



GOT(AST)

LIQUID LINE



AA

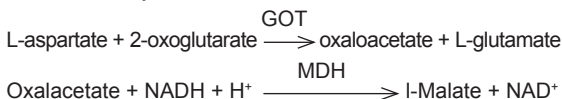
Optimized UV method (IFCC) for the determination of Aspartate Aminotransferase (AST/GOT) in serum or plasma

SUMMARY

Aspartate Aminotransferase 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.

PRINCIPLE

The reaction system is as follows:



PROVIDED REAGENTS

A. Reagent A: TRIS buffer solution, pH 7.8 with L-Aspartate.
B. Reagent B: vials containing 2-Oxoglutarate, reduced NADH, Malate Dehydrogenase (MDH), and Lactate Dehydrogenase (LDH).

Final concentrations

TRIS	100 mmol/l; pH 7.8
L-Aspartate	200 mmol/l
NADH	0.18 mmol/l
MDH	≥ 400 U/l
LDH	≥ 600 U/l
2-Oxoglutarate	12 mmol/l

INSTRUCTIONS FOR USE

Provided Reagents: ready to use. They may be used separately or as **Monoreagent**, mixing 4 parts Reagent A and 1 part Reagent B (e.g. 4 ml Reagent A + 1 ml Reagent B).

WARNINGS

Reagents are for "in vitro" diagnostic use.
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 (2-10°C) until the expiration date shown on the box.
Once opened, do not keep out of the refrigerator for extended periods of time. Avoid contamination.

Monoreagent (premixed): stable in refrigerator for 2 months (2-10°C) from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

The Reagent B may develop a pink brown color that does not affect its performance.
When spectrophotometer has been set to zero with distilled water, absorbance readings of the Monoreagent lower than 0.900 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 or EDTA as anticoagulants.
- 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 30 mg/dl, nor triglycerides up to 500 mg/dl. Hemoglobin significantly interferes increasing the results because of GOT presence in erythrocytes.

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

- Wavelength: 340 nm
 - Reaction temperature: 25, 30 or 37°C. See REFERENCE VALUES corresponding to each temperature.
 - Reaction time: 4 minutes.
 - Sample volume: 100 ul.
- Sample and Reagent volumes may be proportionally changed, without altering the corresponding calculation factors.

PROCEDURE

- A) 30 or 37°C**
- I- MONOREAGENT TECHNIQUE
- In a cuvette at 30-37°C, place:

Monoreagent	1.0 ml
Preincubate for a few minutes. Then add:	
Sample	100 ul
Mix immediately and simultaneously start stopwatch. After 90 seconds record initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes after the first reading. Determine average change in Absorbance/min ($\Delta A/\text{min}$), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.	
II- SEPARATE REAGENTS TECHNIQUE	
In a cuvette at 30-37°C, place:	
Reagent A	0.80 ml
Sample	100 ul
Preincubate for a few minutes. Then add:	
Reagent B	0.20 ml
Mix immediately and simultaneously start stopwatch. After 90 seconds record initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes after the first reading. Determine average change in Absorbance/min ($\Delta A/\text{min}$), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.	
B) 25°C	
Use 250 ul Sample and follow the procedure described in A).	

Low initial absorbance: when the first reading (0 time) is lower than 0.900 O.D. after the serum addition, being the Reagent B 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.

PERFORMANCE

a) Reproducibility: testing replicates of the same sample simultaneously, the following values are obtained:

Level	S.D.	C.V.
58.45 U/l	± 1.67 U/l	2.86 %
148.30 U/l	± 2.85 U/l	1.92 %

b) Sensitivity: the smallest detectable GOT activity distinguishable from zero will be 6 U/l.

c) Dynamic range: the reading range is extended up to 0.345 $\Delta A/\text{min}$ (at 340 nm). If the $\Delta A/\text{min}$ is higher than 0.345, repeat the assay with diluted sample (1:5 or 1:10) with saline solution, correcting the results according to the dilution factor used.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB. PROVIDES

200 ml: - 4 x 40 ml Reagent A
 - 1 x 40 ml Reagent B
 (Cat. N°: 1752360).

250 ml: - 4 x 50 ml Reagent A
 - 4 x 12.5 ml Reagent B
 (Cat. N°: 1009321).

250 ml: - 4 x 50 ml Reagent A
 - 4 x 12.5 ml Reagent B
 (Cat. N°: 1009261).

250 ml: - 4 x 50 ml Reagent A
 - 4 x 12.5 ml Reagent B
 (Cat. N°: 1009619).

REFERENCES

- IFCC - Clin. Chim. Acta 70/2:F19 (1976).
- SSCC - Scand. J. Clin. Lab. Invest. 33:291 (1974).
- DGKC - Z. Klin. Chem. 10:281 (1972).
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- Bergmeyer H.V., Horder, M., Rej R. - J. Clin. Chem. Clin. Biochem. 24:497, 1986.
- Dufour, D.R.; Lott, J.A.; Nolte, F.S.; Gretch, D.R.; Koff, R.S. and Seeff, L.B. - Clin. Chem. 46/12:2027, 2000.
- "Tietz textbook of Clinical Chemistry" - Burtis and Ashwood Editors, 3rd Ed. - Saunders Co., 1999.

CALCULATIONS

GOT (U/l) = $\Delta A/\text{min}$ x factor

In each case, the corresponding calculation factor should be used, depending on the selected reaction temperature:

factor_(30-37°C) = 1,746

factor_(25°C) = 794

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)

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.

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

Symbols

The following symbols are used in the packaging of Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Do not freeze



Calibrator



Biological risks



Control



Volume after reconstitution



Positive Control



Contents




Negative Control



Batch code



Catalog number

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