GOT(AST) UV AA

Optimized UV method (IFCC) for the determination of Aspartate Aminotransferase (AST/GOT) in serum or plasma

SUMMARY

Aspartate Aminotransferase (AST or GOT) is a bilocular (cytoplasmic and mitochondrial) enzyme, and its highest activity is located in the hepatic and heart tissue. Any alteration in these tissues causes an increase in circulating AST levels. In Acute Myocardial Infarction the enzyme shows a moderate increase starting after 6 to 8 hours of onset, reaches its maximum value at about 48 hours and returns to normal values between day 4 and 6. Highest AST levels are found in patients with liver disease, particularly in cases of hepatitis with necrosis. AST testing acquires diagnostic relevance when its values are compared to those of other enzymes of similar tissue origin, allowing to the enzymatic profile of organs such as heart and liver.

PRINCIPLE

The reaction system is as follows:

$$\text{L-aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{GOT}} \text{oxaloacetate} + \text{L-glutamate}$$

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

PROVIDED REAGENTS

A. Reagent A: vials containing 2-Oxoglutarate, reduced NADH, Malate Dehydrogenase (MDH), and Lactate Dehydrogenase (LDH).

B. Reagent B: Tris buffer solution, pH 7.8 (at 30ºC) with L-Aspartate.

Final concentrations (according to IFCC and SSCC)

Tris	80 mmol/l; pH 7.8 (at 30ºC)
L-Aspartate	240 mmol/l
NADH	0.18 mmol/l
MDH	≥ 420 U/l
LDH	≥ 600 U/l
2-Oxoglutarate	12 mmol/l

INSTRUCTIONS FOR USE

Reagent B: ready to use.

Reagent A; reconstitution: add 20 ml Reagent B to a vial of Reagent A. Cap tightly. Shake until complete dissolution. Date.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Reagent B contains azide.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.

Reconstituted Reagent A: stable in refrigerator for 30 days (2-10ºC) or 3 days at room temperature from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero with distilled water, absorbance readings of the reconstituted Reagent A lower than 0.800 O.D. or higher than 1.800 O.D. (at 340 nm), indicate its deterioration.

SAMPLE

Serum or plasma

a) Collection: obtain in the usual way.

b) Additives: when using plasma, only use heparin as anticoagulant.

c) Known interfering substances:

- Samples from hemodialyzed patients or patients with hypovitaminosis of other pyridoxal phosphate deficiencies produce falsely decreased values.
- No interferences are observed by bilirubin up to 60 mg/l, triglycerides up to 5.5 g/l or hemoglobin up to 0.14 g/dl.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: GOT in serum is stable in refrigerator up to 3 days without preservatives. Do not freeze.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes.
- Water bath at temperature indicated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS

(Decrease of absorbance)

- Wavelength: 340 nm (Hg 334 or 366).
- Reaction temperature: 25, 30 or 37ºC. See REFERENCE VALUES corresponding to each temperature.
- Reaction time: 4 minutes.
- Volumes of Sample and Reconstituted Reagent A may be proportionally reduced, without altering the corresponding calculation factors.

PROCEDURE

A) 30 or 37°C

I- MACROTECHNIQUE

In a cuvette at 30-37°C, place:

Reconstituted Reagent A	2 ml
Sample	200 ul

Mix immediately and simultaneously start stopwatch. After 1 minute record initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes after the first reading. Determine average change in Absorbance/min (ΔA/min), substracting each reading from the previous one and averaging these values. Use this mean for calculations.

II- MICROTECHNIQUE

In a cuvette at 30-37°C, place:

Reconstituted Reagent A	1 ml
Sample	100 ul

Mix immediately. Follow the steps described in Procedure A-I.

B) 25°C

MACROTECHNIQUE

Use 500 ul sample. After adding the sample, mix immediately and simultaneously start stopwatch. After 3 minutes record initial absorbance (see PROCEDURE LIMITATIONS) and then follow the steps described in Procedure A-I.

CALCULATIONS

GOT (U/l) = ΔA/min x factor

In each case, the corresponding calculation factor should be used, depending on the selected reaction temperature (30-37°C or 25°C) as shown in the table below:

Temperature / Wavelength	30-37°C	25°C
340 nm	1,740	791
334 nm	1,780	809
366 nm	3,207	1,453

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known aspartate aminotransferase activity.

REFERENCE VALUES

Temperature	25°C	30°C*	37°C*
Men	up to 18 U/l	up to 25 U/l	up to 38 U/l
Women	up to 15 U/l	up to 21 U/l	up to 32 U/l

*Calculated

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

GOT (U/l) x 0.017 = GOT (ukat/l)

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

- Low initial Absorbance: once the serum is added, if the first reading (0 time) is lower that 0.800 O.D., with the Reagent A (substrate) in good conditions, it indicates a sample with a very high GOT activity (that consumes NADH even before this reading) or with a particularly high concentration of endogenous ketoacids. In this case, repeat assay with the sample diluted with saline solution and multiply the results by the dilution performed.
- Moistening deteriorates the Substrate.

PERFORMANCE

a) Reproducibility: precision studies were performed following the guidelines contained in NCCLS (National Committee on Clinical Laboratory Standards) document EP5-A.

Within Run (n = 20)

Level	S.D.	C.V.
37 U/l	± 1.6 U/l	4.4 %
180 U/l	± 2.4 U/l	1.3 %

Run to run (n = 20)

Level	S.D.	C.V.
37 U/l	± 1.8 U/l	4.9 %
180 U/l	± 2.8 U/l	1.6 %

b) Sensitivity: it depends on the photometer used and the wavelength. In spectrophotometers with 1 cm optical length square cuvettes, for a ΔA minimum of 0.001, the smallest detectable activity change will be of 1.8 U/l (at 340 nm and at 30 or 37°C).

c) Dynamic range: the reading range is extended up to 0.200 O.D. ΔA/min (at 340-334 nm). If the ΔA/min is higher than 0.200 O.D. (340-334 nm) or 0.100 O.D. (366 nm), repeat the assay with diluted sample (1:5 or 1:10) with saline solution, correcting the results accordingly.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB. PROVIDES

- 10 x 20 ml (200 ml Reagent B) (Cat. Nº 1751302).

REFERENCES

- IFCC - Clin. Chim. Acta 70/2:F19 (1976).
- SSCC - Scand. J. Clin. Lab. Invest. 33:291 (1974).
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leberkrankheiten. Stuttgart: Georg Thieme Verlag, 1974.
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SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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