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LACTATE DEHYDROGENASE LS* (LDH-L) LIQUID REAGENT

INTENDED USE
Lactate Dehydrogenase LS (LDH-L) liquid reagent is intended to use for the *In Vitro* quantitative kinetic determination of Lactate Dehydrogenase activity in serum.

INTRODUCTION
The enzyme lactate dehydrogenase (LDH-L) is distributed in tissues particularly heart, liver, muscle, and kidney. The enzyme found in circulation is a mixture of five isoenzymes based on their mobility. Elevated serum levels of LDH-L are found in serum in myocardial infarction, liver disease, renal disease, certain forms of anemia, malignant diseases, and progressive muscle dystrophy.[1,2]

LDH catalyzes the following reaction:

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

The LDH-L enzyme activity can be measured in both directions. Using optimal conditions for both directions and taking into account isoenzyme variations, assays in either direction are considered to be equivalent.[3] However, Lactate-to-Pyruvate method offers a number of advantages[4] (1) the rate of reaction is linear over a wide range (2) no pre-incubation is required and (3) better reagent stability.

PRINCIPLE
LDH catalyzes the oxidation of lactate to pyruvate in the presence of NAD, which is subsequently reduced to NADH. The rate of NADH formation measured at 340 nm is directly proportional to serum LDH-L activity.

REAGENT COMPOSITION
After combining R1 and R2 as directed, the reagent contains: Lithium lactate 55 mmol/L; NAD 7.5 mmol/L; Buffer (8.95); and stabilizers and preservatives.

PRECAUTIONS
1. For "in vitro" diagnostic use only.
2. The reagent contains sodium azide as a preservative. Do Not Ingest. Avoid skin and eye contact.
3. All specimens and controls should be handled as potentially infectious, use safe laboratory procedures. (NCCLS M29-T2).

REAGENT PREPARATION
The working reagent is prepared by mixing five (5) volumes of R1 with one (1) volume of R2 in a disposable container.

Example: 25ml R1 + 5ml R2

REAGENT STORAGE
1. Store reagent at 2 - 8°C.
2. The reagents are stable until the expiration date if stored as directed.
3. The "working" reagent is stable for 14 days at 2-8°C.
4. Protect from direct light.
5. Avoid microbial contamination.

REAGENT DETERIORATION
1. If the reagent blank before serum addition has an absorbance that exceeds 0.600 at 340 nm, the reagent may have deteriorated.
2. Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION AND HANDLING
1. Serum with any visible hemolysis cannot be used because of the contamination of this sample with large amount of LDH released from the erythrocytes.[3]
2. Serum should be separated from the clot promptly.
3. Samples should be assayed soon after collection. LDH in serum is reported stable for two to three days at room temperature.[2]
4. The liver LDH is particularly labile and is destroyed if frozen and thawed.[5]

INTERFERENCE
1. Oxalate, oxamates, and EDTA will inhibit LDH.
2. Young, et. al. gave a list of drugs and other substances interfere with the determination of LDH activity.[6]

MATERIALS PROVIDED
Lactate Dehydrogenase LS* Buffer Reagent
Lactate Dehydrogenase LS* Co-Enzyme Reagent

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Test tubes and rack.
3. Timer.
4. Heating bath or block (37°C).
5. Spectrophotometer capable of reading at 340 nm (UV).

AUTOMATED PROCEDURE
Refer to appropriate instrument application instruction.

MANUAL PROCEDURE
1. Prepare the "working" reagent as directed.
2. Pipette 1.0 ml of reagent into appropriate tubes and prewarm at 37°C for at least five (5) minutes.
3. Zero spectrophotometer with water at 340 nm.
4. Add 0.025 ml (25 µl) of sample to reagent, mix and incubate at 37°C for one (1) minute.
5. After one (1) minute, read and record the absorbance and return tube to 37°C. Repeat reading every minute for the next two (2) minutes.
6. Calculate the average absorbance difference per minute (ΔAbs/min.)
7. The absorbance ΔAbs/min. multiplied by 6592 will yield results in U/L.

LIMITATIONS
Samples with values above 1,000 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2) to compensate for the dilution.

CALIBRATION

The procedure is standardized by means of the millimolar absorptivity of NADH, which is 6.22 at 340 nm under the test conditions described.

CALCULATION

Lactate Dehydrogenase activity is expressed as U/L.

Unit definition: One international unit (U/L) is the amount of enzyme that will reduce one micromole of NAD per minute at specific temperature.

$$IU/L = \frac{\Delta \text{ Abs./min} \times TV \times 1000}{d \times \in \times SV} = \frac{\Delta Abs/min. \times 1.025 \times 1000}{1.0 \times 6.22 \times 0.025}$$

$$= \Delta Abs/min. \times 6592$$

Where: Δ Abs/min = Average absorbance change per minute
TV = Total reaction volume (1.025)
1000 = Conversion of U/ml to U/L
d = Light path in cm (1.0 cm)
∈ = Millimolar absorptivity of NADH (6.22)
SV = Sample volume in ml (0.025)

Example:

If the average absorbance change per minute is 0.022, then 0.022 × 6592 = 145 U/L.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with LDH-L values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

EXPECTED VALUES[3]

Males	50-166 IU/L	(30°C)
	80-285 IU/L	(37°C)
Females	60-132 IU/L	(30°C)
	103-227 IU/L	(37°C)

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

1. *Sensitivity:* Based on an instrument resolution of 0.001 absorbance, this procedure has a sensitivity of 3.4 U/L.
2. *Linearity:* Linear from 0 - 1,000 U/L
3. *Comparison:* A group of 54 sera ranging in Lactate Dehydrogenase values from 15 - 992 U/L was assayed by this method and by a similar commercially available reagent. Comparison of the results yielded a correlation coefficient of 0.996 and the regression equation was y = 0.954x - 9.02. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)
4. *Precision*:

	Within-Run	
	Serum 1	**Serum 2**
Mean (U/L)	90	185
Std. Deviation (U/L)	3.37	2.32
C.V. (%)	3.74	1.19

	Run-to-Run	
	Serum 1	**Serum 2**
Mean (U/L)	90	194
Std. Deviation (U/L)	1.7	1.05
C.V. (%)	1.88	0.53

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

REFERENCES

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