台塑生醫科技股份有限公司
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LACTATE DEHYDROGENASE TEST LDH

Kinetic(L-P) Method

NTENDED USE

For the quantitative determination of lactate Dehydrogenase activity in serum.

INSTRUCTIONS AND CLINICAL SIGNIFICANCE

Lactate dehydrogenase (LDH) is widely distributed in mamanilian tissues, being rich in myocardium, kidney, liver and muscle. Determination of serum LDH activity is one of the most frequently performed assays as an aid in the diagnosis of myocardial and pulmonary infarction. Other conditions, such as megaloblastic anemia, extensive carcinomatois, severe shock and hypoxia, granulocytic or aute anemia hemolytic anemia, infectious mononucleosis, progressive musclar dystrophy, hepatitis, cirrhosis, obstructive jaundice, and in delirium tremens are all caused increased activity of LDH.

LDH reversibly catalyzes the conversion of Lactate to pyruvate with the simultaneous reduction of NAD^+ to NADH. The rate of NADH formation is proportional to the LDH activity.

PRINCIPLE

$$\text{Lactic acid} + NAD^+ \xrightarrow{\text{LDH}} \text{pyruvate} + NADH$$

The rate of NADH formation is followed at 340 nm.

SPECIMEN COLLECTION AND PREPARATION

Serum is the choice for the assay since heparin, EDTA reportedly inhibit LDH activity. Hemolysis should be avoided. Serum LDH is reported to be stable for 1 week at room temperature, and for 3 weeks at 2~8℃.

Specimen frozen should be avoided since LDH-4 and LDH-5 significantly decrease their activity after frozen.

REAGENT

1. Package:

R1: 2×40 ml	R2: 2×10 ml	Common
R1: 4×60 ml	R2: 4×15 ml	Hitachi 7170
R1: 4×80 ml	R2: 4×20 ml	Hitachi 7060
R1: 4×50 ml	R2: 2×25 ml	Hitachi 7020

2. Components: Contains lactate, buffer and preservatives. Store at 2~8℃.

PRECAUTIONS

1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety in Microbiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.

PROCEDURE

Wavelength: 340 nm; Cuvette: 1 cm light path; Incubation: 37℃.

Measure against water.

1. Transfer 0.8 ml of R1 and 0.2 ml of R2 reconstituted reagent into a clean Cuvette and bring it to 37℃.
2. Add 0.02 ml of serum.
3. Adjust photometer zero at 340 nm against water and measure the absorbance after 1 minute incubation-Absorbance Ⅰ.
4. After another 1 minute, read the absorbance again-Absorbance Ⅱ.
5. Absorbance II- Absorbance I =△A/min
6. The Hitachi reagents are used directly on Hitachi analyzers in accordant with Hitachi parameters.

RESULT CALCULATION

$$\text{LDH activity (U/L)} = \frac{\triangle A/\text{min} \times 1000 \times 1.02}{6.22 \times 0.02 \times 1.0} = \triangle A/\text{min} \times 8199$$

△A/min: change in absorbance per minute.

1000: convert ml to L

1.02: reagent volume (1 ml) plus serum volume (0.02 ml).

0.02: serum volume.
6.22: millimolar absorptivity of NADH at 340 nm.

EXPECTED VALUES
Male: 37℃ 80~285 U/L; Female: 37℃ 103~227 U/L;
Quality control: Frozen aliquots of pooled sera or commercially available quality control material of known activity should be assayed concurrently with patient specimens to ensure proper performance of the procedure.
Both a low and an elevated control serum must be assayed with each run to assure performance within acceptable limits.

PERFORMANCE
Specificity: Change of 0.001 absorbance over a one minute measuring period will detect approximately 6.6 U/L.
Linearity: LDH activity as high as 1000 U/L can be accurately determined without dilution.
Precision:

		Within run		Between run	
Samples		Level Ⅰ	Level Ⅱ	Level Ⅰ	Level Ⅱ
Number	n	20	20	20	20
Mean	μmol/L	131	367	153	451
SD	μmol/L	9.5	24.6	8.8	22.8
CV	%	7.30	6.70	5.80	5.10

REFERENCES
1. Wroblewski F., La Due J.S., 1955, Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. 90: 210.
2. Martinek R.G. 1972. A rapid ultraviolent spectrophotometric Lactic dehydrogenase assay. Clinical Chem. Acta 40:91.
3. Wroblewski F., 1957. The clinical significance of altemation in Lactic dehydrogenase activity of body fluids. Am. J., Med. Science 234:301.
4. Henry J.B., 1984. Clinical Diagnosis and Management by Laboratory Methods, 17th Ed. P.266-269.