ALT/GPT Liquiform
Insert

Ref.: 108

Intended use . Kinetic system for Alanine Aminotransferase (ALT/GPT) activity determination.

Professional use.

[For in vitro diagnostic use]

Test principle . ALT catalyzes the transfer of the amino group from alanine to ketoglutarate, yielding glutamate and pyruvate. The pyruvate is reduced to lactate by the action of the lactate dehydrogenase (LDH), which oxidizes NADH to NAD^+.
The reduction of the absorbance at 340 nm, as consequence of NADH oxidation, must be photometricaly monitored, and is proportional to the ALT activity in the sample.
Aminotransferase determination involves the following reactions:

$$\text{L-Alanine} + \text{Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

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

Summary . The UV kinetic methodology for measuring ALT activity was introduced by Karmem[1] and improved later by Henry et al.[2]. This measuring procedure was widely accepted for being fast, precise and accurate.

The Reference Procedure proposed by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[3] recommends the use of pyridoxal phosphate to assure the total activation of all the transferase in the serum, avoiding false low results in samples with coenzyme deficiency. The Labtest system is improved following these recommendations.

In order to achieve IFCC Procedure traceable results, is needed the use of the two-reagent method with activation by the pyridoxal phosphate (Reagent 3). When the monoreagent is applied, the pyridoxal phosphate activation is not employed. Thus, the obtained results are not traceable to the IFCC Procedure.

The substances for this reaction are distributed properly in three reagents in order to get more stability in the original liquid form and keep the optimum conditions of operation conditions.

ALT/GPT Labtest uses the kinetic system and is easily applied to most automatic and semi-automatic equipments which are able to measure the absorbance at 340 nm.

Methodology . U.V. Kinetic - IFCC

Reagents

1. R 1 - Reagent 1 - Store at 2 - 8 ºC.
Reagent label bears expiration date. This reagent contains Tris buffer (132.5 mmol/L); L-alanine (687.5 mmol/L); LDH (≥2300 U/L) and sodium azide (0.095%).

2. R 2 - Reagent 2 - Store at 2 - 8 ºC.
Reagent label bears expiration date. This reagent contains Tris buffer (20 mmol/L); NADH (1320 μmol/L); ketoglutarate (82.5 mmol/L) and sodium azide (0.095%).

3. R 3 - Reagent 3 - Store at 2 - 8 ºC.
Reagent label bears expiration date. This reagent contains tris buffer (20 mmol/L); pyridoxal phosphate (11.1 mmol/L) and sodium azide (0.095%).

Precautions and warnings

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

In automatic equipments, the reagents may be contaminated with other reagents or the air, depending on the equipment's characteristic and the work conditions. These can result in stability reduction and calibration modifications.

As it occurs in all enzymatic activity measurement, the incubation time and temperature is important for the quality of the results.

The reagents contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.

Deterioration . Microbial or chemical contamination may decrease reagents stability. ALT Work Reagent and Reagent 1, Reagent 2 and Reagent 3 mixture are not suitable for use if it has an absorbance lower than 1.0 at 340 nm when measured versus water as reference or in case of contaminations signs or if it develops turbidity.

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Specimen collection and preparation

Use serum or plasma (EDTA, Heparin). ALT is reportedly stable in serum or plasma for about 4 days at 2 - 8 ºC and 2 weeks at -10 ºC.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

An increase in serum alanine aminotransferase may occur as a result from prolonged severe exercise (increased muscle activity).

In all women ages, the activity of ALT is lower than in men.

Anabolic steroids, chloramphenicol, chlorothiazide, prolonged use of aspirin, gentamicin and others, may cause an increase of ALT activity.

Bilirubin up to 19 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 650 mg/dL do not interfere significantly.

Hyperlipemic samples and jaundice remarkably increase the absorbance at 340 nm. When the enzyme activity of these kinds of samples is very elevated, the substrate consumption is very fast, without a significant decrease of the absorbance.
In these samples, when a difference of absorbance per minute is very small, the determination should be repeated using a sample diluted with 0.85% NaCl.

Materials required not provided

1. Photometer capable of keeping the cuvette temperature at 37 ºC, and measuring absorbance at 340 nm.
2. Pipetts to measure reagents and samples.
3. Timer.

Manual procedure

See Calculation, Calibration, Measurement/ Reportable Range and Notes.

Enzymatic Activity Determination using the pyridoxal phosphate

In order to achieve traceable results to IFCC Procedure, is needed the use of the two-reagent method, to occur the enzyme total activation by the pyridoxal phosphate.

