LACTATE DEHYDROGENASE (LD)
MANUAL OR AUTOMATED APPLICATIONS
C144-0A; C144-0B

Intended Use
For **IN VITRO** quantitative determination of Lactate Dehydrogenase in serum.

Clinical Significance
Measurements of LD activity are primarily used for diagnosing myocardial infarctions, myocarditis, cardiac failure with hepatic congestion, liver disease, pernicious anemia, megaloblastic anemia and renal disease, as well as for monitoring the causes and treatments. (1)

Method History
The Catachem Lactate Dehydrogenase (LD) method for manual or automated chemistry is based upon the work of Wacker, et al (2) who described a method for the measurement of LD utilizing lactate as substrate and nicotinamide-adenine dinucleotide as cofactor and as the reaction indicator.

Method Principle
Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD oxido reductase) is an enzyme which catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD as a hydrogen acceptor. The reaction is reversible and strongly favors the conversion of pyruvate to lactate (2). The rate of increase in absorbance of the reaction mixture due to the formation of NADH is proportional to the LD concentration present in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.

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

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients will be approximately as follows:

LD Substrate

Each vial contains:	
L-lactic acid lithium salt	50 mmol/L
Buffer	
Nonreactive ingredients and stabilizer	

LD NAD

Each vial contains:	
Nicotinamide Adenine Dinucleotide (NAD)	103 mmol/L
Nonreactive ingredients and stabilizer	

Preparation of Working Reagents
Prepare Catachem LD Working Reagent by adding the contents of the vial containing LD NAD into the bottle containing the LD substrate. Mix and label this reagent "LD Working Reagent".

LD Reagent Storage And Stability
Store the LD reagents (liquid) at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. Store the LD Working Reagent at 2-8˚C. When prepared and stored as directed, the LD Working Reagent is stable for five days. If stored at room temperature, the reagent is stable for eight hours.

Specimen Collection And Preparation
The use of clear, unhemolyzed serum that has been separated from the clot as soon after collection as possible, is recommended.

Precaution
Avoid contact of specimen with skin. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Interfering Substances
A comprehensive discussion has been reported on the effect of interfering substances on various LD methods. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (3)

Expected Values
The range of expected values determined for this method is 120-230 U/L. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for each species under test and the area in which the laboratory is located.

Procedure
Important: Read the entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	To time incubation time
Pipette	1.0 ml for reagent
Pipette	0.050 ml for sample
Pipette or graduated cylinder	25 or 50 ml for reagent

Materials Provided: LD Reagents

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Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	zero-order rate, direct
Reaction Time	2.0 min. 37˚C
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-Reagent Ratio	1:21

Assay Procedure

1. Into separate cuvettes, pipette 1 ml Working Reagent.
2. Pipette 0.05 ml of "Control" or "Sample" into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for exactly 30 seconds. Read the "Control" and each "Sample" absorbance at 340nm at 90 or 150 seconds.

Quality Control

To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of normal and abnormal control serum.

Calibration

A reference calibration is not required to calibrate the LD procedure. Obtain results by using the given formula.

Calculations And Results

$$\text{LD U/L} = \frac{\Delta \text{ A/min} \times 1.05 \text{ ml} \times 1000}{6.22 \times 1 \text{ cm light path} \times 0.05 \text{ ml}}$$

LD U/L = A/min x 3376

A/min = Change in absorbance per minute
1.05 ml = Total assay volume in ml
1000 = Converts u/ml to u/L
6.22 = Absorbance coefficient of NADH at 340nm
Light Path = 1 cm
0.05 ml = Sample volume
3376 = Factor derived from constants in the equation

Example:
Sample A/min = 0.03
LD U/L = 0.03 x 3376 = 101 u/L

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.0002 absorbance units per U/L.

Linear Range: No significant nonlinearity over the range of 0-600 U/L.

Precision Study

LD	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
61	0.90	1.40	1.20	1.80
391	3.40	1.20	2.90	0.90
546	5.70	1.00	3.70	0.60

Correlation

A comparison of the Catachem LD method using an automated analyzer was carried out against a reference LD procedure based upon the same principle as the Catachem LD method resulted in the following regression statistics:

Range	=	100 - 431 U/L
N	=	123
Y	=	0.999x + 0.08
r	=	0.999
Sy.x	=	3.7

References

1 Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. W.B. Saunders Co. pp 652-660 (1967).
2. Wacher Wec, Ulmer Vallee. Metaloenzymes and myocardial infarction. New England J Med 225, 449 (1956).
3. Young, Pestaner, Gibberman. Effects of drugs on clinical laboratory tests. Clin Chem 21 No 5 (1975).

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