ENZYMATIC LACTATE
Instruction for use

Ref.: 138

Intended use . Enzymatic system for quantitative determination of the plasma lactate (fluoride) and cephalorachidian liquid.

Professional use.

[For in vitro diagnostic use.]

Principle . The lactate is determined in accordance with the following reactions:

$$\text{L - Lactate} + O_2 \xrightarrow[\text{Oxidase}]{\text{Lactate}} \text{Pyruvate} + H_2O_2$$

$$2\,H_2O_2 + \text{TOOS} + 4\,\text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

In presence of oxygen, the lactate oxidase catalyzes oxidation of the lactic acid, thus promoting creation of pyruvate and hydrogen peroxide. Then, a coupling reaction takes place between the hydrogen peroxide, 4-aminoantipyrine and TOOS, catalyzed by peroxidase, thus producing a quinoneimine that has maximum absorbance in 550 nm.

The reaction product color intensity is directly proportional to concentration of the lactate in the sample.

Summary[1,2] . The system uses the enzyme lactate oxidase to determine the concentration of lactate present in a sample. The enzymatic method is highly specific and simple to execute.

The repeatability and reproducibility data show that the method is capable of providing results that meet the desirable specifications of maximum bias, based on the biological variation components.

Measurement in 550 nm minimizes interference of bilirubin and hemoglobin.
Concentrations of triglycerides up to 1100 mg/dL, bilirubin up to 30 mg/dL and hemoglobin up to 300 mg/dL do not significantly interfere the reaction.

The product may be applied in manual, semi-automatic and automatic procedure.

Methodology . Enzymatic -Trinder

Reagent

1. R1 - Reagent 1 - Store between 2-8ºC.
It contains buffer 50 mmol/L pH 7.2; 4-aminoantipyrine ≥0.05 mmol/L; peroxidase ≥1000 U/L stabilizers, solubilizer, surfactante and preservative.

2. R2 - Reagent 2 - Store between 2-8ºC.
It contains buffer 50 mmol/L pH 7.2; lactate oxidase ≥1000 U/L; N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS) ≥1.0 mmol/L, stabilizers, solubilizer, surfactante and preservative.

3. CAL - Standard - 40 mg/dL. Store between 2-8ºC.
It contains lithium lactate and sodium azide 0,095%.
After handling, store it well-sealed so as to avoid evaporation.

The reagents, when stored under the conditions indicated, are stable until the expiration date printed on the label. During handling, the reagents are subject to chemical and microbial contaminations that may cause stability reduction.

Precautions and warnings

In order to preserve performance, the reagents should remain out of the fridge only for the time required to obtain the volume to be used. Avoid direct sunlight exposure.

The customary safety care should be applied when handling the reagent. Do not use the reagents when they are turbid or with contamination signs.

The standard contains sodium azide that is toxic. Do not ingest and, in case of contact with the eyes, immediately wash with plenty of water and look for medical assistance. The azide may form highly explosive compounds with lead and copper tubes. Therefore, use large volumes of water to dispose of the reagents.

Materials required not provided

1. Water bath maintained at constant temperature (37ºC).
2. Photometer able to accurately measure the absorbance at 550 nm (530-570).
3. Pipettes to measure samples and reagent.
4. Stop watch.

Sample[3-7]

Use plasma (fluoride) or cephalorachidian liquid.
Fluoride inhibits glycolysis, i.e., it is responsible for stabilization of the blood lactate.

Recommendations for patient preparation, obtaining and processing of the sample . Previously to carrying out the blood collection procedure, the patient should remain resting for at least 30 minutes.

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Blood collection should be preferably carried out without tourniquet or immediately after application of the tourniquet. If collection is not completed right after application of the tourniquet, it should be removed and collection should only be initiated again after 2 minutes. The patient should avoid exercising the hands and arms immediately before and during the collection procedure.

Separation by centrifugation should be made within at most 15 minutes. After separation, the plasma is stable 8 hours between 20-25ºC, 14 days between 2-8ºC or 1 month at -20ºC.

The cephalorachidian liquid should be centrifuged and used without additional changes.

Since no known assay may ensure that blood samples do not transmit infections, all of them should be considered as potentially infectious. Therefore, when handling them, one should follow the standards established for biosecurity.

In order to dispose of the reagents and the biological material we suggest application of the local, State or federal environmental protection standards.

