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:

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

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

PROVIDED REAGENTS

A. Reagent A: vial containing NADH.

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

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

Reagent A: add to a Reagent A vial the volume of Reagent B indicated on the label. Cap tightly and shake gently by inversion until complete dissolution. Date.

Reagent B: ready to use.

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 (2-10°C) until the expiration date shown on the box.

Reconstituted Reagent A: stable in refrigerator (2-10°C) for 21 days or 3 days at room temperature from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When the spectrophotometer has been set to zero with distilled water, absorbance readings of the reconstituted Reagent A 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 in the usual way. Separate from clot within 2 hours. Plasma can also be used.

b) Additives: when using plasma, use heparin as anticoagulant.

c) Known interference substances: samples with visible or intense hemolysis should not be used as they produce falsely increased values.

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 to measure the indicated volumes.
- Adequate volumetric material.
- Water bath at the temperature indicated in the procedure to follow.
- Stopwatch.

ASSAY CONDITIONS

(Absorbance decrease)

- Wavelength: 340 nm (Hg 334 or 366)
- Reaction Temperature: 25, 30 or 37°C. See REFERENCE

VALUES corresponding to each temperature.
- Reaction Time: 3 minutes and 30 seconds.
- The sample and reagent volumes can be proportionally decreased without altering the calculation factors.

PROCEDURE

A) 25°C

In a cuvette at the desired working temperature, place:

Reconstituted Reagent A	3 ml
Pre-incubate for a few minutes, then add:	
Sample	100 ul

Mix immediately and simultaneously start the stopwatch. Wait 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) 30-37°C

I- MACROTECHNIQUE
Use 50 ul Sample, following the procedure indicated in A).

II- MICROTECHNIQUE
Use 20 ul Sample and 1.0 ml reconstituted Substrate following the procedure indicated in 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 (micro or macrotechnique) as shown in the table below:

Temperat. / Wavelength	25°C	30-37°C	
		I	II
340 nm	4921	9683	8095
334 nm	5016	9871	8253
366 nm	9118	17941	15000

REFERENCE VALUES

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

(*) calculated

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

SI SYSTEM UNITS CONVERSION

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

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.

PROCEDURE LIMITATIONS

See known interference 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 substrate (Reagent A) 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.
- Moistening deteriorates the Reagent A.

PERFORMANCE

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

Level	S.D.	C.V.
438 U/l	± 5.14 U/l	1.17 %
683 U/l	± 7.99 U/l	1.17 %

b) Detection Limit: it depends on the spectrophotometer and the wavelength. According to the sensitivity required, in spectrophotometer at 340 nm (Hg 334 or 366), with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light, ≤ 8 nm pathlength, for a ΔA minimum of 0.001, the smallest detectable change of activity will be of 5 U/l (at 340 nm and 25°C).

c) Dynamic Range: the reading range is extended up to 0.200 O.D. ΔA/min (at 340 nm and 25°C). If the ΔA/min is higher than 0.200 O.D. (340-334 nm and 25°C) or 0.100 O.D. (366 nm and 25°C), repeat the assay, dilute the sample 1/5 or 1/10 with saline solution, correcting the results accordingly.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB PROVIDES

- 3 x 20 ml (Cat. 1521303).

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 the packaging for Wiener lab. diagnostic reagent 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:


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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