Lactate

LO-POD. Enzymatic colorimetric

Quantitative determination of lactate

IVD

PACKAGING

Ref.: 101-0565	Cont.: 9 x 10 mL

Store at 2 - 8º C

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.

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].

REAGENTS

R 1 Buffer	PIPES pH 7.5	50 mmol/L
	4- Chlorophenol	4 mmol/L
R 2 Enzymes	Lactate oxidase (LO)	800 U/L
	Peroxidase (POD)	2000 U/L
	4- Aminophenazone (4-AP)	0.4 mmol/L
LACTATE CAL	Lactate aqueous primary standard 10 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

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 - 8º C or 1 week at room temperature (15 – 25º 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 - 8º 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 505 nm ≥ 0.18.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or heparinzed plasma. Free of hemolysis[1].

Serum or plasma must be placed on a refrigerator and separated of the blood cells within 15 min; the reason is that blood cells will metabolise glucose to lactic acid. Once is separated, lactate is stable.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

LACTATE CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

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

	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 - 25º C).
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

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

Conversion factor: mg/dL x 0.1123= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

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 0.39 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.

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Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	11.1	21.8	11.4	22.1
SD	0.24	0.25	0.36	0.54
CV (%)	2.14	1.16	3.12	2.47

Sensitivity: 1 mg/dL = 0.01 A.

Accuracy: Results obtained using CHRONOLAB 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) 0.998.
Regression equation: y= 0.9979x + 1.2518.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
Intravenous injection of epinephrine, glucose, bicarbonate, or ather 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].

BIBLIOGRAPHY
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