Interference[1]

Concentrations of triglycerides up to 1100 mg/dL, bilirubin up to 30 mg/dL and hemoglobin up to 300 mg/dL do not significantly interfere the reaction.

Samples with bilirubin, hemoglobin and triglycerides in concentrations larger than those referred to above should be diluted into NaCl 150 mmol/L (0.85%) before the assays. Multiply the result obtained by the dilution factor.

Preparing the work reagent . The set of one flask with Reagent 1 and one flask with Reagent 2 allows for preparing the Work Reagent. Transfer the contents of a flask with Reagent 2 to a flask with Reagent 1 and slightly homogenize. Identify the Work Reagent flask and note down the expiration date.
Studies show that the work reagent has high stability. After preparation, one should consider that the expiration date of the work reagent is the same as the reagents composing it, provided that it is kept between 2 and 8ºC, in a sealed flask and when there is no chemical or microbial contamination. Development of slightly pink color in the Work Reagent is normal and does not affect performance.
Optionally, a lower volume of the Work Reagent may be prepared by using the proportion 4 volumes of the Reagent 1 and 1 volume of the Reagent 2. In order to prepare the volume of reagent required to carry out an assay, mix 0.8 mL of the Reagent 1 and 0.2 mL of the Reagent 2.

In order to preserve its performance, the reagent should remain out of the fridge only for the time required to obtain the volume to be used. Avoid direct sunlight exposure.

Procedure . See Notes 1 and 2.

Identify 3 test tubes and proceed as described as follows:

	Blank	Unknown	Standard
Sample	-----	0.01 mL	-----
Standard	-----	-----	0.01 mL
Water	0.01 mL	-----	-----
Work Reagent	1.0 mL	1.0 mL	1.0 mL

Homogenize and place in water bath at 37ºC during 5 minutes. The water level in water bath should be greater than the level of the reagents in the test tube. Determine absorbance of the test and standard in 550 nm (530 - 570), regulating the zero with the white. Color is stable for 30 minutes.

The procedure suggested for measurement is appropriate for photometers which minimum volume of solution for reading is equal to or lower than 1.0 mL. The requirement for adjusting the volume for the photometer used should be checked. The sample and reagent volumes may be proportionally changed, without prejudice to performance of the assay, and the calculation procedure shall remain unchanged. In case of volume reduction, it is essential that the minimum volume required is observed for photometric reading. Sample volumes lower than 0.01 mL are critical in manual applications and should be used cautiously because they increase inaccuracy of the measurement.

Calculations . See linearity.

$$\text{Lactate (mg/dL)} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times 40$$

The result may also be obtained by using the calibration factor:

$$\text{Calibration factor} = \frac{40}{\text{Standard Absorbance}}$$

Lactate (mg/dL) = Test Absorbance x Calibration factor

Examples

The data presented as follows are illustrative.

Test Absorbance: 0.450
Standard Absorbance: 0.757

$$\text{Lactate (mg/dL)} = \frac{0.450}{0.757} \times 40 = 24$$

or

$$\text{Calibration factor} = \frac{40}{0.757} = 52.84$$

Lactate (mg/dL) = 0.450 x 52.84 = 24

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Calibration

Manual calibrations . obtain the calibration factor when using a new batch of reagents or when the internal quality control states so.

Automatic systems
Blank of reagent: water or sodium chloride solution 150 mmol/L (0.85%);
Use calibrator Calibra H - Labtest.

Calibration intervals
Calibration of 2 points when changing the batch;
Calibration of 2 points when the quality control states.

Linearity

Reaction is linear up to 150 mg/dL. For values greater than 150 mg/dL, dilute the sample into NaCl 150 mmol/L (0.85%), effect new determination and multiply the result obtained by the dilution factor.

Internal quality control[2,8,9] . The laboratory should keep an internal quality control program clearly defining the applicable regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective measures and registration of the activities. Control materials should be used so as to monitor inaccuracy of the measurement and calibration deviations. The specifications for the variation coefficient and total bias are suggested to be based on the biological variation components (VB).

Use of the products from the line Qualitrol - Labtest is suggested for internal quality control in clinical chemistry assays.

Reference interval[10,11] . These values should be used only as guidelines. Each laboratory is recommended to establish, in the population served, their own range of reference values.