Preparing the reagent . Add 0.300 mL of the Reagent 3 to a bottle of Reagent 1 (24 mL) and mix gently. Stability: 21 days at 2-8ºC and 24 hours at 15-25ºC when no chemical or microbial contamination occurs. Optionally, a lower volume of the mixture (Reagent 1 + Reagent 3) may be prepared by using one part of the Reagent 3 to 80 parts of Reagent 1.

Procedure

1. In a test tube labeled "Test" or "Calibrator", add 0.800 mL of the mixture Reagent 1 + Reagent 3.

2. Add 0.100 mL of the sample or enzymes calibrator, homogenize and incubate in a water-bath at 37 ± 0.2 ºC. Wait five minutes. After this incubation it is possible wait until 30 minutes to start the kinetic determination with the addition of the Reagent 2.

3. Perform a water blank measurement at 340 nm.

4. Add 0.200 mL of the Reagent 2, homogenize and transfer immediately to a cuvette at 37 ± 0.2 ºC. Wait one minute.

5. Measure the initial absorbance (A_1), and start simultaneously the timer. Measure the absorbance again after 2 minutes (A_2).

In order to verify the reaction linearity, it is recommended to measure in 1 minute as well, and check if the difference of absorbance in each minute is constant.

Enzymatic Activity Determination without the pyridoxal phosphate

Preparing the working reagent . Transfer all the contents of one Reagent 2 bottle to one Reagent 1 bottle and mix gently.
The Working Reagent is stable 14 days at 2 - 8 ºC and 24 hours at 15 - 25 ºC, when no chemical or microbial contamination occurs. Optionally, a lower volume of the Working Reagent may be prepared by using the volume proportion 4:1 of the Reagent 1 and Reagent 2, respectively.

Procedure

1. In a test tube labeled "Test" or "Calibrator", add 1.0 mL of the Work Reagent.

2. Perform a water blank measurement at 340 nm.

3. Add 0.100 mL of the sample or enzymes calibrator, homogenize and transfer immediately to a cuvette at 37 ± 0.2ºC. Wait one minute.

4. Measure the initial absorbance (A_1), and start simultaneously the timer. Measure the absorbance again after 2 minutes (A_2).

In order to verify the reaction linearity, it is recommended to measure in 1 minute as well, and check if the difference of absorbance in each minute is constant.

Initial absorbance (A_1) equal to or lower than 0.8 indicates that the sample has elevated ALT activity. In this case, dilute the sample and measure again (see Measurement/Reportable Range).

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Calibration

Manual Calibrations . Use Labtest Calibra series. ALT activity is traceable to reference material ERM-AD454/IFCC and the IFCC reference method[3].

Calibration frequency
Two or three point calibration after reagent lot change;
Two or three point calibration when the internal quality control indicates.

Automatic Systems
Reagent Blank: reagent water or 0.85% NaCl.
Use Labtest Calibra series. ALT activity is traceable to reference material ERM-AD454/IFCC and the IFCC reference method[3].

Calibration frequency
Two or three point calibration after reagent lot change;
Two or three point calibration when the internal quality control indicates.

Quality control . For quality control use Qualitrol Level 1 and Qualitrol Level 2 or other suitable control material. The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Calculations . It is a usual procedure calculates the enzymatic activity results using a theoretical factor achieved in reaction optimum conditions, described below:
Wavelength: 340 nm.
Cuvette at 37 ± 0.2 ºC, 10 mm light path.
Pass band ≤2 nm.
Sray light ≤0.1%.

If one of the correlated parameters is modified, it is recommended to apply an enzymes calibrator indicated by the reagent manufacturer. Labtest Diagnostica recommends Calibra series to perform the ALT/GPT system calibration.

$$\Delta A/\text{minute (test or Calibrator)} = \frac{A_1 - A_2}{2}$$

$$\text{Factor: } \frac{\text{Calibrator activity}}{\Delta A/\text{minute Calibrator}}$$

ALT activity (U/L) = ΔA/minute (test) x Factor

If all the correlated parameters are fulfilled, the theoretical factor (1746) can be applied.

Measurement/reportable range

From 3.5 up to 400 U/L.
If ALT concentration exceeds 400 U/L, the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor.

EXPECTED VALUES[3,7] . These values should be used as guidance only. Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Age	Men (U/L)	Women (U/L)
1-30 days	20 - 54	21 - 54
1-6 months	26 - 55	26 - 61
7-12 months	26 - 59	26 - 55
1-3 years	19 - 59	24 - 59
4-11 years	24 - 49	24 - 49
12-15 years	24 - 59	19 - 44
Adults	11 - 45	10 - 37

Conversion: Conventional Unit (U/L) x 16.7 = Unit IS (nkat/L).

