LACTATE

Lactate

LO-POD. Enzymatic colorimetric

Quantitative determination of lactate
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

Lactate is oxidized by lactate oxidase (LO) to pyruvate and hydrogen peroxide (H_2O_2), which under the influence of peroxidase (POD), 4-aminophenazone (4-AP) and 4-chlorophenol form a red quinone compound:

$$\text{L-Lactate} + O_2 + H_2O \xrightarrow{LO} \text{Pyruvate} + H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{4-Chlorophenol} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the lactate concentration in the sample[1].

CLINICAL SIGNIFICANCE

Lactate is a metabolic intermediary, originated in the lactic fermentation from glucose, which accumulates during high intensity exercise as a result of the associated increase in glycolytic activity. The formation of ATP is linked to the generation of lactate and H^+.
If fatigue develops, the increased levels of lactate correlate with the reduction of force[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	PIPES pH 7,5 4- Chlorophenol	50 mmol/L 4 mmol/L
R 2 Enzymes	Lactate oxidase (LO) Peroxidase (POD) 4- Aminophenazone (4-AP)	800 U/L 2000 U/L 0,4 mmol/L
LACTATE CAL	Lactate aqueous primary standard	10 mg/dL

PREPARATION

Working reagent (WR): Dissolve (→) the contents of one vial R 2 Enzymes in 10 mL of R 1 Buffer.
Cap and mix gently to dissolve contents.
The reagent is stable after reconstitution 1 month at 2-8ºC or 1 week at room temperature (15-25º).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC 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 505 nm ≥ 0,18.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Plasma. Free of hemolysis1. As anticoagulants use glycolytic inhibitors: fluoride/oxalate or fluoride/heparin.
Plasma must be placed on a refrigerator and separated of the blood cells within 15 min; the reason is that blood cells will metabolism glucose to lactic acid.
Once is separated, lactate is stable in plasma 8 hours at 20 – 25ºC and 14 days at 2-8ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . .505 nm. (490-550)
   Cuvette: . . . . . 1 cm. light path
   Temperature: . . . . . .37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette: (Note 3)

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard(Note 1,2) (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min. at 37ºC or 10 min. at room temperature (15-25ºC).
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A)\text{Sample} - (A)\text{Blank}}{(A)\text{Standard} - (A)\text{Blank}} \times 10 \text{ (Standard conc.)} = \text{mg/dL lactate in the sample}$$

Conversion factor: mg/dL x 0,1123= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

0,5-2,2 mmol/L ≅ 4,5-19,8 mg/dL
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range*:* From *detection limit* of 0,099 mg/dL to *linearity limit* of 150 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	13,8	31,7	14,3	32,1
SD	0,07	0,18	0,34	0,64
CV (%)	0,53	0,56	2,36	2,00

Sensitivity*:* 1 mg/dL = 0,013 A.
Accuracy: Results obtained using SPINREACT 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 : $(r)^2$: 0,998.
Regression equation: y= 1,1488x – 0,9688
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Intravenous injection of epinephrine, glucose, bicarbonate, or other infusions that modify the acid-base balance, causing an elevation in lactate. Avoid using hemolyzed samples[1].
A list of drugs and other interfering substances with lactate determination has been reported by Young et. al[2,3].

NOTES

1. LACTATE CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

1. Gau N. Lactic acid. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1040-1042 and 418.
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.

PACKAGING

Ref: 1001330	Cont.	R1: 1 x 50 mL, R2: 5 → 10 mL, CAL: 1 x 5 mL

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