Pointe Lactate Dehydrogenase Reagent Set

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Intended Use
For the *in vitro* quantitative kinetic determination of lactate dehydrogenase activity in serum using the Yumizen C230 and Yumizen C240 analyzers.
Rx Only.

Clinical Significance
Increased levels of LD are associated with myocardial infarction. Levels reach a maximum approximately 48 hours after the onset of pain and persist about ten days. The degree of elevation is of value in assessing the extent of damage and in developing a prognosis. LD elevations are also observed in liver disease, pernicious anemia, in some cases of renal disease, and in some cases of skeletal muscle trauma.[1]

Method History
Wroblewski and Ladue[2] published the first UV kinetic method for the determination of LDH activity in serum in 1955. Their method was based on the classic Kubowitz and Ott[3] assay (1943) utilizing the pyruvate to lactate reaction. In 1956, Wacker et al[4] described a procedure that followed a lactate to pyruvate reaction. The lactate to pyruvate reaction became the preferred reaction[5], even though the slower of the two, because of a wider linear range[6] and no pre-incubation requirement[7]. The present method follows the forward reaction and has been optimized for greater sensitivity and linearity as outlined by Gay et al.[8]

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

Lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate with simultaneous reduction of NAD to NADH. The rate of NAD reduction can be measured as an increase in absorbance at 340nm. This rate is directly proportional to LD activity in serum.

Reagent Composition
After combining R1 and R2 the reagent contains: NAD 5.8 mM, L-Lactate 55 mM, Buffer pH 8.95. Non-reactive stabilizers and sodium azide (0.1%) as preservative.

Reagent Preparation
Reagents are supplied as ready to use liquids.

Reagent Storage and Stability
Reagents are stable until stated expiration if stored as directed. Protect from light. Avoid microbial contamination.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. The reagents contain sodium azide (0.1%) as a preservative. Do not ingest. Avoid skin and eye contact. Sodium azide may react with lead and copper plumbing fixtures giving rise to explosive metal azides. Flush with large volumes of water when disposing of the reagent.
3. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

Specimen Collection and Storage
1. Non-hemolyzed serum is recommended. Red cells contain large concentrations of LD.[5]
2. The serum should be removed from the clot promptly.
3. Samples should be assayed soon after collection. LD in serum is reported stable for two to three days at room temperature.[9]
4. Do not freeze or expose the serum to high temperatures (37°C) as this may inactivate thermolabile LD isoenzymes.[10]
5. Specimen collection should be carried out in accordance with NCCLS M29-T2.[11] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all samples should be considered potentially infectious.

Interferences
1. Certain drugs and substances affect LD activity. See Young, et al.[12]
2. Bilirubin to the level of 20 mg/dl has been found to exhibit negligible interference (≤ 5%) in this assay.
3. Hemolysis has been shown to significantly interfere with the assay at levels as low as 100 mg/dl.

Materials Provided
Lactate Dehydrogenase Buffer (R1) Reagent
Lactate Dehydrogenase Co-Enzyme (R2) Reagent

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	LDH	Chemistry:	Lactate Dehydrogenase
Chemistry No.:	223	Print Name:	LDH
Reaction Type:	Kinetic	Reaction Direction:	Positive
Pri. Wave:	340 nm	Sec. Wave:	405 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	3 11
Unit:	U/L	Incubation Time:	3

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	11	uL	uL	uL	180	uL	uL
Decreased;		uL	uL	uL	45	uL	uL
Increased;		uL	uL	uL			

Linearity Range (Standard);	0-1000		Linearity Limit:	0.3
Linearity Range (Decreased):			Substrate Depletion:	25000
Linearity Range (Increased):			Mixed Blank Abs.:	-40000 40000
R1 Blank Abs.:	-40000	40000	On-board Stability:	30 Day (s)
Blank Response	-40000	40000	Reagent Alarm Limit:	5
Twin Chemistry:				

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	U/L

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe Liquid Lactate Dehydrogenase Reagent Set

Calibration Setup Parameters

Chem: LDH
Calibration Setting
Math Model: K Factor
Factor: 3907.000 Replicates: 2
Acceptance Limits
Cal Time: 24 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	

Limitations

1. Hemolyzed serum will cause falsely elevated serum LD levels.
2. Samples that exceed the linearity limit (1000 U/L) should be diluted with an equal volume of saline and re-assayed. Multiply the results by two to compensate for the dilution.

Calibration

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

Quality Control

The validity of the reaction should be monitored by use of control samples with known normal and abnormal LD values. These controls should be run at least with every working shift in which LD assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Calculation (Example)

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$IU/L = \frac{(A_2 - A_1) \times 1.050 \times 1000}{1 \times 6.22 \times .050\ ml} = (A_2 - A_1) \times 3376$$

Where:

(A_2-A_1) = Change in absorbance
1.050 = Total reaction volume in ml
1000 = Conversion of U/ml to U/L
1 = Light path in cm
6.22 = Millimolar absorptivity of NADH
0.050 = Sample volume in ml

Example: If initial reading $(A_1) = 0.450$
Final reading $(A_2) = 0.480$
$(A_2-A_1) = 0.03$
Then $0.03 \times 3376 = 101$ U/L

Note: For SI units (nkat/L), multiply result by 16.76.

Expected Values[5]

Male	50-166 U/L (30°C)	80-285 U/L (37°C)
Female	60-132 U/L (30°C)	103-227 U/L (37°C)

Due to a wide range of conditions (dietary, geographical, age, etc.) known to affect reference ranges, it is recommended that each laboratory establish its own reference range.

Performance

1. Assay: 0-1000 U/L. Samples that exceed 1000 U/L should be diluted with an equal volume of saline, re-assayed and results multiplied by two.
2. Correlation: A study was performed between the Yumizen 200 series analyzers and a similar analyzer using this method, resulting in a correlation coefficient of 0.999 with a regression equation of y=1.013x + 4.1.
3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[12]

Within Run			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
131.6	4.4	3.4	114.4	2.3	2.0
331.5	6.4	1.9	331.3	7.0	2.1

4. Sensitivity: The sensitivity for the Liquid LD reagent was investigated by reading the change in absorbance at 340nm for a deionized water sample, and serum samples with known LD activities. Ten replicates of each sample were performed. The results of this investigation indicated that on the analyzer used, the Liquid LD reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, a change in absorbance of 0.0001 was approximately equivalent to 1 U/L of LD activity.

References

1. Tietz, N.W., editor, Fundamentals of Clinical Chemistry, 3rd Ed., W.B. Saunders Co., 391 (1987).
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3. Kubowitz,F., Ott, P., Biochem. 314:94 (1943).
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6. Amador, E., et al, Clin. Chem. 9:391 (1963).
7. Buhl, S.N., et al, Clin. Chem. 23:1289 (1977).
8. Gay, R.J., McComb, R.B., Bowers, G.N., Clinical Chemistry, 2nd Ed., W.B. Saunders Co., 657 (1976).
9. Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., W.B. Saunders Co., 657,(1976).
10. Kreutzer, H.H., et al, Clin. Chim. Acta 9:64 (1964).
11. NCCLS Document M29-T2, 2nd Ed. (1991).
12. Young, D.S., et al, Clin. Chem., 21:1D (1975).
13. NCCLS Document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-L7572-100

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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