GPT(ALT) UV AA

Optimized UV method (IFCC) for the determination of Alanine Aminotransferase (GPT/ALT) in serum or plasma

SUMMARY

Alanine Aminotransferase (ALT or GPT) is an unilocular (cytoplasmic) enzyme, and its highest activity is located in the hepatic tissue. The destruction or any change in the permeability of the cellular membranes causes the releasing of ALT into the blood stream. The highest increases of ALT activity in serum are caused by hepatic alterations.

In the case of viral hepatitis, the increase of ALT precedes jaundice, reaching the maximum after this symptom is observed. If the values remain high after 6 weeks, the possibility of an active hepatitis or the beginning of a chronic hepatitis must be inferred, that is why the serial determinations of the enzyme are very useful.

ALT determination becomes of diagnostic importance when its values are compared with the values of other enzymes of similar tissue origin, allowing to complete the enzymatic profile of organs such as the liver.

PRINCIPLE

The reaction system is as follows:

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

PROVIDED REAGENTS

A. Reagent A: vials containing 2-Oxoglutarate, reduced Nicotinamide Adenine Dinucleotide (NADH) and Lactate Dehydrogenase (LDH).

B. Reagent B: Tris buffer solution, pH 7.5 (at 30ºC) with L-Alanine.

Final concentrations (according to IFCC and SSCC)

Tris	100 mmol/l, pH 7.5 (at 30ºC)
L-Alanine	500 mmol/l
NADH	0.18 mmol/l
LDH	≥ 1,200 U/l
2-Oxoglutarate	15 mmol/l

INSTRUCTIONS FOR USE

Reagent B: ready to use.

Reagent A: add 20 ml Reagent B to a Reagent A vial. Cap tightly and 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 (2-10ºC) for 30 days or 3 days at room temperature from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When the 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 interference substances:

- Samples with visible or intense hemolysis should not be used as they produce falsely increased results.
- Samples from hemodialyzed patients or patients with hypovitaminosis or other pathologies associated with pyridoxal phosphate deficiencies, produce falsely increased values.

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

d) Stability and storage instructions: GPT in serum is stable up to 3 days in refrigerator (2-10ºC) without preservatives. Do not freeze.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at the temperature indicated in the procedure to follow.
- Stopwatch.

ASSAY CONDITIONS

(Absorbance decrease)

- Wavelength: 340 nm (Hg 334 or 366).
- Reaction Temperature: 25, 30 or 37ºC. See REFERENCE VALUES corresponding to each temperature.
- Reaction Time: 4 minutes
- Sample and Reconstituted Reagent A volumes can be proportionally reduced without varying the 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 the stopwatch. After 1 minute record the initial absorbance and then at 1, 2 and 3 minutes from the first reading. Determine average change in Absorbance/min (ΔA/min) subtracting each reading from the previous one and averaging these values. Use this means for the 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 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 the follow the steps described in Procedure A-I.

CALCULATIONS

GPT (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	1740	791
334 nm	1780	809
366 nm	3207	1453

QUALITY CONTROL METHOD

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

REFERENCE VALUES

Temperature	25°C	30°C*	37°C*
Men	up to 22 U/l	up to 29 U/l	up to 41 U/l
Women	up to 17 U/l	up to 22 U/l	up to 31 U/l

*Calculated

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

SI SYSTEM UNITS CONVERSION

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

PROCEDURE LIMITATIONS

See Known interference substances under SAMPLE.

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

Moistening deteriorates the Reagent A.

PERFORMANCE

a) Reproducibility: when replicates of a sample are assayed at the same time, the following results are obtained:

Level	S.D.	C.V.
19.8 U/l	± 1.11 U/l	5.63 %
118 U/l	± 2.02 U/l	1.71 %

b) Detection Limit: it depends on the photometer and the wavelength. According to the sensitivity required, with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light, ≤ 8 nm pathlength, 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 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

Kit for 10 x 20 ml (200 ml Reagent B) (Cat. 1761302).

REFERENCES

- I.F.C.C. - Clin. Chim. Acta 105:147 F (1980).
- S.S.C.C. - Scand. J. Clin. Lab. Invest. 33:291 (1974).
- D.G.K.C. - Z. Klin. Chem. 10:281 (1972).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

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

Σ — Conteins 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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