Newborns, children and teenagers

Plasma (fluoride)

	mg/dL	mmol/L
0 to 90 days	9 - 32	1.0 - 3.5
3 to 24 months	9 - 30	1.0 - 3,3
2 to 18 years	9 - 22	1.0 - 2.4

Adult

Plasma (fluoride)

	mg/dL	mmol/L
Venous	4.5 - 19.8	0.5 - 2.2
Arterial	4.5 - 14.4	0.5 - 1.6

Concentration of lactate in the liquor is usually similar to the blood levels. However, in biochemical changes to the SNC, the lactate values in the liquor are changed regardless of the blood values.

Conversion . Conventional units (mg/dL) x 0.111 = SI Units (mmol/L)

Performance characteristics[12]

Recovery studies . In two samples with lactate concentrations equal to 9.8 mg/dL and 19.7 mg/dL, different amounts of the analyte were added, and the following results were obtained:

Concentration (mg/dL)				Recovery percentage
Initial	Added	Expected	Found	
9.8	40.8	38.2	39.5	103.4%
19.7	10.2	76.4	77.1	100.9%

The total estimated systematic bias is equal to 0.3 mg/dL for the level of 13.6 mg/dL, 0.8 mg/dL for the level of 37.1 mg/dL and 1.78 mg/dL for the level of 82.8 mg/dL. Average of the bias is lower than the analytic systematic bias of the desirable specification based on the VB components which is ≤±8,0%.

Method comparison studies . Accuracy of the method was checked by comparison to another enzymatic method, and the following results were obtained:

	Comparative Method	Enzymatic Lactate
Number of samples	40	40
Interval (mg/dL)	6.7 to 135.1	6.5 to 147.3
Regression equation	Enzymatic Lactate = 1,060 + 1,38 mg/dL	
Correlation coefficient	0.999	

By using the regression equation, the estimated systematic bias is equal to 2.8% for the level of 15.7 mg/dL and 2.9% for the level of 44.8 mg/dL. Average of the bias is lower than the analytical systematic bias of the desirable specification based on the VB components which is ≤±8.0%.

Accuracy studies . The accuracy studies were carried out by using two samples.

Repeatability - Imprecision within-assay

	N	Average (mg/dL)	DP	CV (%)
Sample 1	80	15.7	0.11	0.71
Sample 2	80	44.8	0.62	1.39

Reproducibility - Total imprecision

	N	Average (mg/dL)	DP	CV (%)
Sample 1	80	15.7	0.72	4.61
Sample 2	80	44.8	1.26	2.81

Imprecision found meets the desirable specification for total imprecision based on the VB components which is ≤13.6%.

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Total estimated bias (random bias + systematic bias) is equal to 10.39% for the level of 15.7 mg/dL and 7.55% for the level of 44.8 mg/dL. Results point out that the method meets the desirable specification for total bias (≤30.4%) based on the VB components.

Methodological sensitivity . A sample containing 15 mg/dL lactate was used to calculate the test detection limit, and a value equal to 0.4 mg/dL was found, equivalent to three standard deviations of an analytical run with 20 replicates.

By using the standard absorbance as parameter, it was verified that the photometric detection limit is 0.05 mg/dL, corresponding to an absorbance equal to 0,001.

Matrix dilution effects . A sample with concentration equal to 151 mg/dL was used to assess the system response in matrix dilution with NaCl solution 150 mmol/L (0.85%). By using the dilution factor from 2 to 16 the average recovery of 102.5% corresponding to an average systematic bias equal to 2.5% was found.

Clinical meaning[10] . Lactate is the final product of glucose degradation in absence of oxygen (anaerobic glycolysis) and is produced by reduction of the pyruvate. The lactate is produced in all tissues; however, the skeletal muscle, the brain and the erythrocytes are responsible for production of most part of the organism lactate. Normal lactate production is 1 mmol/Kg/hour. Concentration of lactate in the blood depends on the production and metabolism rate in the liver and kidneys. Approximately 30% of the total basal production of lactate is used by the liver with predominance in the gluconeogenesis.

Increased lactate concentration in the blood indicates a decrease of the blood flow for the tissues, with consequent decrease in oxygen provision.

Reduced tissue oxygenation, arising out of hypovolemia, chock or left ventricular insufficiency may induce the individual to lactic acidity (type A or hypoxic), in which status there is increased concentration of the blood lactate.
Increase of blood lactate is also noted in type B or metabolic lactic acidity. The latter is associated with diseases such as *Diabetes Mellitus*, neoplasia and hepatic disease or with drugs and/or toxins, such as ethanol, methanol and salicylate.

