PYRUVIC TRANSAMINASE
Insert

Ref.: 53

Intended use . System for measuring the activity of pyruvic transaminase (GPT) in blood sample by kinetic method of fixed time and measurement of end point.

Test principle . Pyruvic transaminase promotes the transference of the amino group of alpha-amino acids to alpha-keto acids.

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{Glutamate}$$

Pyruvate is measured by the hydrazone formation what has intense color in alkaline solution.

Summary . Pyruvic transaminase Labtest System contains all reagents needed to a safe determination, using a four steps process and allowing performing the photometry in any photometer that has filters with emission at 490 and 540 nm.

Reagents are rigorously standardized and established towards to rigid maintenance and great conditions for enzymatic action.

Sodium hydroxide is prepared and tittered in conditions of rigorous control what eliminates the presence of carbonate and assures a correct action of reagent.

Methodology . Reitman and Frankel.

Reagents

1. R 1 - GPT substrate - Store at 2 - 8 ºC.
Reagent label bears expiration date. Buffer pH 7.4 (67 mmol/L), α-ketoglutaric acid (2.0 mmol/L), L-Alanine (100 mmol/L) and sodium azide (15.4 mmol/L).

2. R 2 - Color Reagent - Store at 2 - 8 ºC.
Reagent label bears expiration date. Do not freeze. 2,4-dinitrofenilhydrazine (1.0 mmol/L) and chloride acid (1.0 mol/L).

3. R 3 - NaOH stock - Store at 2- 25 ºC.
Sodium hydroxide (1.25 mmol/L). Corrosive reagent.

4. CAL - Standard - Store at 2 - 8 ºC.
Reagent label bears expiration date. Sodium pyruvate (2 mmol/L). Keep the bottle tightly closed in order to avoid evaporation.

Precautions and warnings

For in vitro diagnostic use.

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

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

NaOH stock contains sodium hydroxide what is corrosive and may skin burn.

The GPT substrate contains 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.

Cares regarding reaction time, temperature of incubation and pipetting are extremely important in order to obtain correct results. The difference of one minute in the incubation time induces an error of 3.3% in the results.

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.

Specimen collection and preparation

Use serum or plasma (EDTA, heparin) and cerebrospinal fluid. The enzymatic activity is reportedly stable in serum 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 GPT is lower than in men.
Anabolic steroids, androgens, chloramphenicol, chlorothiazide, prolonged use of aspirin, gentamicin and others, may cause an increase of GPT activity.

Bilirubin up to 5 mg/dL, hemoglobin up to 90 mg/dL and triglycerides up to 250 mg/dL do not interfere significantly.

Hemoglobin values up to 180 mg/dL and triglycerides values between 250 and 750 mg/dL provide positive interference which can be decreased by using the blank of the sample.

Bilirubin values over 5 mg/dL, and triglycerides values over 750 mg/dL yield false increased results. In this case, the blank of sample is not applicable.

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Minimizing the interferences

Blank of the sample . Mixture 3.0 mL of 150 mmol/L NaCl (0.85%) with 0.05 mL of the sample. Measure the absorbance in 505 nm or green filter (490 - 540 nm), against distilled or deionized water. Subtract the obtained absorbance from the test absorbance and obtain the oxaloacetic transaminase activity using a calibration curve. This correction system is applied only in case of sample photometric interference.

Materials required not provided

1. A constant temperature water bath (37 ºC).
2. Photometer capable of measuring absorbance at 490 - 540 nm.
3. Pipettes to measure reagents and samples.
4. Timer.

Preparing the working reagent

See notes 1 and 2.

NaOH in use . Transfer the content of the bottle nº 3 (160 mL) to a graduated cylinder, complete to 500 mL with distilled or deionized water free of CO_2 and homogenize. Stable for 12 months in plastic bottle at 15 - 25 ºC.

Water must have resistivity ≥1 megaohm, or conductivity ≤1 microsiems and silicates concentration must be <0.1mg/L.

Calibration curve . Reitman-Frankel system of measurement (U/mL) does not follow Beer's Law, thus it is impossible to use the factor method for calculation. It is necessary to prepare the calibration curve.

Set up five tubes and proceed as follows:

	Tube nº (mL)				
	1	2	3	4	5
Standard (nº 4)	----	0.05	0.1	0.15	0.2
GPT Substrate (nº 1)	0.5	0.45	0.4	0.35	0.3
Distilled or deionized water	0.1	0.1	0.1	0.1	0.1
Color reagent (nº 2)	0.5	0.5	0.5	0.5	0.5

Mix and let it reach room temperature for 20 minutes.

