Lactate

For lactate determination in plasma and cerebrospinal fluid

SUMMARY

Lactic acid, an intermediate in the anaerobic metabolism of carbohydrates, comes mainly from skeletal muscle, brain, skin, renal medulla and erythrocytes. Lactate concentration in blood depends on the balance between its production in the tissues and its metabolism in the liver and kidneys. Approximately 65% of the lactate produced is used mainly by the liver in the gluconeogenesis process. When lactate concentration is greater than 18 mg/dL (2 mmol/L) liver lactate clearance is saturated by increasing its blood level. A specific example occurs during prolonged exercise in which lactate levels may increase significantly. The increase in blood lactate associated with a decrease in blood pH is called lactic acidosis. The decrease in tissue oxygenation (hypoxia) is the most common cause of lactic acidosis, for example secondary hypoxia to different clinical conditions such as shock, pneumonia, acute hemorrhage, pulmonary edema and congestive heart failure. There have also been cases of lactic acidosis in hepatic necrosis, neoplasms, lymphoma, various forms of leukemia, thiamine deficiency and in diabetic ketoacidosis. Other causes of lactic acidosis include intravenous infusion of substances such as fructose, sorbitol, epinephrine, and increased intake of alcohol and/or acetaminophen.

Lactate levels in the cerebrospinal fluid (CSF) are similar to its levels in blood, but in the presence of CNS pathologies lactate concentration in CSF varies independently. It is observed an increase of lactate levels in cerebrospinal fluid (CSF) in bacterial meningitis, hypocapnia, hydrocephalus, brain abscess, cerebral ischemia and/or any clinical condition associated with a reduced oxygenation of the brain, inflammation and/or increased intracranial pressure.

PRINCIPLE

The lactate from the sample is oxidized by the lactate oxidase specific enzyme (LOD). The hydrogen peroxide formed in this reaction is then used by the peroxidase (POD) to generate a chromogen.

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + H_2O_2$$

$$H_2O_2 + \text{TOOS} + \text{4-AAP} \xrightarrow{\text{POD}} \text{chromogen} + 2\,H_2O$$

The color intensity of the complex formed is directly proportional to the concentration of L-lactate in the sample and is determined by measuring the increase in absorbance at 540-550 nm.

PROVIDED REAGENTS

A. Reagent A: 3.5 mm TOOS, ascorbate oxidase (cucumber) ≥ 30 U/mL; 100 mM phosphate buffer pH 7.8, sodium azide < 0.1%.

B. Reagent B: 5 mM 4-aminoantipyrine, lactate oxidase (microorganisms) ≥ 10 U/mL peroxidase (horseradish) ≥ 24 U/mL 100 mM phosphate buffer pH 7.8, sodium azide <0 .1%.

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus**.
- Saline solution (9 g/L NaCl).

INSTRUCTIONS FOR USE

Reagent A: ready to use.
Reagent B: ready to use.

WARNINGS

The reagents are for "in vitro" diagnostic use.
Use the reagents keeping standard work precautions in the clinical chemistry laboratory.
All reagents and samples must be discarded according to local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: Unopened reagents are stable in refrigerator (2-10ºC) until the expiration date shown on the box.

SAMPLE

Plasma and CSF

a) Collection: the collected sample may be plasma or cerebrospinal fluid. Do not use serum.

Blood samples must be collected from a vein free from stasis, although a minimal hemostasis (less than 30 seconds) does not affect lactate levels. Avoid where possible the use of tourniquet.

In the case of plasma is collected, centrifuge within 15 minutes after obtaining the sample.

Collection systems for plasma samples from different manufacturers may contain different materials, which in some cases may affect the results. If the samples are processed in primary tubes (sample collection system for plasma) follow the instructions of the tubes manufacturer.

The CSF may be used directly without being processed

b) Additives: in case plasma is collected, use fluoride/EDTA (Wiener lab. **Anticoagulante G**), fluoride/heparin and fluoride/oxalate. If plasma is obtained without inhibitors of glycolysis (fluoride), store the whole blood in ice and separate plasma from cells within 15 minutes after collection.

c) Known interfering substances: no interference is observed by triglycerides up to 1400 mg/dL, bilirubin up to 32 mg/dL, hemoglobin up to 1000 mg/dL, heparin up to 55 IU/L and ascorbic acid up to 50 mg/dL.
Refer to the literature of Young to see the effects of other interfering substances in this method.
d) Stability and storage instructions: plasma samples should be rapidly processed; otherwise they must be stored at 2-10°C or frozen at -20°C because the lactate increases 20% in 3 minutes and 70% in 30 minutes at 25°C. Samples are stable for 8 days at 2-10°C and for 4 weeks at -20°C.

