ALAT/ALT/GPT

(KINETIC)

4 + 1

VITRO SCIENT.

INTENDED USE

Vitro ALT reagent is intended for the in vitro quantitative determination of alanine aminotransferase (EC 2.6.1.2) activity in serum on both automated and manual systems.

METHOD

Kinetic UV method according to IFCC specifications.

Liquid stable reagent.

BACKGROUND

Alanine aminotransferase (glutamate pyruvate transaminase) belongs to the group of transaminases, which catalyze the conversion of amino acids to the corresponding α-keto acids via the transfer of amino groups; they also catalyze the reverse process. Although higher activities exist in the liver, minor activity can also be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction[1]. Although both serum aspartate aminotransferase AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT persist longer than elevations of AST activity.

ASSAY PRINCIPLE

ALT methods using U.V. procedures were first described by Henley[2] in 1955. Wroblewski and LaDue[3] in 1956 described a method for determining ALT using lactate dehydrogenase (LDH) and nicotinamide adenine dinucleotide, reduced (NADH). This method was later modified by Henry[4] and Bergmeyer[5] to optimize substrate conditions and eliminate side reactions. The Vitro ALT reagent is based on the recommendations of the IFCC[6]. The series of reactions involved in the assay system is as follows:

1. The amino group is enzymatically transferred by ALT present in the specimen from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.
2. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD.

L-Alanine+2-Oxoglutarate $\xleftrightarrow{ALT/GPT}$ **Pyruvate+L-Glutamate**

Pyruvate+NADH+H$^+$ $\longleftrightarrow$ **L-Lactate+NAD$^+$**

The rate of oxidation of the coenzyme NADH is proportional to the ALT activity in the specimen. It is determined by measuring the decrease in absorbance at 334 / 340 / 365 nm correspondingly. Endogenous specimen pyruvate is rapidly and completely reduced by LDH during the initial incubation period so that it does not interfere with the assay.

EXPECTED VALUES

	Male	Female
25^{0}C	Up to 22 U/l Up to 0.37 µkat/l	Up to 17 U/l Up to 0.28 µkat/l
30^{0}C	Up to 29 U/l Up to 0.48 µkat/l	Up to 22 U/l Up to 0.37 µkat/l
37^{0}C	Up to 41 U/l Up to 0.68 µkat/l	Up to 31 U/l Up to 0.52 µkat/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALT results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Tris buffer pH 7.8	100	mmol/l
	L-Alanine	500	mmol/l
	LDH	1700	U/l
R_2	NADH	0.18	mmol/l
	2-Oxoglutarate	18	mmol/l

Reagent Preparation & Stability

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

Prepare the working solution by as follows:

- *2 ml of **R_2** to one vial of **R_1** (8 ml vials)*
- *4 ml of **R_2** to one vial of **R_1** (16 ml vials)*
- *10 ml of **R_2** to one vial of **R_1** (40 ml vials)*

- *Mix well, do not shake. the working solution is stable*
  3 days at 20–25 ^{0}C.
  4 weeks at 2 – 8 ^{0}C

SPECIMEN

Serum, EDTA or heparinized plasma. Avoid hemolysis

Specimen Preparation & Stability For serum specimen

Separate serum/plasma from clot/cells within 8 hours at room temperature or 48 hours at 2–8^{0}C.

Freezing of the samples is not recommended.

PROCEDURE

- **Manual Procedure**

Wavelength	340,334,365 nm
Cuvette	1 cm light path
Temperature	25, 30 or 37 ^{0}C
Zero adjustment	against air

Pipette into test tube or cuvette	
Working solution	1000 µl
Serum or plasma	100 µl

Mix, incubate for 1.0 minute, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

4 Automated Procedure

User defined parameters for different autoanalyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the ALT/GPT activity by using the following formulae:

U/l = 1780 × ΔA 334 nm/min

U/l = 1746 × ΔA 340 nm/min

U/l = 3235 × ΔA 365 nm/min

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{{}^*\varepsilon \times SV \times LP}$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*ε	millimolar absorptivity of NADH
LP	Cuvette pathlength in cm
1000	Conversion of U/ml to U/l

* millimolar absorptivity of NADH
at 334 nm= 6.18,
at 340 nm= 6.22, and
at 365 nm= 6.40

- **Unit conversion**
  U/l x 16.67 x 10^{-3} = µkat/l

Medical Device Safety Services
MDSS GmbH
Burckhardtstr. 1
30163 Hannover, Germany
Tel.: +49 511 6262 8630
Fax: +49 511 6262 8633

Article # :121 -EN
Date of Revision : 10/2012

CE

- **Temperature correction**
  Multiply the result by 1.31 if the assay performed at 25^{0}C but is to be reported at 30^{0}C.
  Multiply the result by 1.91 if the assay performed at 25^{0}C but is to be reported at 37^{0}C.
  Multiply the result by 1.43 if the assay performed at 30^{0}C but is to be reported at 37^{0}C.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established ALT/GPT values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.

If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Fluoride and citrate inhibit the enzyme activity. The only accepted anticoagulants are heparin and EDTA.
- **Bilirubin:**
  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- **Drugs:**
  Young[7] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Erythrocyte contamination may elevates results, since ALT/GPT activities in erythrocytes are three to five times higher than those in normal sera.
- **Lipemia:**
  Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.
- **Pyruvate:**

High levels of serum pyruvate may interfere with assay performance

WARNING & PRECAUTION

- Vitro ALT reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid or if the absorbance is less than 1.0 at 340 nm.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	33	128
SD (U/l)	0.72	1.45
CV (%)	3.12	1.42

	Between Day	
Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	31.5	130
SD (U/l)	0.95	1.62
CV (%)	3.05	1.26

Method Comparison

Comparison studies were carried out using a similar commercially available ALT(GPT) reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.
Number of sample pairs 45
Range of sample results 8 - 188 U/l
Mean of reference method results 47 U/l
Mean of ALT(GPT) results 38 U/l
Slope 0.80
Intercept -0.03 U/l
Correlation coefficient 0.998

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable ALT/GPT activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 2 U/l or 0.03 μkat/l

LINEARITY

When run as recommended, the assay is linear up to 450 U/l or 7.46 μkat/l

If result exceeds 450 U/l or 7.46 mkat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

BIBLIOGRAPHY

1. **Zilva, JF, Pannall, PR (1979):** Plasma enzymes in Diagnosis in Clinical Chemistry in Diagnosis and treatment. Lioyd-Luke London. 1979: Chap 17:338.
2. **Henley, KS, Pollard, HM (1955):** J. Lab Clin. Med., Vol. 46, p. 785.
3. **Wroblewski, F, LaDue, JS (1956):** Proc Sec Exp Biol and Med 1956; 34:381.
4. **Henry, RJ, et al. (1960):** Am J Clin Path 1960; 34: 381.
5. **Bergmeyer, HU, et al. (1978):** Clin chem. 1978; 24: 58-73.
6. **IFCC Export panel on enzymes (1986):** part 3. J Clin Chem Clin Biochem 1986; 24:481-95.
7. **Young, Ds (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1251	10 X 10 ml
1252	5 X 20 ml
1253	3 X 50 ml
1254	10 X 20 ml

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
info@vitroscient.com

orders:
+202 26439698
order@vitroscient.com

Article # :121 -EN
Date of Revision : 10/2012

CE