GPT

Colorimetric test. Reitman-Frankel

IVD *For in vitro diagnostic use only*

2°C 8°C Store at 2-8ºC

INTENDED USE

For the determination of GPT concentration in human serum.

PRINCIPLE

The glutamic transaminase enzymes, serum glutamic oxalacetic (GOT) and serum glutamic pyruvic (GPT), catalyze the transfer of the amino group of glutamic acid to oxalacetic acid and pyruvic acid in reversible reactions. The transaminase activity is proportional to the amount of oxaloacetate pyruvate formed over a definite period of time and is measured by a reaction with 2.4-dinitrophenylhydrazine (DNPH) in alkaline solution.

REAGENTS

Materials Provided		
	Content	**Concentration**
Reagent -1	Substrate GPT (ALT) DL-Alanine Ketoglutarate	 200 mmol/L 2 mmol/L
Reagent -2	Colour reagent 2.4-dinitrophenylhydrazine	 1 mmol/L
Standard	Pyruvic standard.	1.2 mmol/L

Materials Required But Not Provided	
Content	**Concentration**
Soduim Hydroxide (NaOH)	4N

SAMPLES

Serum, free of hemolysis.

PROCEDURE

	GPT
Substrate GPT (R.1)	0.5 ml
Preincubate for 5 min at 37°C.	
Serum	100 µL
Mix. Return to bath for 30 min	
DNPH R.2	0.5 ml
Mix. Allow to stand for 20 min at room temperature.	
NaOH 0.4 N	5.0 ml
MIX. Let stand for 15 min at room temperature. Read at 505 nm against a water blank. The colour is stable at least 60 minutes.	

CALCULATIONS

From absorbencies, read units of GPT from corresponding curves.

CALIBRATION (mL)

	1	2	3	4	5	6
Water	0.2	0.2	0.2	0.2	0.2	0.2
GOT R.1	1.0	0.9	0.8	0.7	0.6	0.5
Pyruvic stand.	--	0.1	0.2	0.3	0.4	0.5
DNPH R.2	1.0	1.0	1.0	1.0	1.0	1.0
Mix. Allow to stand for 20 min at room temperature.						
NaOH 0.4 N	10	10	10	10	10	10
MIX. Allow to stand for at least 15 min. Read against water blank at 505 nm. Plot a calibrator curve of the absorbances found vs. the corresponding units, on a graph paper, according to the following concentrations:						
GPT WU/mL	0	25	50	83	126	--
U/L	0	12	24	40	62	--

LINEARITY

When GPT exceeds 126 WU/Ml (61 u/l) repeat test using a 1:10 dilution of serum with saline sol.(9g/L). Multiply the result by 10.

UNITS

1 WU/mL = 1U/L x 2.07
1 U/L = 1WU/mL x 0.483

NORMAL VALUES

GPT/ALT	= 5-30 WU/ml (2.4 -14.5 U/L)

REFERENCES

1. Reitman S., Frankel S.,Am. Clin. Pathol., 28,56 (1957)
2. Tietz, NW.,Fund of Clinical Chem., 446 (1970)
3. Schmidt, E., Enzymology Biol.Clin., 3,1 (1963)

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REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		

Atlas Medical

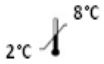
CE

GPT (ALT)

NADH. Kinetic UV. IFCC Liquid.

Quantitative determination of alanine aminotransferase GPT (ALT)

IVD *For in -vitro diagnostic use only.*



Store at 2-8ºC.

PRINCIPLE OF THE METHOD

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutmate 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.

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

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

This test has been formulated according the standardized method described by IFCC.

REAGENTS

R 1 ALT Substrate	TRIS buffer pH 7.3	150 mmol/L
	Lactate dehydrogenase (MDH)	>1350U/L
	L-Alanine	750 mmol/L
R2 ALT coenzyme	NADH	1.3 mmol/L
	2-oxoglutarate	75 mmol/L

PREPARATION

- Working reagent (WR): Mix: 4 ml of (R1) + 1ml (R2)
- Stability: 4 weeks at 2-8°C. Protect from light

STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8 ° C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.
- Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.000 in 1cm cuvette.

SAMPLES

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

INTERFERENCES

- Lipemia (intralipid >15 g/L) does not interfere.
- Bilirubin (>30 mg/dL) does not interfere.
- Hemoglobin (>10g/dL) does not interfere.
- Other drugs and substances may interfere.

EQUIPMENTS NEEDED BUT NOT PROVIDED

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

PROCEDURE

1. Peincubate 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	37°C	30°C
WR (mL)	1.0ml	1.0ml
Sample (µL)	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 minutes (ΔA/min).

CALCULATIONS

U/L= ΔA/min x 3333 (37°C)

U/L= ΔA/min x 1746 (30°C)

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 the results are to be expressed as SI units apply :

U/L x 0.01667=µkat/L.

REFERENCE VALUES

Adults	37°C	Up to 40U/L(0.67 µkat/L.)
	30°C	Up to 25U/L(0.42 µkat/L.)

Levels approximately twice the adult level are seen in neonates and infants; these decline to adult level approximately 6 months of age.

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: ATLAS H Normal and Pathologic. If control values are found outside the defined range, check the instrument, reagents and technique for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNFINANCE

The group of enzymes called transaminase exist in tissues of many organs. Necrotic activity in these organs causes a release of measured. Since heart tissue is rich in AST increased serum levels appear in patients after myocardial infraction, as well as in patients with muscle disease. Muscular dystrophy and dermatomyositis .