REQUIRED MATERIAL (non-provided)
- Micropipettes for measuring stated volumes.
- Automated analyzer

PROCEDURE
(Automated analyzer)
A general procedure for determination of lactate in automated analyzers is detailed below. When implementing the technique on a particular analyzer, follow the same work instructions. In a cuvette maintained at the required temperature, place:

Sample or Calibrator	2 uL
Reagent A	175 uL

Incubate for 120 seconds at 37°C. Read absorbance at 540-550 nm (sample blank)

Reagent B	35 uL

Incubate for 300 seconds at 37°C. Read absorbance at 540-550 nm (lactate concentration).
Wiener lab analyzers automatically calculate lactate concentration for each sample.

CALIBRATION
Calibrator A plus is processed in the same way as the samples and the corresponding factor is calculated from it. The lactate concentration values vary batch to batch. Check the values in the package insert of Wiener lab's **Calibrator A plus.** We recommend using two-point calibration after changing reagent batch and when required by Quality Control.

QUALITY CONTROL METHOD
Process 2 levels of quality control material (**Standatrol S-E 2 niveles**) with known concentrations of lactate.

REFERENCE VALUES
Plasma
Venous blood: 4.5 - 19.8 mg/dL
Arterial blood: <11.3 mg/dL

CSF
Newborns: 10 - 60 mg/dL
3 to 10 days old: 10 - 40 mg/dL
> 10 days old and adults 10 to 25 mg/dL

UNITS CONVERSION
1 mg/dL x 0.111 = 1 mmol/L

PROCEDURE LIMITATIONS
See Known Interfering Substances under SAMPLE.
Lactate levels increase rapidly after physical activity. The time required to return to baseline depends on the individual but usually 30 minutes of rest is enough.
Once the process of glycolysis is activated, lactate levels begin to increase rapidly. Since plasma cells favor this process, it is essential to separate the plasma from the globular package quickly in order to obtain accurate concentrations of lactate. Lactate increases 20% in 3 minutes and 70% in 30 minutes at 25°C.
To preserve the integrity of the reagents avoid any kind of contamination, using only perfectly clean and dry micropipettes for measurement. The use of Wiener lab's **Standatrol S-E 2 niveles** as quality control material is recommended. Due to the use of controls from other trademarks values outside the specified range may be obtained, since they depend on the method or system used.

PERFORMANCE
a) Reproducibility: simultaneously processing replicates from the same sample, the following values may be obtained:

Level	S.D.	CV_{wr}	CV_t
11.6 mg/dL	± 0.15 mg/dL	1.3%	2.7%
21.9 mg/dL	± 0.26 mg/dL	1.2%	2.6%
38.5 mg/dL	± 0.50 mg/dL	1.3%	2.4%

b) Linearity: the reaction is linear up to 130 mg/dL. For higher values dilute the sample 1+2 parts with saline solution (NaCl 9 g/L), repeat the determination and multiply the result by the dilution factor.
c) Detection limit: 0.7 mg/dL.
d) Quantification limit: 1.8 mg/dL.

WIENER LAB PROVIDES
72 mL: 1 x 60 mL Reagent A
1 x 12 mL Reagent B
(Cat. Nº 1999795)

72 mL: 1 x 60 mL Reagent A
1 x 12 mL Reagent B
(Cat. Nº 1009380)

24 mL: 1 x 20 mL Reagent A
1 x 4 mL Reagent B
(Cat. Nº 1009370)

24 mL: 1 x 20 mL Reagent A
1 x 4 mL Reagent B
(Cat. Nº 1009668)

REFERENCES

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SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.


This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP Authorized representative in the European Community

IVD "In vitro" diagnostic medical device


Contains sufficient for <n> tests


Use by


Temperature limitation (store at)


Do not freeze


Biological risks

Volume after reconstitution

Cont. Contents

LOT Batch code


Manufactured by:


Harmful


Corrosive / Caustic


Irritant


Consult instructions for use

Calibr. Calibrator

CONTROL Control

CONTROL + Positive Control

CONTROL − Negative Control

REF Catalog number

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