LDH-L

For the determination of lactate dehydrogenase in serum, plasma and cerebrospinal fluid

SUMMARY

The determination of lactate dehydrogenase activity has a wide variety of clinical uses. As an intracellular enzyme, its increase indicates tissue damage with its consequent release to the blood stream. The damage can range from simple anoxia with small cell damage and cytoplasm loss to severe cellular necrosis causing various degrees of enzyme activity increase.
In Acute Myocardial Infarction, the total LDH activity (along with that of CK and AST) constitutes an important diagnostic element. The activity starts increasing 12-24 hours after the infarction and reaches a peak between 48-72 hours, remaining high up to the seventh or tenth day.
On the other hand, an LDH activity increase is observed in patients with hepatic necrosis (produced by toxic agents or acute infections such as viral hepatitis) even accompanying renal tubular necrosis, pyelonephritis, etc.
In blood tumors like leukemia and lymphoma increased levels of LDH are also observed.
In the cerebrospinal fluid (CSF) normal value is approximately 10% of its value in serum, markedly increasing its value in bacterial meningitis. In viral meningitis, LDH increases its value only in 10% of cases.

PRINCIPLE

The method is based on the following reaction scheme:

$$\text{L-Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

The rate of NADH formation is directly proportional to the LDH catalytic activity and is determined by measuring the increased absorbance at 340 nm.
Test concentrations are optimized according to the reference procedures for measuring the enzyme catalytic activities at 37ºC described by the International Federation of Clinical Chemistry (IFCC).

PROVIDED REAGENTS

A. Reagent A: 400 mM methylglucamine (MEG) and 61 mM lactate solution; pH 9.4 at 37ºC.
B. Reagent B: solution containing 61 mM NAD.

NON-PROVIDED REAGENTS

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

INSTRUCTIONS FOR USE

Reagent A and B: ready to use.

WARNING

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
All reagents and samples should be discarded according to current regulations.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. Once opened, they should not remain uncapped or outside the refrigerator for extended periods of time. Avoid contamination. Protect from direct light.

SAMPLE

Serum, plasma or CSF
a) Collection: obtain serum in the usual way, free from hemolysis. It can also be use plasma or CSF. The plasma must be free from hemolysis and cells since platelets have high concentrations of LDH. The plasma collected in primary tubes, according to the manufacturer's instructions, may contain cells in suspension, producing falsely increased results. Transferring the plasma into a secondary tube and centrifuging is recommended.
b) Additives: when using plasma, heparin is recommended as anticoagulant. EDTA or citrate could also be used as anticoagulant. When EDTA is used, it has shown to decrease the activity of LDH up to 10%.
c) Known interfering substances: no interference has been observed with triglycerides up to 1400 mg/dl and bilirubin up to 30 mg/dl. Hemoglobin significantly interferes in its full range of concentrations, thus it is recommended to use samples free from hemolysis.
Refer to Young, D.S. in references for drugs' effect on the present method.
d) Stability and storage instructions: separate serum or plasma from clot or cells and perform the test immediately. In case the test cannot be performed immediately, sample could be stored for up to 7 days at 20-25ºC, for up to 4 days at 2-10ºC or for up to 6 weeks at -20ºC.

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring the stated volumes
- Automated analyzer

PROCEDURE

(Automated analyzer)

Below is a general procedure for **LDH-L** in automated analyzers. When implementing the technique in a particular analyzer follow its work instructions. In a cuvette kept at the selected temperature, place:

Sample or Calibrator	4 ul
Reagent A	100 ul
Incubate for 300 seconds at 37°C	
Reagent B	20 ul

Incubate for 120 seconds at 37°C.
[LDH] = (ΔA/min) x factor; $\varepsilon_{NAD/NADH}$ = 6230 M^{-1} cm^{-1}
Wiener lab analyzers calculate automatically the LDH activity of each sample.

CALIBRATION
LDH-L method was standardized against the IFCC's original formula used as reference. Calibrador A plus should be processed in the same way as samples and the corresponding factor is calculated based on it.
We recommend using a two-point calibration after changing reagent lot and when required by the quality control.

QUALITY CONTROL METHOD
Process 2 levels of quality control material (**Standatrol S-E 2 niveles**) with known activities of lactate dehydrogenase for each determination.

REFERENCE VALUES
Adults: 135 -240 U/l
Children (2 -15 years old): 120 -300 U/l
Newborns (4-20 days): 240 - 600 U/l

It is recommended that each laboratory establish its own reference values, taking into account sex, age, eating habits, medications and other population factors.

SI SYSTEM UNITS CONVERSION
LDH (U/L) x 0.0167 = LDH (ukat/l)

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.
Plasma samples should be centrifuged before testing.
To preserve reagents' integrity avoid all forms of contamination, using only perfectly clean and dry micropipettes for measurement. We recommend Wiener lab.'s **Standatrol S-E 2 niveles** as quality control material, since different values than the specified range may be obtained with controls from other trademarks, because they depend on the method or system used.

PERFORMANCE
a) Reproducibility: simultaneously processing replicates of the same sample, the following values were obtained:

Level	S.D	C.V.
150 U/l	± 2.69 U/l	1.8 %
250 U/l	± 3.75 U/l	1.5 %

b) Linearity: the reaction is linear up to 1000 U/l. For higher values dilute sample 1+4 parts with saline (9 g/L NaCl), repeat the assay and multiply the result by the dilution factor.
c) Detection limit: 4 U/l
d) Quantification limit: 20 U/l

PARAMETERS FOR AUTOMATED ANALYZERS
For programming instructions, refer to the corresponding applications for LDH-L method of the Wiener lab automated analyzers. For calibration use Wiener lab's **Calibrador A plus**.

WIENER LAB PROVIDES
120 ml: - 1 x 100 ml Reagent A
- 1 x 20 ml Reagent B
(Cat. Nº 1999726)

120 ml: - 2 x 50 ml Reagent A
- 2 x 10 ml Reagent B
(Cat. Nº 1009213)

150 ml: - 2 x 60 ml Reagent A
- 2 x 15 ml Reagent B
(Cat. Nº 1009364)

150 ml: - 2 x 60 ml Reagent A
- 2 x 15 ml Reagent B
(Cat. Nº 1009625)

REFERENCES

- Conmutable Calibrador with Value Assigned by the IFCC Reference Procedure. Clinical Chemistry 54/8:1349-1355, 2008.
- IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C. Clin. Chem. Lab. Med. 40/6:643-648, 2002.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI EP6-A Vol. 23 Nº 16, 2003.
- Method Comparison and Bias estimation Using Patient Samples; Approved Guideline - Second Edition. CLSI EP9-A2 Vol. 22 Nº 19, 2002.
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI EP17-A Vol. 24 Nº 34, 2004.
- J. Vázquez, M. Adducci, D. Monzón, K. Iserson - Journal of Emergency Medicine, 37/1:93-97, 2009.

Symbols

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

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

Authorized representative in the European Community

"In vitro" diagnostic medical device

Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Contents

Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibrator

Control

Positive Control

Negative Control

Catalog number

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