Reactivos GPL

Barcelona, España

CE

LACTATE

- Lactate - LO-POD. Enzimatic-colorimetric

Store at: +2+8ºC.

Presentation:
Cod. SU024 CONT: *R1 1 x 100 mL + R2 10 x 10 mL.+ CAL 1 x 5 mL.*

Procedure

Quantitative determination of lactate.
Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

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 COMPOSITION

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 calibrator	10 mg/dL.

REAGENT PREPARATION AND STABILITY

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. (WR) is stable 1 month at 2-8ºC or 1 week at room temperature (15-25ºC).
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use.
Do not use reagents over the expiration date.
Lactate Cal: Once open is stable up to 1 month when stored tightly closed at 2-8ºC, protected from light and contamination prevented during their use.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm. ≥ 0.18

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

SPECIMEN

Heparinzed plasma. Free of hemolysis[1].
Specimen must be placed on a refrigerator and separated the plasma within 15 min; because the blood cells will metabolise glucose to lactic acid. Once the plasma is separated, lactate is stable.

MATERIAL REQUIRED BUT NOT PROVIDED

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

General laboratory equipment.

TEST PROCEDURE

1. **Assay Conditions**
- Wavelenght : ..... 505 nm.
- Cuvette: ..... 1 cm light path.
- Temperature ..... 37º / 15-25ºC.

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
WR (mL.)	1.0	1.0	1.0
Calibrator[(note1-2)] (µL.)	--	25	--
Sample (µL.)	--	--	25

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

CALCULATIONS

$$\text{Lactate (mg/dL.)} = \frac{(A)\,\text{Sample}}{(A)\,\text{Standard}} \times 10\ (\text{Calibrator conc.})$$

Conversion Factor. mg/dL. x 0.111 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Normal and Pathological.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

4,5 – 19,8 mg/dL. 0.5-2,2 mmol/L.

(These values are for orientation purpose).
It is suggested that each laboratory establish its own reference range.

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.

REAGENT PERFORMANCE

– *Measuring Range*:
From detection limit 0f 0.39 mg/dL. to linearity limit of 150 mg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.

– *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. = 0.01A
- Accuracy: Results obtained GPL 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.

INTERFERING SUBSTANCES

- 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].

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.

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