Performance characteristics[8]

Recovery studies . In two samples with alanine aminotransferase concentrations of to 100 and 227 U/L were added different quantities of the enzyme. Subsequent analyses provided recoveries ranging from 92.2 to 102.6%. The mean proportional systematic error at 80 U/L decision level was 0.7 U/L and at 281 U/L decision level was 2.5 U/L.

Method Comparison . The proposed method was compared with IFCC[3] reference method, obtaining the follow results:

Tests performed using the pyridoxal phosphate

	Comparison Method	Labtest Method
N	40	40
Range (U/L)	8.2 - 256.2	8.0 - 262.7
Mean (U/L)	102.4	105.5
Regression analysis	Labtest Method = 1.036 x Comparison Method - 0.589	
Correlation coefficient	0,999	

The total systematic error (bias) is 2.9% in a decision level of 89 U/L and 3.3% in a decision level of 272 U/L.

Tests performed without the pyridoxal phosphate

	Comparison Method	Labtest Method
N	40	40
Range (U/L)	8.2 - 256.2	10.8 - 243.3
Mean (U/L)	102.4	99.4
Regression analysis	Labtest Method = 0.958 x Comparison Method - 1.284	
Correlation coefficient	0.998	

The total systematic error (bias) is 2.6% in a decision level of 80 U/L and 3.7% in a decision level of 282 U/L.

Imprecision

Tests performed using the pyridoxal phosphate

Imprecision - within run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	89	1.43	1.6
Sample 2	20	272	2.61	1.0

Imprecision - run-to-run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	89	2.31	2.6
Sample 2	20	272	7.40	2.7

Tests performed without the pyridoxal phosphate

Imprecision - within run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	80	1.20	1.5
Sample 2	20	282	2.19	0.8

Imprecision - run-to-run

	N	Mean (U/L)	SD (U/L)	(%) CV
Sample 1	20	80	1.40	1.8
Sample 2	20	282	3.31	1.2

The total imprecision obtained for the samples meets the optimum specification for total imprecision based on the biological variation that is 6.1%.

Total error estimate . The total error (random error + systematic error) estimated at 89 U/L is equal to 7.2% and at 272 U / L is equal to 7.8% in the tests performed with pyridoxal phosphate. For tests performed without pyridoxal phosphate the estimated total error of 80 U/L is 5.5% and 282 U/L is 5.7%. The results indicate that the method meets the optimal specification for total error (≤16%) based on the components of the Biological Variation (BV).

Analytical sensitivity . Detection limit: 1.75 U/L. The detection limit represents the lowest measurable ALT concentration that can be distinguished from zero.

Effects of matrix dilution . Two samples with values 408 and 459 U/L were used for evaluating the system response on dilution with 0.85% NaCl. Using dilution factors from 2 to 16, the mean recovery found was 100.3%.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The water in the laboratory to prepare reagents and use in the measurements, must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be ≤0.1mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

3. It is suggested to consult: http:/www.fxol.org in order to review physiopathological source and drugs interference in results and methodology.

References

1. Karmen A. J Clin Invest 1955;34:131.

2. Henry RJ, Chiamori N, Golub O, Berkman S. Amer J Clin Path 1960; 34:381.

3. IFCC Reference Procedure for the Measurement of Catalytic Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002; 40 (7):718-24.

4. Sociedad Española de Bioquímica Clínica y Patología Molecular, Base de Datos de Variación Biológica. Disponível em: <http://www.seqc.es/article/articleview/330/1/170> (access in 2006/04).

5. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.

6. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem 1981; 27:493-501.

7. Soldin SJ, Brugnara C, Wong EC. Pediatric Reference Intervals, 5.ed. Washington: AACC Press, 2005. p. 3-4.

8. Labtest: data on file.

9. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, 2.ed. Philadelphia: W.B. Saunders Company, 1994. p. 788-96.

Presentation

Product	Reference	Contents	
ALT/GPT Liquiform	108-4/30	R 1	4 X 24 mL
		R 2	4 X 6 mL
		R 3	1 X 1,5 mL
	108-2/100	R 1	2 X 80 mL
		R 2	2 X 20 mL
		R 3	1 X 2,2 mL
ALT/GPT Liquiform Labmax 560/400	108-4/49	R 1	4 X 39 mL
		R 2	4 X 10 mL
		R 3	1 X 2,5 mL

The number of tests in automated instruments depends on the programmed parameters.

Application procedures using ALT kinetic system are available for various automated instruments.

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

CE

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Período post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		Ref.: 140214

Labtest