The liver is especially 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 billary tree by the ademato us pancreas and the presence of associate hepatic disease may contribute to elevated AST levels 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 ampicilin.

ANALYTICAL PERFORMANCE

-**Detection Limit**: 7.95 U/L.

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

-**Precision**:

(U/L)	Within-run		Between-run	
Mean	32.4	140.8	32.4	140.8
SD	0.79	1.41	0.97	2.77
CV (%)	2.43	1.00	3.49	1.97
N	10	10	10	10

-**Sensitivity**: 0.280 mA/min/U/L GPT.

-**Correlation**: this assay (y) was compared with similar commercial method (x).

The result were :

N=50 r=0.99 y=1.041 x + 1.447

The analytical performances have been generated using on automatic instrument. Results may very depending on the instrument.

Notes:

1. The method may be used with different instruments. Any application to an instrument should be validated to demonstrate method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result. But should integrate both clinical and laboratory data.

REFERENCES

1. Winn-Deen E.S ,David H, Singer G, and Chavez R. Clin Chem 1988;34:2005.
2. International Federation of clinical Chemistry (IFCC). Clin Chem Lab 1998;36:185.
3. Young DS. Effect of drugs on clinical laboratory tests. 5th ed . AACC Press,2000.
4. Tietz. Textbook of Clinical Chemistry, 2 edition. Burtis CA, Ashwood ER.W.B.Saunders Co. 1994.

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REF	Product Reference No.	IVD	For in-vitro diagnostic use.
	Caution.	2°C 8°C	Store at 2 - 8°C.
	Read product insert before use.		Manufacturer.
LOT	Lot (batch) number.		Manufacturer telephone number.
	Expiry date.		Manufacturer fax number.

Atlas Medical

CE

ATLAS GPT (ALT) TEST (Kinetic)

IVD *For in -vitro diagnostic use only.*

2°C 8°C Store at 2-8°C

INTENDED USE

For the determination of GPT (ALT) concentration in human serum or plasma.

INTRODUCTION

The ALT is a cellular enzyme, found in highest concentration in liver and kidney. High levels are observed in hepatic disease like hepatitis, diseases of muscles and traumatisms, its better application is in the diagnosis of the diseases of the liver.
When they are used in conjunction with AST aid in the diagnosis of infarcts in the myocardium, since the value of the ALT stays within the normal limits in the presence of elevated levels of AST.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Alanine aminotranferase (ALT) o Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and piruvate.
The piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

Alanine + α-Ketoglutarate $\xrightarrow{ALT}$ Glutamate + Piruvate

Piruvate + NADH + H $\xrightarrow{LDH}$ Lactate + NAD'

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample'.

REAGENTS

R 1 Buffer	TRIS pH 7.8 L-Alanine	100 mmol/L 500 mmol/L
R 2 Substrate	NADH Lactate dehydrogenase (LDH) α-Ketoglutarate	0.18 mmol/L 1200 U/L 15 mmol/L

EQUIPMENTS NEEDED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25°C, 30°C, 37° C (± 0.1°C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PREPARATION

- Working reagent (WR):
- Dissolve one tablet of R2 Substrate in 2mL of R1.cap and mix gently to dissolve contents.
- Stability: 21 days at 2-8°C or 72 hours at room temperature (15-25°C).

STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
- Do not use the tablets if appears broken.
- Do not use reagents over the expiration date.
- Signs of reagent deterioration:
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 340 nm < 1.00.

SAMPLES

Serum or plasma': Stability 7 days at 2-8°C..

PROCEDURE

1. Assay conditions:
   Wavelength: .....340 nm
   Cuvette :.....1 cm light path
   Constant temperature .....25°C / 30°C / 37°C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR (mL)	1.0
Sample (µL)	100

4. Mix, incubate for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1-minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 1750 = U/L of ALT

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to 25°C	30°C	37°C
25°C	1.00	1.32	1.82
30°C	0.76	1.00	1.39
37°C	0.55	0.72	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable **tolerances.**

REFERENCE VALUES[4,5]

	25°C	30°C	37°C
Men	up to 22 U/L	29 U/L	40 U/L
Women	up to 18 U/L	22 U/L	32 U/L

Normal newborns have been reported to show a reference range of up to double the adult, attributed to the neonate's hepatocytes. These values decline to adult levels by approximately 3 months of age.
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range:
From *detection limit* of 1,20 U/L to *linearity limit* of 262 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.

Precision:

	Intra-assay (n=20)	
Mean U/L	34 .9	11 8.4
SD	0.64	1.17
CV (%)	1.84	0.99

Inter-assay (n=20)	
34.1	118.3
1.03	1.53
3.04	1.29

Sensitivity:

1 U/L = 0.000557 ΔA / min.

Accuracy:

Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99.

Regression equation: y= 0.98x + 0.38.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Anticoagulants currently in use like heparin, EDTA, oxalate and fluoride do not affect the results. Hemolysis interferes with the assay[1].

A list of drugs and other interfering substances with ALT determination has been reported by Young et Al.

REFERENCES

1. Murray R. Alanine aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1088-1090.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed *AACC* Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed *AACC* 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed *AACC* 1995.

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REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
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