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LDH-L REAGENT SET (KINETIC PROCEDURE)

INTENDED USE
For the quantitative determination of lactate dehydrogenase activity in human 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
(Concentrations based upon reconstitution): L-Lactate 75 mM; NAD 5.5 mM; Buffer 80 mM; pH 9.0 $\pm$ 0.1 (30°C); Non-reactive stabilizers and fillers.

PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION
Reconstitute reagent with volume of distilled water stated on the vial label. Invert gently to dissolve.

REAGENT STORAGE
1. Store reagent at 2 - 8°C.
2. Reconstituted reagent is stable for fourteen (14) days when refrigerated at 2 - 8°C and for eight (8) hours at room temperature (18 - 30°C).

REAGENT DETERIORATION
1. If the reagent blank before serum addition has an absorbance that exceeds 0.60 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
LDH-L 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).

PROCEDURE (AUTOMATED)
Refer to appropriate instrument application instruction.

PROCEDURE (MANUAL)
1. Reconstitute reagent according to instructions.
2. Pipette 1.0 ml of reagent into appropriate tubes and pre-warm at 37°C for three (3) 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 IU/L.
8. Samples with values above 800 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two (2).

NOTE: 1. If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate readings, 3 ml of reagent, and 0.1 ml (100 µL) of sample should be used the factor for this condition is 4984.

2. If the spectrophotometer being used is equipped with a temperature-controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

CALCULATION

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

$$IU/L = \frac{\Delta\ Abs/min \times TV \times 1000}{d \times \in \times SV} = \frac{\Delta Abs/min \times 1.025 \times 1000}{1 \times 6.22 \times 0.025}$$

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

Where: Δ Abs/min = Average absorbance change per minute
TV = Total reaction volume (1.025)
1000 = Conversion of IU/ml to IU/L
d = Light path in cm (1.0)
∈ = 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 $0.022 \times 6592 = 145$ IU/L.

PROCEDURE LIMITATION

1. The procedure measures total lactic dehydrogenase irrespective of its tissue or organ of origin.
2. The reaction temperature must be maintained to within ± 0.1°C, during the assay.

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 reagent deterioration, instrument malfunction, or procedural errors.

TEMPERATURE CORRECTION[3]

1. If the assay is performed at 37°C but is to be reported at 30°C; multiply the results by 0.6.
2. If the assay is performed at 30°C but is to be reported at 37°C; multiply the results by 1.7.

NOTE: Temperature factors give only an approximate conversion and therefore it is suggested that values be reported at the temperature of measurement.

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

1. Linearity: 800 IU/L
2. Comparison: Studies between the present method and a similar method yield a correlation coefficient of 0.98 and a regression equation of y = l.06x - 10.68
3. Precision studies:

Within Run		
Mean (mg/dl)	S.D.	C.V.%
129.7	6.3	4.8
365.4	27.7	7.5
Run-to-Run		
Mean (mg/dl)	S.D.	C.V.%
151.6	5.5	3.6
449.7	23.5	5.2

REFERENCES

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L535: 12/2017

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