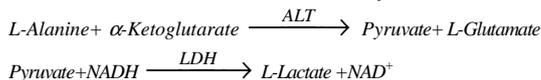


Reagent kit for the determination of the alanine aminotransferase (ALT) activity in serum based upon IFCC recommendations.

ALT is found in the cytosol of cells, it is a non-tissue specific soluble enzyme. ALT catalyses the transfer of amino groups during the transformations of aminoacids and alpha-ketoacids. Pyridoxal phosphate activates the process. The enzyme found in the serum is principally derived from the liver and kidney. The serum enzyme activity is increased during various hepatic disease states including hepatitis.

Principle

ALT catalyses the transformation of L-Alanin and 2-Oxoglutarate at optimal pH. The Pyruvate released in the reaction is transformed by Lactate dehydrogenase (LDH) in the presence of NADH /NAD⁺ coenzyme to L-lactate, while the NADH/NAD⁺ oxidoreductive process shows a decrease in absorbance at 340 nm. The change in absorbance correlates with serum ALT activity.



Reference values

ALT activity: < 40 U/l (<0,67 µkat/l)

It is recommended that each laboratory should assign its own normal range.

Reagents

1.Reagent (R1)

Tris buffer, pH:7.50	110 mmol/l
L-Alanine	600 mmol/l
LDH	1500 U/l
NADH	240 µmol/l

2.Reagent (R2)

α-Ketoglutarate	16 mmol/l
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Precaution

Discard cloudy reagent. These reagents contain 0.1% sodium azide. Avoid contamination by using clean laboratory materials (pipettes, plastic vials for analyzers, ...). To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Samples

Serum free of haemolysis. Haemolysis, lipaemia interfere with the test.

PROCEDURE

Preparation and stability of working reagent

- One-reagent procedure:

Mix 2 volumes of reagent 1 with 1 volume of reagent 2.

Stability:	at 20-25 °C:	5 days
	at 2-8 °C:	2 weeks

- Two-reagent procedure: reagents are ready for use.

If the absorbance of working reagent is lower than 1.1 at 334 nm the reagent can not be used.

Assay conditions

Wavelength :	340 (334-365) nm
Temperature :	37°C
Cuvette :	1 cm light path
Read against:	distilled water
Method:	kinetic (decreasing)

One-reagent procedure

Working reagent	1 ml
Sample or Control	100 µl

Mix and after a 2-minute incubation, measure the change of absorbance per minute (ΔA/min) during 2 minutes.

Two-reagent procedure

R1	1 ml
Sample or Control	150 µl

Mix, incubate for one minute 37 °C and add:

R2	500 µl
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Mix and after a 2-minute incubation, measure the change of absorbance per minute (ΔA/min) during 2 minutes.

Calibration(37°C, IFCC without pyridoxal-phosphate)

- S1: Distilled water
- S2: Roche C.F.A.S. (Calibrator for automated system) or Randox Calibration Serum Level I or Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended

- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance, C = Concentration

Calculation using factor

340 nm: Activity (U/l) = ΔA/min. x 2200; (µkat/l) = ΔA/min. x 36,67

334 nm: Activity (U/l) = ΔA/min. x 1950; (µkat/l) = ΔA/min. x 32,50

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 400 analyzer (37°C).

Linearity

The test is linear up to 450 U/l (7,50 µkat/l) GPT activity.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 1.95 U/l (0,033 µkat/l) GPT activity at 334 nm.

Precision

	Reproducibility		
	Average activity (U/l)	SD	CV%
sample I	34.2	0.80	2.35
sample II	136	0.80	0.59

	Repeatability		
	Average activity (U/l)	SD	CV%
sample I	14.5	0.158	1.09
sample II	318	3.032	0.95

Correlation

Comparative studies were done to compare our reagent with another commercial GPT reagent.

The results from these studies are detailed below.

Correlation coefficient: r=0.9997

Linear regression: y (U/l)= 1.001x+0.199

(x= other commercial reagent, y= own reagent).

Specificity

Hemoglobin 1.6 µmol/l (10 mg/dl), bilirubin 257 µmol/l (15 mg/dl), lipid 300 mg/dl, glucose 55.5 mmol/l (1000 mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.

Note

The test doesn't contain pyridoxal-phosphate.

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only.

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Expert Panel on enzyme of the IFCC, Clin. Chem. Acta, 1976. 70:F19.