ALT/GPT BR opt. CE

CONTENTS			
REF	1105000	ALT/GPT BR opt.	2 x 50 mL
	1105010	ALT/GPT BR opt.	3 x 100 mL
For *in vitro* diagnostic use only			

ALT/GPT BR

IFCC
UV enzymatic method
KINETIC

PRINCIPLE

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).
The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+, proportional to the activity of ALT present in the sample.

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

$$\text{Pyruvate + NADH + } H^+ \xrightarrow{\text{LDH}} \text{Lactate + } NAD^+$$

The method follows the proposed optimised formulation of the IFCC[1].

REAGENT COMPOSITION

R1 **ALT substrate**. TRIS buffer 150 mmol/L pH 7.3, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L.

R2 **ALT coenzyme**. NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L. Biocides.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 4 weeks at 2-8ºC. Protect from light.
Discard the reagent if presents an absorbance below 1.200 at 340 nm against distilled water or if it fails to recover the declared values of control sera.

SAMPLES

Serum and EDTA or heparinized plasma free of hemolysis.
ALT is stable in serum or plasma 24 hours at room temperature and for 1 week at 2-8ºC.

INTERFERENCES

- Samples from patients under hemodialysis, severe vitamine B deficiency or with related pathologies, lead to an underestimation of ALT values.
- As a result of the high levels of ALT in red cells hemolyzed samples are not suitable for testing.
- Lipemic samples (triglycerides up to 2 g/L) and icteric samples (bilirubin >20 mg/dL) do not interfere.
- Other drugs and substances may affect the ALT values.[2]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37ºC, capable to read at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	37ºC	30ºC
Working reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

U/L = ΔA/min x 3333 (37ºC)
U/L = ΔA/min x 1746 (30ºC)

Samples with ΔA/min exceeding 0.160 at 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L

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REFERENCE VALUES[3]

Serum, plasma

Adults	37ºC	up to 40 U/L (0.67 µkat/L)
	30ºC	up to 25 U/L (0.42 µkat/L)

Levels approximately twice the adult level are seen in neonates and infants; these decline to adult level by approximately 6 months of age.
It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of ALT. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of ALT. Assayed.

CLINICAL SIGNIFICANCE

The group of enzymes called transaminase exist in tissues of many organs. Necrotic activity in these organs causes a release of abnormal quantitaties of enzyme into the blood where they are measured.

Since heart tissue is rich in AST increased serum levels appear in patients after myocardial infarction, as well as in patients with muscle disease, muscular dystrophy and dermatomyositis.

The liver is specially rich in ALT, being this enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated AST levels in these patients.

Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 500 U/L
- ***Precision***

U/L	Within-run		
Mean	75	165	540
SD	1,25	1,89	5,6
CV%	1,67	1,15	1,04
N	10	10	10

Replicates: 10 for each level.
Instrument: CECIL CE 2001

- ***Sensitivity.*** Using this reagent and method an ΔA/min of 0.00´ read at 340 nm is equivalent to 2.3 U/L of GPT activity.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
N = 25 r = 0.998 y = 0.96x – 1.32

REFERENCES

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