NaOH in use	5.0	5.0	5.0	5.0	5.0

Mix and let it reaching room temperature for 5 minutes. Determine the absorbance or T% in 505 nm or green filter (490 – 540) against distilled or deionized water. The color is stable during 60 minutes.

Drawing the calibration curve . Draw the calibration curve correlating the readings obtained with the U/mL values expressed on the table below, using linear paper (for absorbance) or monolog (for T%).

	Tube nº				
	1	2	3	4	5
GPT (Units/mL)	Zero	28	57	97	150

Procedure

See note 3.

Set up one tube and proceed as follows:

	Unknown
GPT Substrate (nº 1)	0.25 mL

Incubate at 37 ºC during 2 minutes.

Sample	0.05 mL

Incubate at 37 ºC during 30 minutes.

Color Reagent (nº 2)	0.25 mL

Mix and let at room temperature during 20 minutes.

NaOH in use	2.5 mL

Mix and wait 5 minutes. Measure the absorbance or T% at 505nm or green filter (490 - 540) against distilled or deionized water. The color is stable during 60 minutes.

Obtain the GPT value using the calibration curve (see System Performance).

The suggested measurement procedure is appropriated to photometer of which the minimal volume of solution for reading is equal or lower than 3.0 mL. It should be done a verification of the necessity of volume adjustment for the photometer to be used. Sample and reagent volume may be modified proportionally without affecting the test performance and the calculation procedure. In case of volume reduction is important to observe the minimum volume needed to the photometric reading. Volume of sample lower than 0.01 mL is critical in manual applications and should be avoided because it increases the measurement imprecision.

System performance . When a value equal to or higher than 150 U/mL, dilute the sample with 150 mmol/L NaCL (0.85%), perform new measurement and multiply the obtained result by the dilution factor. Dilute the sample so that the obtained value is around 50 and 120 U/mL.

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.

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Serum . 4 to 32 U/mL

IU = U/mL x 0.482

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Performance characteristics[8]

Recovery studies . In two samples with pyruvic transaminases concentrations of 9 and 75 U/mL were added different quantities of the enzyme. Subsequent analyses provided recoveries ranging from 94 to 104%. The mean proportional systematic error at 60 U/mL decision level was 1.8 U/mL or 3.0 %.

Method comparison . A group of 80 sera were assayed by the proposed method and a similar technique. Serum GOT values ranged from 2 - 140 U/mL. The comparisons yielded a correlation coefficient of 0.99 and regression equation was y = 0.817x - 0.241. The mean proportional systematic error at 60 U/mL was 11 U/mL or 18 %.

Imprecision - Within Run

	N	Mean (mg/dL)	SD (mg/dL)	(%) CV
Sample 1	20	21	0.91	4.3
Sample 2	20	43	1.24	2.9

Imprecision - Run-to-Run

	N	Mean (mg/dL)	SD (mg/dL)	(%) CV
Sample 1	20	22	1.42	6.5
Sample 2	20	42	2.20	5.3

Analytical sensitivity . Detection limit: 3.0 U/mL. The detection limit represents the lowest measurable GPT activity that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without oxaloacetic transaminases.

Matrix dilution effects . Two sample with values equal of 112 and 154 U/mL were used to evaluate the system response in the matrix dilution with 150 mmol/L NaCl (0.85%). Recoveries were found a range of 91 and 100 %, using dilution factors that vary from 2 to 4.

Notes

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

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

3. It is suggested to consult "Young DS. Effects of Drugs on Clinical Laboratory Tests, 3rd Edition, Washington: AACC Press, 1990." in order to review physiopathological source and drugs interference in results and methodology.

References

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2. Reitman S, Frankel S. Am J Clin Path 1957;28:56.
3. Tietz NW. Fundamentals of Clinical Chemistry, W.B. Saunders Co, 1970.
4. Tonks DB. Quality Control in Clinical Laboratories. Warner Chilcott Labopratories, Diagnosis Reagents Division. Scarborough, Canada, 1972.
5. Varley H. Pratical Clinical Biochemistry, Willian Heinemann Medical Book Ltd. 1967.
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7. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem. 1981, 27:493-501.
8. Labtest: data on file.

Presentation

Product	Reference	Contents	
Pyruvic Transaminase	53-200	R 1	1 X 50 mL
		R 2	1 X 50 mL
		R 3	1 X 160 mL
		CAL	1 X 4 mL

Consumer 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.

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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

Ref.: 170309

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