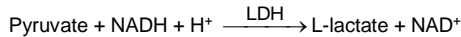


Quantitative determination of lactate dehydrogenase (LDH) IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate by NADH, according the following reaction:


 The rate of decrease in concentration of NADPH, measured photometrically, is proportional to the catalytic concentration of LDH present in the sample¹.

CLINICAL SIGNIFICANCE

Lactate dehydrogenase (LDH) is an enzyme with wide tissue distribution in the body.

The higher concentrations of LDH are found in liver, heart, kidney, skeletal muscle and erythrocytes.

 Increased levels of the enzyme are found in serum in liver disease, myocardial infarction, renal disease, muscular dystrophy and anemia^{1,4,5}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

Reagent 1	Imidazol	65 mmol/L
Buffer	Pyruvate	0,6 mmol/L
Reagent 2	NADH	0,18 mmol/L
Substrate		

PREPARATION

Working reagent (WR):

Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 15 days at 2-8°C or 5 days at 15-25°C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm <1,00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25°C, 30°C or 37°C (± 0,1°C)
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

 Serum¹. Separated from cells as rapidly as possible. Do not use oxalates as anticoagulants since they inhibit the enzyme.

Do not use haemolysed samples. Stability: 2 days at 2-8°C.

PROCEDURE

- Assay conditions:
 Wavelength: 340 nm
 Cuvette: 1 cm light path
 Constant temperature: 25°C /30°C / 37°C
- Adjust the instrument to zero with distilled water or air.
- Pipette into a cuvette:

	25° - 30°C	37°C
WR (mL)	3,0	3,0
Sample (µL)	100	50

- Mix, incubate for 1 minute.
- Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
- Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

$$25^\circ - 30^\circ\text{C} \quad \Delta A/\text{min} \times 4925 = \text{U/L LDH}$$

$$37^\circ\text{C} \quad \Delta A/\text{min} \times 9690 = \text{U/L LDH}$$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25°C	30°C	37°C
25°C	1,00	1,33	1,92
30°C	0,75	1,00	1,43
37°C	0,52	0,70	1,00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. CONTROL Normal and Pathologic.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

25°C	30°C	37°C
120-240 U/L	160-320 U/L	230-460 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 3,42 U/L to linearity limit of 1600 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (U/L)	SD	CV (%)	
Mean (U/L)	400	785	392	773
SD	3,15	10,97	6,23	9,93
CV (%)	0,79	1,40	1,59	1,28

Sensitivity: 1 U/L = 0,00009 ΔA/min.

Accuracy: Results obtained using Audit Diagnostics 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)²: 0,98382.

Regression equation: y= 0,8988x + 2,583.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Haemolysis interferes with the assay.

 Some anticoagulants such as oxalates interfere with the reaction¹.

 A list of drugs and other interfering substances with LDH determination has been reported by Young et. al^{2,3}.

NOTES
Audit Diagnostics has instruction sheets for several automatic analyzers.
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