LIQUID LINE

LDH-P UV AA

Optimized UV method (SFBC) for the determination of lactate dehydrogenase (LDH) in serum or plasma

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 reaction system is as follows:

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

Assay concentrations are optimized according to the Societê Français de Biologie Clinique (SFBC).

PROVIDED REAGENTS

A. Reagent A: Tris buffer solution pH 7.2, containing pyruvate and sodium chloride.
B. Reagent B: vial containing NADH.

Final concentrations (according to SFBC)

Tris	80 mM, pH 7.2
Pyruvate	1.6 mmol/l
NADH	0.2 mmol/l
ClNa	200 mmol/l

INSTRUCTIONS FOR USE

Provided Reagents: ready to use. They could be used separately or as **Monoreagent**, mixing 4 parts Reagent A with 1 part Reagent B (e.g.: 4 ml Reagent A + 1 ml Reagent B).

WARNINGS

Reagents are for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator (2-10ºC) until the expiration date shown on the box. Once opened, they should not remain uncapped and outside the refrigerator for extended periods of time. Avoid contamination.
Monoreagent (premixed): stable in refrigerator (2-10ºC) for 1 month from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When the spectrophotometer has been set to zero with distilled water, absorbance readings of the premixed Monoreagent lower than 0.800 O.D. or higher than 1.800 O.D. (at 340 nm) indicate its deterioration.

SAMPLE

Serum or plasma
a) Collection: obtain serum as usually. Separate serum from clot within 2 hours from collection. Plasma can also be used.
b) Additives: when using plasma, use heparin as anticoagulant.
c) Known interference substances: no interferences are observed from triglycerides up to 570 mg/dl, bilirubin up to 18 mg/dl, hemoglobin up to 180 mg/dl (in samples with normal levels of LDH) or up to 350 mg/dl (in samples with elevated levels of LDH) and heparin up to 50 IU/ml.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: use fresh samples. LDH is stable in refrigerator up to 24 hours. Do not freeze.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Adequate volumetric material.
- Water bath at the temperature stated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS

(Absorbance decrease)
- Wavelength: 340 nm (Hg 334 or 366)
- Reaction Temperature: 25, 30 or 37ºC. See REFERENCE VALUES corresponding to each temperature.
- Reaction Time: 3 minutes and 30 seconds.

The sample and reagent volumes may be proportionally modified without altering the calculation factors.

PROCEDURE

I- MONOREAGENT TECHNIQUE

A) 30-37°C

In a cuvette at the desired working temperature, place:

Monoreagent	1 ml
Pre-incubate for a few minutes, then add:	
Sample	20 ul

Mix immediately and simultaneously start the stopwatch. Wait for 30 seconds. Read initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes from the first reading. Determine the average change in absorbance/min (ΔA/min), subtracting each reading from the previous one and averaging the values. Use this mean for calculations.

B) 25°C

Use 100 ul Sample and 3 ml Monoreagent, following the procedure indicated in I-A).

II- SEPARATE REAGENTS' TECHNIQUE

A) 30-37°C

In a cuvette at working temperature, place:

Reagent A	1 ml
Sample	20 ul
Pre-incubate for a few minutes, then add:	
Reagent B	0.25 ml

Mix immediately and simultaneously start the stopwatch. Wait for 30 seconds. Read initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes from the first reading. Determine the average change in absorbance/min (ΔA/min), subtracting each reading from the previous one and averaging the values. Use this mean for calculations.

B) 25°C

Use 3 ml Reagent A with 100 ul Sample and 0.75 ml Reagent B, following the procedure indicated in II-A).

CALCULATIONS

LDH (U/l) = ΔA/min x factor

In each case, the corresponding calculation factor should be used according to the selected reaction temperature (30-37°C or 25°C) and the technique used (Monoreagent or separate reagent) as shown in the table below:

MONOREAGENT TECHNIQUE

Temperat. / Wavelenght	25°C	30-37°C
340 nm	4,921	8,095
334 nm	5,016	8,253
366 nm	9,118	15,000

SEPARATE REAGENTS TECHNIQUE

Temperat. / Wavelenght	25°C	30-37°C
340 nm	6,111	10,080
334 nm	6,230	10,275
366 nm	11,324	18,675

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known lactate dehydrogenase activity.

REFERENCE VALUES

Temperature	25°C	30°C	37°C
Values (U/l)	120-240	160-320	230-460

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

LDH (U/l) x 0.017 = LDH (ukat/l)

PROCEDURE LIMITATIONS

See known interfering substances under SAMPLE.

Low initial absorbance: once the serum is added, if the first reading (0 time) is lower than 0.800 O.D., with the Reagent B in good conditions, it indicates a sample with a very high LDH activity (that consumes NADH even before this reading). In this case, dilute the sample 1/10 with saline solution, repeat the assay, and multiply the result by the dilution performed.

PERFORMANCE

a) Reproducibility: when simultaneously processing replicates of one sample, the following results were obtained:

Level	S.D.	C.V.
439 U/l	± 3.64 U/l	0.8 %
919 U/l	± 11.41 U/l	1.2 %

b) Linearity: the linearity range is extended up to 1000 U/l. If ΔA/min is higher than 0.120 O.D. (340-334 nm and 37°C), repeat the assay, dilute the sample 1/5 or 1/10 with saline solution, correcting the results accordingly.

c) Quantification limit: the lower quantifiable lactate dehydrogenase activity is 24 U/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB PROVIDES

100 ml (Cat. Nr. 1521304): - 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B

100 ml (Cat. Nr. 1009267): - 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B

125 ml (Cat. Nr. 1009315): - 5 x 20 ml Reagent A
- 2 x 12.5 ml Reagent B

150 ml (Cat. Nr. 1009626): - 2 x 60 ml Reagent A
- 2 x 15 ml Reagent B

REFERENCES

- Societé Française de Biologie Clinique (SFBC) - Ann. Biol. Clin. 40:160, 1982.
- Sociedad Española de Química Clínica - Comité Científico, Comisión de Enzimas - Quim. Clin. 57-61, 1989.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

SYMBOLS

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

CE	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:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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