = 3529

Monoreagent procedure

340 nm	f = 1746
334 nm	f = 1780
365 nm	f = 3235

Note:
As the factor "f" used to calculate results depends on several variables (wavelength, temperature, sample volume, reaction volume…), it is recommended to use commercial calibration sera to asset the instruments.

REFERENCE VALUES

Male: 10÷50 U/l
Female: 10÷35 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

		Within Run		Between Run	
Sample	**Mean (U/l)**	**SD**	**%CV**	**SD**	**%CV**
Serum 1	44.7	0.69	1.5	2.23	5.0
Serum 2	132.4	2.00	1.5	6.74	5.1

Linearity
The assay is linear up to 440 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 1 U/l.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

y = 1.0457x – 0.8281 U/l r = 0.9853

Interferences

Bilirubin	> 40 mg/dl
Triglycerides	> 2000 mg/dl
Ascorbic acid	> 30 mg/dl
Hemoglobin	The presence of hemoglobin in serum indicates destruction of erythrocytes with release of AST, producing high interference.

PRECAUTIONS IN USE

Reagent A is harmful.
Refer to Safety Data Sheet.
Reagent B is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY

1. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
3. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for the determination of four enzymes in blood. Scand J Clin Lab Invest 1974; 33:291-306.
4. Penttilä IM, Jokela HA, Viitala AJ, Heikkinen E, Nummi S, Pystynen P et al.: Activities of aspartate and alanine aminotransferases and alkaline phosphatase in sera of healthy subjects. Scand J Clin Lab Invest 1975; 35: 275-84.
5. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. Biochimica Clinica, 1994; 18, 10:673.
6. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
7. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

AMS S.p.A. – Registered Office and Plant
Via E. Barsanti 17/A
00012 Guidonia (Rome) – Italy
Ph. + 39 0774 354441 r.a.
Fax + 39 0774 578035
www.ams-analyzers.com
info@ams-analyzers.com

Diagnostics Manufacturing
Via Galileo Galilei, 38
Seggiano di Pioltello (MI) - Italy
Ph. + 39 02 929189.1
Fax + 39 02 929189.39
www.gdsrl.com
infodiagnostics@ams-analyzers.com

Met. GA491000.0 eng
Edition: 2011/10/04