LACTATE DEHYDROGENASE (LD)

OSR6127	**4 x 40 mL** **4 x 20 mL**	**R1** **R2**
OSR6227	**4 x 51 mL** **4 x 26 mL**	**R1** **R2**

Intended Use
System reagent for the quantitative determination of Lactate Dehydrogenase activity (EC 1.1.1.27) in human serum on Beckman Coulter AU analyzers.

Summary
Elevations in serum lactate dehydrogenase (LD) occur from myocardial infarction, liver disease, pernicious and megaloblastic anemias, pulmonary emboli, malignancies, and muscular dystrophy.[1] A combined analysis of LD and CK (creatine kinase) isoenzymes provides a definite diagnosis of acute myocardial infarction.[2]

Methodology
The LD procedure employs a modification of the method of Wacker et al.[3,4] which utilizes the forward reaction of lactate to pyruvate. Lactate and NAD are converted to pyruvate and NADH catalyzed by LD. NADH strongly absorbs light at 340 nm, whereas NAD does not. The rate of change of absorbance at 340 nm is directly proportional to the LD activity in the sample.

$$\text{Lactate} + NAD^+ \xrightarrow{LD} \text{Pyruvate} + NADH + H^+$$

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final Concentration of Reactive Ingredients:

AMP Buffer, pH 8.9 (37°C)	230 mmol/L
Lactate	70 mmol/L
NAD^+	10 mmol/L

Also contains preservatives.

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation Of Reagents
The LD reagent is ready for use. No preparation is required.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum or heparinized plasma, free from hemolysis, are the recommended specimens. Remove serum from clot as soon as possible to minimize hemolysis.[5] Citrate and oxalate anticoagulants interfere with the assay.

Sample Storage and Stability
The stability of LD in serum at different storage temperatures has been studied by various investigators. Although the results are inconsistent, it has been established that heart isoenzymes are more stable than liver isoenzymes. In any event, it is essential that a standard collection and storage procedure be adopted to ensure consistent results. It is better not to refrigerate or freeze specimens, but to hold it at room temperature (15 – 25°C) with analysis done as soon as possible.[5]

Interfering Substances
Results of studies[6] show that the following substances interfere with this LD procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Lipemia: No significant interference up to 1000 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

Even minimal hemolysis causes significant increase in LD due to high levels of this enzyme in red cells.[1]

Lactate Dehydrogenase (LD)

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[7] for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
LD Reagent

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
Calibration of this lactate dehydrogenase procedure on the AU400/400^e and AU600/640/640^e, is based upon the bichromatic extinction coefficient for NADH, which has a molar absorptivity of 4960 at 340/380 nm. On the AU2700/5400/680/480 it is based on experimental determination of the molar absorptivity at 340/660nm.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in U/L at 37°C.

Dynamic Range

The LD procedure is linear from 25 to 1200 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Expected Values

Adults:[5] 140 – 271 U/L
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the LD Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[9]
Estimates of precision, based on CLSI recommendations,[8] are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines above.

N = 60	Within run		Total	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
101	0.7	0.7	2.2	2.2
417	2.4	0.6	7.5	1.8

Method Comparison[9]
Patient samples were used to compare this LD Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	AU600
Slope	1.04
Intercept	- 0.8
Correlation Coeff. (r)	0.9998
No. of Samples (n)	182
Range (U/L)	48 – 821

Sensitivity
Typical change in absorbance per minute for 1 U/L of LD is 0.09 mAbsorbance at 340/380nm and 0.12 mAbsorbance at 340/660nm**.**

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1987.
2. Roe, C.R., Limbird, L.E., Wagner, G.S. and Nerenberg, S.T., J Lab Clin Med, 80: 577, 1972.
3. Wacker, W. E. C., Ulmer, D.D. and Vallee, B.L., N. Eng J Med, 255: 449, 1956.
4. Amador, E., Dorfman, L. E. and Wacker, W.E.C., Clin Chem, 9: 391, 1963.
5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
6. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
7. Young, D.S., Effects of Drugs in Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
8. CLSI/NCCLS Evaluation Protocol EP5-T2, 1992.
9. Data is on file for specific AU analyzers.

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