After long-duration physical exercise, the lactate production may significantly increase. In order to assess the capacity of exercise and monitor intensity of training of the athletes, determination of the blood lactate may be made.

Lactate concentration in the liquor is usually similar to the blood levels. However, in biochemical changes at SNC, the lactate values in the liquor are changed regardless of the blood values. Increased levels at the liquor are observed in brain vascular accidents, intracranial hemorrhage, bacterial meningitis, epilepsy and other SNC disturbances.

Notes

1. Appropriate cleaning and drying of the material used are core factors for stability of the reagents and obtainment of correct results.

2. The clinical laboratory is aimed at providing accurate and precise results. Use of inappropriate quality water is a potential cause of analytical bias. The water used in the laboratory should have the appropriate quality for each application. Thus, to prepare reagents, use in the measures and for use in the final rinsing of the flasks, the water should have resistivity ≥1 megaohm.cm or conductivity ≤1 microsiemens/cm and silicate concentration <0,1 mg/L. When the deionizing column is with its capacity saturated, occurs release of several ions, silicates and substances with large oxidation or reduction power that deteriorate the reagents in a few days or even hours, thus changing the results unpredictably. Consequently, it is essential to establish a quality control program for the water.

Referências

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Presentation

Product	Reference	Content	
Enzymatic Lactate	138-1/50	R 1	1 X 40 mL
		R 2	1 X 10 mL
		CAL	1 X 5 mL
Enzymatic Lactate Labmax 560/400	138-2/20	R 1	2 X 16 mL
		R 2	2 X 4 mL
		CAL	1 X 5 mL
Enzymatic Lactate Linha CS400/CS800	138-1/88	R 1	1 X 68 mL
		R 2	1 X 20 mL
		CAL	1 X 5 mL
Enzymatic Lactate Linha Audmax i	138-1/50	R 1	1 X 40 mL
		R 2	1 X 10 mL
		CAL	1 X 5 mL

For information about other commercial presentations, consult the website www.labtest.com.br or contact SAC.

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.

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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 > ensaios** Contenido suficiente para < n > ensayos Sufficient content for < n > trials		**Risco biológico** Riesgo biológico Biological risk
	**Prazo de validade (aaaa-mm-dd ou mm/aaaa)** Fecha de expiración (aaaa-mm-dd o mm/aaaa) Expiration date (yyyy-mm-dd or mm/yyyy)		**Corrosivo** Corrosivo Corrosive
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limit (store at)		**Tóxico** Tóxico Poison
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Marca CE** Marcado CE CE Mark
	**Carcinogênico/mutagênico e/ou sensibilizante à respiração** Carcinogénico/mutagénico y/o sensibilizante respiratorio Carcinogenic/mutagenic and/or respiratory sensitizer		**Atenção** Atención Attention
	**Tóxico para os organismos aquáticos** Tóxico para los organismos acuáticos Toxic for aquatic organisms		**Data de fabricação** Fecha de fabricación Date of manufacture
	**Gases/líquidos comburentes** Gases/liquidos oxidantes Oxidizing gases/liquids		**Fabricante** Fabricante Manufacturer
	**Substância inflamável** Sustancia inflamable Flammable substance		**Uso veterinário** Uso veterinario Veterinary use
	**Período após abertura** Período post-abertura Period after-opening	LYOPH	**Liofilizado** Liofilizado Lyophilized
	**Produto de uso único** Producto de un solo uso Single use product	LOT	**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use	REF	**Número do catálogo** Número de catálogo Catalog Number
	**Instalar até** Instalar hasta Install before	CONTROL	**Controle** Control Control
CAL	**Material Calibrador/Padrão** Material Calibrador/Estándar Calibrator/Standard Material	CONTROL –	**Controle negativo** Control negativo Negative control
IVD	**Dispositivo médico de diagnóstico in vitro** Dispositivo médico para diagnóstico in vitro In vitro diagnostic medical device	CONTROL +	**Controle positivo** Control positivo Positive control
M	**Reagente contendo microparticulas** Reactivo con microparticulas Reagent with microparticles	R R	**Reagente** Reactivo Reagent
UDI	**Identificador único do dispositivo** Identificador único del dispositivo Unique device identifier	PT	**Pré-Tratamento** Pretratamiento Pre-Treatment

Ref.: 160125

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